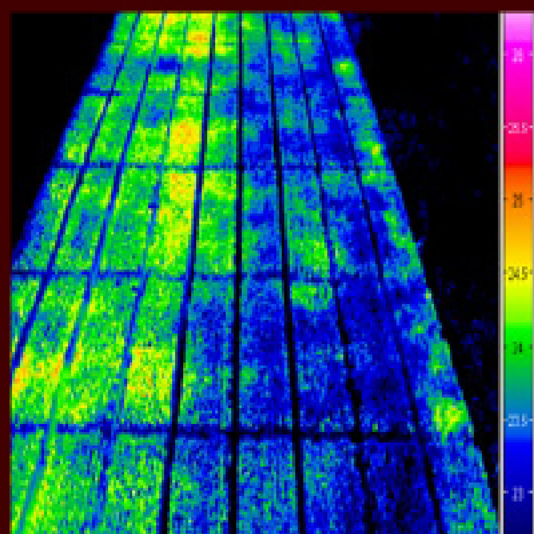
INTEGRATED NEMATODE MANAGEMENT

State-of-the-Art and Visions for the Future



Edited by

Richard A. Sikora
Johan Desaeger
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CABI

Integrated Nematode Management: State-of-the-art and visions for the future

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Dedication

This book is dedicated to the nematologists who went before us, those active today, and to coming generations working to improve integrated nematode management.

Foreword: The need

Cropping systems have changed a lot over the past decades as growers have responded to market demands for increased production of high-quality cheap food and feed. To match this demand, short-term profitability has often been prioritized over the long-term productivity and health of the soil. This has particularly been the case on rented land in many countries where sound agronomic practices are not always implemented. Crop rotations are often short or not used at all due to market demands which has led to a build-up of many soilborne pathogens and pests that are limiting the yield potential in the long term. Crop protection products can in many cases minimize the problems, but particularly for nematodes it has been more and more difficult due to many nematode species being hosts on multiple crops and in some cases also on weed species. Nematode infestations are complex and often difficult to eradicate once they are established in a field. Living below ground and often without visual symptoms above ground these pathogens can be detrimental to the yield and quality of many crops.

Nematode awareness and nematology have developed together over the past hundred years with agricultural trends such as monoculture increasing the yield limiting impact nematodes have had on important crops. Worldwide, nematology expertise was gained and multiplied by universities and independent research organizations as well as private companies. Considering that multiple factors impact on nematode biology, a holistic understanding of agricultural systems is essential to implement management strategies. On one side, the change in research (funding) focus from more applied and holistic research topics to a stronger focus on the mechanistic aspects at molecular and gene level, means that holistic and applied knowledge is more and more hard to find or even worse, lost. On the other hand, new technologies and computer-assisted modelling allow analytics that were not possible or very labour intensive in the past. It is an important goal to pass on the knowledge gained on nematode biology within the agricultural cultivation systems over the past century and to connect it with the new tools we now have in molecular biology, computing and sensing. This provides a very powerful approach to improve nematode damage prediction, nematicide application techniques (including targeted application), resistance management and improving soil health overall. This book is a treasure providing exactly this. It is a collection of state-of-the-art nematology expertise and management knowhow together with ideas and suggestions on how to connect this 'nemato*know*logy' with new tools and capabilities for the future. The editors selected key nematologists from all regions and major crops of the world as authors for this book. Together they share their knowledge in short and to the point chapters, with practical management advice and personal visions on the future of their research area. To fully deploy the technologies, we have available now and in the future, management of nematodes will be even more knowledge intensive than it was in the past. Therefore, capturing and bringing together the knowledge in applied nematology and new technologies is key to resolve the challenges nematode control will face us with in coming years.

Dieter Hofer, Global Product Biology Seedcare Lead
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Steve West, Head Crop Protection Development
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Foreword: An optimistic vision of integrated nematode management

The forward-looking organization of this book makes it especially important at this time.

Nematode management, much like the world as a whole, is at a crossroads, with global changes increasingly becoming apparent. A lack of access to the resources needed to sustain communities in many parts of the world, once evident mainly by localized famine and extreme poverty, now threatens people everywhere with increasing conflict, mass migration and zoonotic plagues. A recurring consensus among authors throughout this book that rapid climate change will further destabilize access to arable land and water, increase nematode population growth and crop loss, and rearrange soil food webs including the nematode pests faced by farmers, lends further urgency to the need to optimize farm management with respect to impediments and opportunities that will transform cropping systems.

Integrated Nematode Management: state-of-the-art and visions for the future, is exactly what it says: a practical guide to nematode management, not a comprehensive review of management research. It is remarkable for its scope, 65 chapters, each carefully edited to focus on current integrated nematode management tactics and strategies combined with a vision of targeted research needed to better manage nematodes now and in the future.

The strategies detailed here across so many cropping systems reinforce the fact that management principles have changed little over the decades. To the extent possible in small-plot, low-value, or organic cropping systems, exclusion, resistance/tolerance and crop rotation remain the preferred means to mitigate nematode losses. Few direct costs are incurred by the farmer, nor hidden costs by the environment – with the critical exception of habitat loss to compensate for modest yields in some of these systems. The higher returns from each hectare gained by the regional monocultures and high-value systems described in the book, continue to require expensive on-farm inputs that are less sustainable in terms of soil quality, water purity, biodiversity and many natural resources. There are abundant practical suggestions (e.g. increased marker assisted breeding and gene stacking, greater reliance on nematode detection and crop loss/systems modelling, improved seed treatments, refinement of site-specific management tactics and increased field validation research) for near-term improvements to low and high input systems. And an emphasis in many chapters on the extraordinary pace at which genetic and computational sciences continue to advance, reinforces the belief that truly innovative changes to low-input integrated nematode management tactics are no longer futuristic dreams. Unravelling the nature of nematode–host communications that modulate susceptibility and resistance is already increasing the discovery and utilization of resistance genes. Increased application of metagenomic tools and artificial intelligence to current research programmes will provide growers with faster, more accurate plant parasitic nematode identification and decision tools, by revealing the food web in its entirety, the evolutionary relationships of its inhabitants and the myriad interactions comprising mechanisms by which soil suppressiveness operates.

The unambiguous need to accelerate food production without further depleting uncultivated habitats bodes well for achieving the improved integrated nematode management programmes envisioned in this book. Important nematological deficiencies noted by many authors and summarized by the editors in the final chapter, such as insufficient focus on resistance development beyond a few crops and the insufficiency of nematology positions, can be resolved in the main by increased funding. The cost to wealthy countries for international development that can adequately mitigate the global crises mentioned above will eventually reach levels that demand close scrutiny to identify the most profitable investment targets. Agricultural development will top that list and integrated nematode management will increasingly resemble many of the programmes envisioned in these chapters.

Professor Larry Duncan, University of Florida, Gainesville, Florida, USA
President IFNS – International Federation of Nematology Societies

Preface

Our first goal in producing this book was to make known the magnitude of plant parasitic nematode induced crop loss facing the agricultural community. The second objective was to present currently used and recommended integrated nematode management (INM) practices and the third to outline future improvements to management and finally to anticipate the future of INM in the expert's area of interest.

We believe it is indisputable that there is a lack of understanding of the broad dynamics of INM practices either being applied, or not being applied, to agricultural crops on a global scale.

A number of excellent books have been written in the past that gave in-depth reviews of the principles of nematode management and that described in detail many of the then available technologies for nematode management. Since their publication, new nematode problems have emerged and abiotic and biotic drivers affecting damage have come to light. In addition, major technological advances have been made that will alter the design of INM to meet the challenges of the future.

A modern scientific book that gives a comprehensive state-of-the-art overview of the losses caused and the management approaches used to reduce nematode damage on crops globally is presently unavailable.

As new technological advances evolve and are refined, new tools are made available for improvement of INM. The incorporation of innovative technology into integrated management programmes has and will continue to modernize phytonematology at a speed not anticipated in the recent past. We attempted to demonstrate how this process will strengthen the impact INM has in improving agricultural production and plant health. With this book we hope to present scientifically based information that fills these knowledge gaps.

Vision is important in the development of INM in both applied and basic fields of nematology science as well as from an industrial perspective. Anticipating future changes in monitoring nematode distribution, population dynamics, pathogenicity, cropping systems, resistance breeding, pesticide use patterns, food habits, social norms, and the impact of environmental factors will all influence how INM is conducted in the future. In this book we asked the experts to anticipate where INM needs to be strengthened until the year 2050 and beyond.

The editors invited leading experts in the field of nematology based on their qualifications and their contributions to INM. We did our best to develop a broad consortium of experts from different parts of the world working on a broad array of crops and species of plant parasitic nematodes. We hope this broad spectrum of expertise demonstrates the differences in loss caused by nematodes, contrasts in INM approaches worldwide and the different ways being used for management. We realize there are many excellent specialists working with INM who could have added even more information to the book and we are sorry we could not include everyone in this endeavour.

The editors decided that the book would avoid the traditional literature review approach, used in many books, and request the authors to: (i) write short and focused chapters; (ii) keep literature citations to a minimum; (iii) present currently used INM practices; and (iv) outline recommendations for improvement. Our goal is to combine practical field experience and research expertise while recognizing from the start that the book would never be all-inclusive.

We selected authors working with economically important food, feed and industrial crops grown in the temperate, subtropical and tropical climatic zones across five continents. The plant parasitic nematodes selected included different forms of parasitism, including ecto-migratory, endo-migratory as well as sedentary parasites in an attempt to expose the readers to the diversity of nematode problems facing growers worldwide. The book is divided into nine distinct sections: principles of INM, field crops, legumes, fruit and nuts, vegetable crops, roots and tubers, emerging technologies, constraints and conclusion. Because reference was made to the impact of climate change on INM in almost all the chapters we attempted to look at the complexity of climate change and climate volatility and how it will affect nematodes and INM in the future. The book ends with an outlook chapter written by the editors that attempts to bring together the main highlights presented in the chapters.

Although the majority of chapters deal with medium to large holder agriculture, we have also included chapters dealing with the problems facing the 580 million smallholder farmers in many countries around the world. In the end, 82 authors, from five continents, and 19 countries have outlined the state-of-the-art of nematode management in important nematode–crop interrelationships in their area of expertise. We want to thank them for their commitment and for their efforts in making this book possible.

We believe the knowledge presented in the chapters in this book is highly relevant to INM worldwide and important for the food and value chain and food security globally. We hope the book will influence decision makers who we believe need to maintain a balance between applied and basic nematology in the future structuring of departments devoted to plant health. Above all, we hope that those working in the field in INM, as well as students and experts working in industry and as extension experts will find inspiration in the shared experiences and ideas described in the very diverse chapters in order to further develop INM.

The Editors

Acknowledgements

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Sincere thanks goes to scientists from around the world who have worked on integrated nematode management and whose information and insights were used by the authors and editors in developing the chapters in this volume.

Many thanks also go to the staff of CAB International, especially Rebecca Stubbs for their support and patience during the complex process of compiling this book in what we feel is record time.

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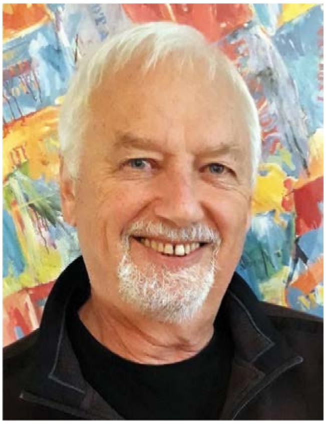
About the Editors



Richard A. Sikora is a University of Bonn, Professor Emeritus, who has published numerous books on nematology and plant protection including the recent third CABI edition of *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture* in 2018. He has worked in nematology for 53 years as a teacher and researcher. He has published, together with his team of students, over 300 peer reviewed publications and has edited five scientific books. Richard has trained over 150 PhD and MS students in basic and applied nematology with many coming from the tropics and subtropics. During his career he focused on integrated nematode management and the development of innovative tools to improve management including: biological and chemical control, remote sensing and different aspects of resistance and induced resistance. Richard has collaborated globally with nematologists in over 30 countries on INM in a wide array of crops and in promoting capacity building.

Johan Desaeger is currently a nematologist at the University of Florida. He started his nematology career in 1994 in western Kenya working at the International Center for Research in Agroforestry where he was responsible for nematode research in subsistence farms. After completing his PhD in Belgium in 2001, he moved to the US to work on nematode management at the Universities of Florida and Georgia. He made the leap to industry in 2005 when he joined the late DuPont company to start up a new nematology programme and develop new nematicides. Johan came back to Florida in 2016 to join the Department of Entomology and Nematology faculty at the University of Florida and start a new research team at the university's Gulf Coast Research and Education Center near Tampa, Florida. His research, extension and educational work now focuses on integrated nematode management in fruit, vegetable and other high-value crops.

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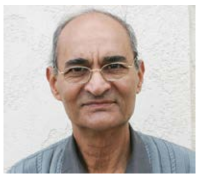

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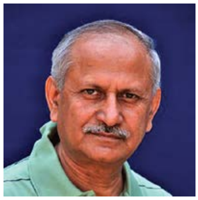

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SECTION I:

Introduction

1 Integrated nematode management and crop health: Future challenges and opportunities

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Introduction

Agroecosystem drivers and constraints

It is now estimated that the world needs to increase food production by at least 2% every year across all crops to ensure an adequate food supply for the world's growing population. With the biotic and abiotic factors impacting food production now and in the future, all forms of crop production improvement will be relevant for food security for the generations to come. Those working in nematology have an important role to play in securing an adequate food supply for the world.

Drivers and constraints, both natural and human induced, impact agroecosystems in many ways. Drivers are usually natural phenomena that can cause major shifts in agricultural ecosystems and include factors such as human population dynamics, climate, water and minerals availability, energy usage and globalization. Drivers are for the most part, not easily influenced or managed. Constraints on the other hand, are often induced by humans and are to some extent manageable (fertility, soil quality, tillage, pest management amongst others). Both drivers and constraints impact world agricultural production and exert more pressure than ever on the issues facing food security and human well-being (Sikora *et al.*, 2020). Insects, pathogens, weeds and nematodes are serious constraints, and integrated pest management (IPM) and integrated nematode management (INM) need to play a major role in improving food security in the future. Plant parasitic nematodes are major constraints and part of the solution to sustainable food production. INM has contributed extensively to world food security by ensuring higher yields on a broad array of crops. Nematology has made major advancements in improving food production and will remain important as the above constraints affect crop health and risks to the farmers we serve.

The big giveaway

Nematology as a science needs to have a strong INM response to these constraints as they become more severe and human demand for food expands. The dilemma between uncontrollable and manageable drivers and constraints will

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require a high degree of scientific vision for the development of technologies for risk improvement in INM. Adaptation of INM to confront these constraints will be the major challenge of future nematological research.

Plant parasitic nematodes are part of what has been called the 'big giveaway' with regards to global food insecurity (Sikora *et al*., 2020). Farmers around the world feed, unwillingly, a large proportion of the food they produce to insects, nematodes and diseases without any compensation (Savery *et al*., 2019). Plant parasitic nematodes have been calculated to reduce up to 10% of the world's agricultural output, causing economic losses valued at over US$125 billion each year (Chitwood, 2003).

Our inability to adequately control many nematodes makes them a significant part of the 'big giveaway'. It is to some extent incomprehensible that phytonematology, which evolved as a science to reduce nematode losses to food crops through research and management in the late 1800s, has still not reached this goal for many crop–nematode interactions after 20 decades of intense research. Reliance on chemical control and even fumigation is still common in many high-value crops. Of course, major strides have been made in reducing crop losses caused by a number of nematodes, but there is still much to do to shift from what could be called a direct control mode to a true INM mode in many crops.

Integrated pest management and integrated nematode management

The term integrated pest management was explicitly defined by FAO in 1968 as 'Integrated pest control is a pest population management system that utilizes all suitable techniques in a compatible manner to reduce pest populations and maintain them at levels below those causing economic injury' (Smith and Reynolds, 1966). Today there are a multitude of definitions, but all have the same target – crop health.

IPM is an umbrella term that includes management of insects, diseases, weeds, nematodes, crop production and social economics (Bajwa and Kogan, 2002). Of course, the word integration means different things to different groups: economics, environment, pest populations, control, tactics and ecology in that order were most frequently found in the different definitions. Kogan (1998) defined IPM as: 'a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts on producers, society, and the environment'. Duncan and Noling (1998) stated that IPM is an integral part of sustainable agriculture, and this combination of actors is more important now than ever when the drivers and constraints listed below are reflected upon.

Integrated nematode management

INM was defined by Bird (1981) as the integration of research, development, technology transfer and implementation needed to integrate two or more control procedures to manage one or more nematode species. The definition gives a clear outline of the concept of INM. INM is often expanded to include specific crops, control methods, production practices and one or more plant parasitic nematodes either alone or present concomitantly within a cropping system. Nematode management, however, is not static but changes as external drivers and constraints impact crop growth, as nematode problems evolve, and as grower needs shift in importance with the development of new technologies (Sikora and Roberts, 2018). The concepts and components of INM have been reviewed in depth in earlier publications (Brown and Kerry, 1987; Luc *et al*., 1990; Barker *et al*., 1998; Whitehead, 1998; Chen *et al*., 2004).

INM now and in the future

In this book we have attempted to move away from the approaches taken in earlier texts and tried to direct attention to what we believe is practical INM. The chapters outline INM approaches used by growers on a daily basis across a wide spectrum of food crops that are parasitized by economically important nematodes around the world.

The authors of the chapters were asked to present state-of-the-art approaches to INM on a wide range of crops in an attempt to show shifts in INM problems and current solutions available. They were also asked to present their vision of the future of INM. The visions they presented and those of the editors are discussed in the outlook chapter (Chapter 65) at the end of this book.

Stimulating anticipation was also part of the challenge given to the authors. We wanted to capture the visions of the experts on anticipated changes in nematode–crop interactions and driver induced constrains to production. We also asked them to outline needed structural changes in INM through 2050 and beyond. The same challenges were given to the authors of the chapters covering advanced technologies.

INM – Pillars of strength

INM is a complex system of both independent and interdependent measures of management presented as pillars with specific management tools in Fig. 1.1. The five major pillars of INM used here are: crop rotation, cultivar choice and establishment, soil management, targeted control, and monitoring and evaluation. Each pillar is constructed of numerous control tools that prevent, reduce populations or enhance plant tolerance to nematode damage as well as provide tactical tools to make INM more effective. Many of the pillars and tools listed here are also discussed in the reviews and books mentioned above. The number of tools in each pillar and their applicability for use varies with nematode–crop interactions, between production regions and with farm financial status around the world as seen in many of the chapters in this volume (see Chapter 63).

Building blocks – complexity at work

The building blocks of INM that make up the pillars have been important in the development of management strategies in the past and their importance will increase as fumigants and some non-fumigant nematicides are gradually removed from the market due to their negative impact on the environment. The building blocks of INM are shown in different colours connected to the respective central pillars of INM, and are discussed below with their advantages and limitations.

Prevention

The most effective nematode control tool is prevention of introduction and spread of plant parasitic nematodes. Certified seed and planting material is a highly effective tool used to prevent invasive nematode species from expanding their range. Quarantine policies are aimed at keeping new species out of an area for as long as possible. However, the strict regulations that require every positive detection of a nematode on a quarantine list in a field to be reported to the authorities has had the opposite effect. Growers and their advisers want to avoid a formal infestation for as long as possible. They will even stop intensive sampling if notification of an infestation is required, which results in a more rapid spread of the problem. A better approach to ensure maximum delay of nematode dispersal would be a system whereby growers detect infestations early through intensive soil sampling so that they can take immediate action. This approach is not part of most governmental plant protection organizations' policies.

Crop rotation

Policy makers often live under the assumption that nematode problems are preventable by expanding the length of rotations with non-host crops. This is true only for nematode species with narrow host ranges such as cyst nematode species. For polyphagous species such as those of the genera *Meloidogyne* and *Pratylenchus*, it is not the frequency of cultivation of a crop that is decisive but the makeup of the cropping sequence. Farmers also tend to overlook the fact that some green manure crops are good hosts. This fact needs to be taken into account when constructing rotation schemes.

Cultivar selection

Crop cultivars with resistant or tolerant traits are available and offering a good option to build an effective INM system. However, most breeders search for resistance in crops in a rotation with known nematode problems. They usually overlook potential resistance in other crops in the rotation

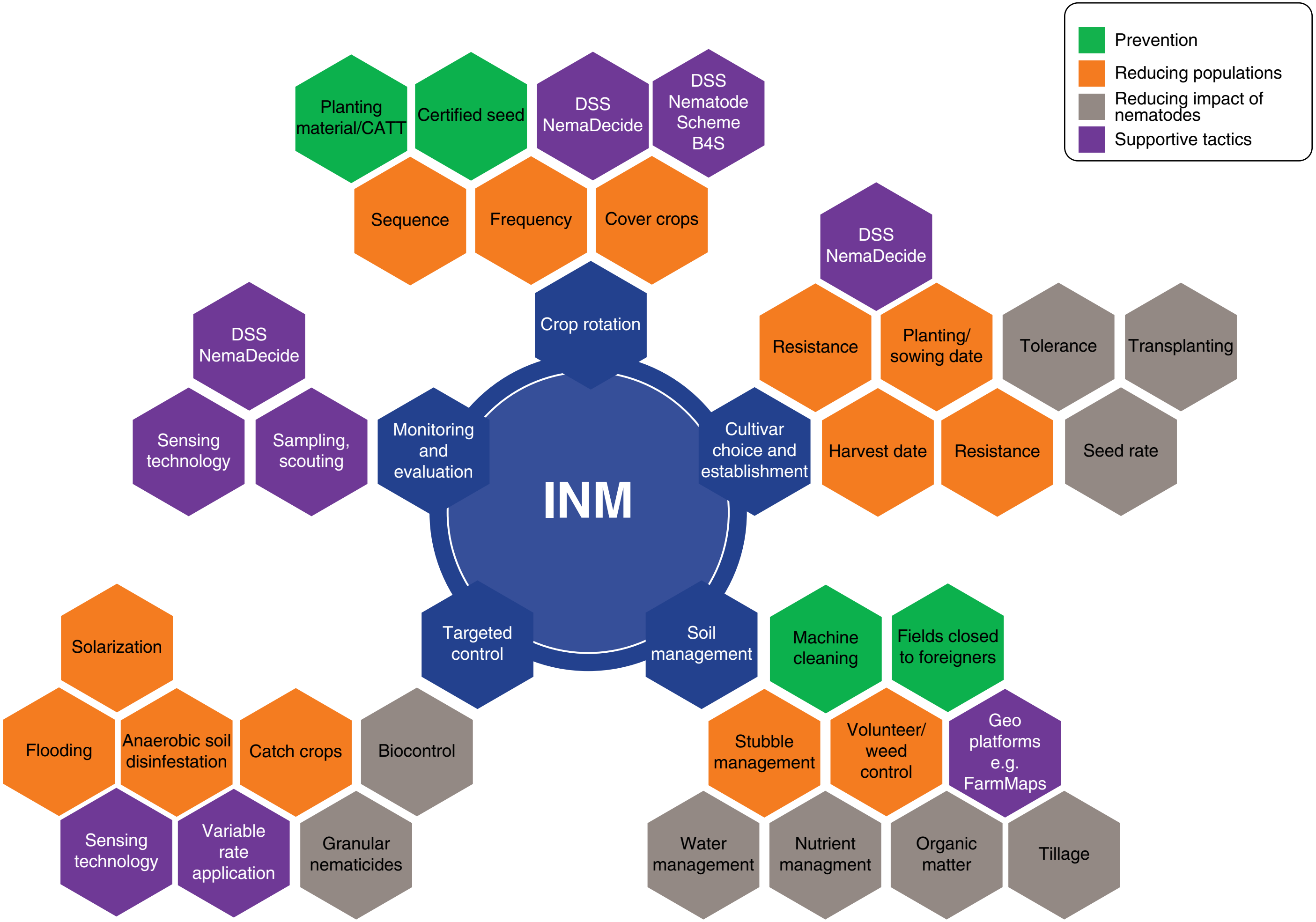

Fig. 1.1. The five pillars and management tools available for integrated nematode management. The pillars of INM are rotation, cultivar choice, soil management, targeted control, and monitoring and evaluation (blue). The management tools making up the pillar are divided in their mode of impact: prevention of nematode introduction (green); reduction of nematode population densities (orange); improvement of crop tolerance (grey); and supportive tools and tactics (purple). Figure courtesy of Wageningen University & Research, Field Crops.

sequence planted prior to the main susceptible crop. Unfortunately, the concepts of resistance and tolerance cause much confusion among farmers. Resistance means that a nematode species cannot reproduce, or that reproduction is significantly decreased when compared to a susceptible cultivar. Conversely, tolerance describes the damage a crop suffers as a result of a nematode infection. A tolerant cultivar shows little damage compared to a sensitive cultivar. All combinations of the two are possible: there are cultivars that allow reproduction but do not allow yield loss, as well as cultivars that show significant drops in yield but do not allow nematode reproduction. A tolerant crop can be a potential danger in a farming system because high nematode infestation levels develop undetected. It is important for growers to understand the definition of crop resistance versus crop tolerance as it relates to a nematode–crop interaction. The use of resistant/tolerant cultivars should be coupled with resistance management to prevent the development of virulent races or pathotypes. Sometimes damage can be prevented by choosing a sowing and harvesting date to escape early root infection of a nematode species or to avoid development of late season generations, through early host removal.

Soil management

Optimal growing conditions help crops to endure nematode damage. Therefore, proper soil management is an important pillar of INM. Nutrient and water management, proper tillage and organic matter content are all basic conditions for a healthy crop, a condition that is not standard around the world. A strong plant is more resilient to pest and disease impacts.

Targeted control

In some situations it is necessary to intervene in a targeted way. In such a case the standard tools of INM as presented in the pillars are not effective and targeted control is necessary. In the past, synthetic nematicides were the starting point of targeted control in INM. This view is no longer tenable when we aim to build a sustainable agricultural system. Nematicides are still an important measure within INM, but they are no longer the starting point but rather the safety net in difficult situations. Nematicides should no longer be used preventatively, but only used in situations where nematode populations have exceeded damage threshold levels as determined through sampling or crop damage observations. Targeted variable rate application within the field where necessary is increasingly becoming the favoured approach. In addition to chemical and biological agents, there are also physical techniques such as solarization, inundation and anaerobic soil disinfestation that can be utilized.

Monitoring and evaluation

The monitoring and evaluation pillars are at the same time the first and final step in an INM system. Knowing what species are present at what infestation levels make it possible to begin to develop an INM plan and to check whether the results of INM are satisfactory or need restructuring. Historical information of a field is a good starting point. Once a nematode species has been introduced into a field, it will typically stay there below or above detection levels.

Sampling

Evaluation of the soil and observation on the health of root systems are other important sources of information. During harvest of root crops like potato, carrots and sugar beet, a great deal of information can be gathered by looking for symptoms on the harvested product as well as on the root system. During the growing season, plant canopy development can tell the farmer that there is a problem developing under the soil surface. Examination of the root system of randomly selected plants for nematode damage is therefore a valuable tool in INM. Canopy surveillance by satellite, drones or other techniques for remote sensing is becoming increasingly important in farming operations.

Decision support systems (DSS)

There are very few DSS on the market or in the public domain that can be used by farmer extension agents for keeping track of the large amount of data accumulated for proper selection of the tools needed at any specific time. Useable DSS programmes and trained extension agents for complex cropping programmes will be needed (see Chapter 60).

Communication

Integrated nematode management and knowledge transfer go hand-in-hand. INM is knowledge intensive so effective knowledge exchange is crucial. It is important to share 'success stories' within the farm community. Communication should be interactive and traditional methods such as on-farm demonstrations and field training should be supplemented with websites, blogs and webinars. Data visualization tools and easy to use DSS such as smartphone apps help to transfer validated information and discover knowledge gaps or misunderstandings. The use of geographic information systems to visualize the available information on a farm map improves the farmer's understanding of the situation (see Chapter 60). Economic analysis of different measures in different cropping systems will help to judge if a given measure fits in the economic goals of the farm. Communicating with farmers without a realistic outlook on cost/benefits is a waste of time. By presenting the right data and combining it with relevant knowledge, a farmer can make well-founded decisions.

INM rated by degree of integration

In the real world, INM in the field is often decoupled from the goals set in the definition. In other words, there is a difference between what the term INM suggests and the actual on-farm approaches utilized by growers. In many cases a simple monoculture or two-crop rotation is considered an integrated system. What is recommended by scientists is also not always what farmers incorporate into their production system. This is the case for many crop–nematode combinations, especially for smallholder agriculture on a worldwide scale. The term *one size does not fit all* needs to be considered when recommending INM to large diverse farm communities (see Chapter 63). There have been major advances in the science behind INM. Some of these technologies might even justify using the alternative term *intelligent nematode management*. However, nematode management in the future will require advances over the present state-of-the-art systems and will require the incorporation of advanced technologies into nematode management programmes. Some of these new technologies have been outlined in a number of chapters in this volume.

Biotic equilibrium versus equilibrium shifts

The physical and chemical characteristics of a soil determine its quality/fertility, whereas the biological characteristics determine the impact of soil biodiversity on root and plant health. In other words: soils don't get sick – plants get sick!

Crop health from a plant pathology viewpoint is complex and starts with root health. The biotic equilibrium and shifts in biological balance between nematodes and antagonists in the rhizosphere are important control mechanisms regulating soil and root infection levels. Soil is not just a stacking of mineral parts mixed with organic matter but is full of life; it is a complete ecosystem in itself. Nematodes that cause soil-borne diseases are mostly in the minority in this ecosystem, which also includes numerous species of fungi, bacteria, insects and protozoa. Because these species also interact with each other in ways that could lead to even more severe plant damage, it is important to develop a strategy that attempts to manipulate the biotic equilibrium toward improved pest and disease suppressiveness as outlined in a number of chapters.

Soil quality usually refers to nutrient equilibrium, whereas the biotic component regulates pest and disease impact on root health. The latter component, that is the antagonistic potential in a soil, is often sufficiently active to reduce nematodes and diseases below damage threshold levels in what is generally called suppressiveness. Developing and implementing an INM strategy that takes natural nematode suppression into consideration is urgently needed. INM must be coupled with maintaining soil quality, suppressiveness and fertility. An imbalance in these factors is behind problems with root health and crop loss. A lack of awareness and knowledge of this complex interrelationship can lead to a reflex or reactive 'management per incident' approach, which can result in the improper use of biological or chemical inputs.

At the crossroads of change

We are at a crossroads when it comes to the future of INM. Agricultural production is being confronted with new constraints that did not exist a few years ago. Public awareness, the need to improve the agricultural north–south production discrepancy, the loss of biodiversity, short-term impacts of climate volatility and long-term impact of climate change were factors seldom considered in the past when developing INM. As stated above, the pillars have limitations. These limitations need to be offset with new alternatives, developed through nematological research, as presented in some of the chapters in this book. The development of new technologies offers great potential to improve INM for a wide array of crops. Research is needed to adapt and integrate them into existing or new INM programmes. Intelligent or creative management is the direction in which farmers, extension workers, students and scientists are directing their efforts to find new solutions for nematode management. Many examples can be found in the chapters to come.

The future holistic approaches and collaboration

In some cases there are major differences in what is recommended by nematologists and what farmers ultimately incorporate into their management programmes. The term INM, therefore, is often used when integration either does not exist or is insignificant in degree.

With this said, we believe the future priority of nematology needs to be directed at placing greater emphasis on the development of new innovative and environmentally safe management tools and the integration of these tools into DSS as outlined in Chapters 56 to 62. The present and future impact of climate variability on nematodes and crop production will require new approaches and farmer support systems (see Chapter 64).

Economic analysis of INM tools used in different cropping systems will help to judge if a given measure fits in the economic goals of a farm operation. Convincing farmers to incorporate risk-management concepts that target nematode population levels over time is needed to offset the impact of abiotic stresses such as severe drought stress or oppressive heat brought on by climate change and variability. Developing INM for risk management will be a major challenge to nematology in the future.

The complexity of nematode interactions in the soil with soil biota and with each other causes severe problems. Their control requires persistence, motivation, and solid collaboration between actors in the food production chain and also between fundamental and applied research, so as to completely understand the underlying mechanisms of this complexity. This interaction is becoming rare as scientific fields become more specialized. Support for applied research and an increase in extension personnel is needed in many places in the world to accomplish these goals. Practical knowledge on INM across a broad array of crops and within different countries adds knowledge to the system as outlined in this book.

Finally, it is our conviction that INM needs to be amalgamated with other management programmes including weed, pathogen, insect, soil and farm management to finally attain the goal of a sustainable food production system (Fig. 1.2). The need to move INM towards a more holistic agroecosystem DSS as discussed

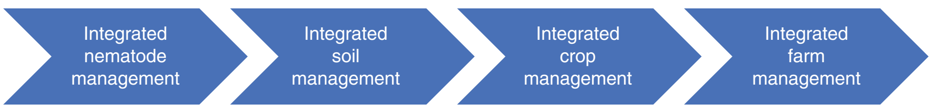

Fig. 1.2. The different levels of integration making up integrated farm management programmes. Author's own figure.

by others is needed (Duncan and Noling, 1998; Sikora and Roberts, 2018). Practical examples of DSS that are holistic in approach are presented in this volume. These types of approaches need to be developed for other crops in the future.

The chapters in this volume that cover nematodes, management approaches, crops and climatic regions, while certainly not all-inclusive, will give the reader an overview of the diversity and complexity of cropping systems and nematode problems farmers around the world are confronting. It was our goal to provide stakeholders involved in INM with practical and personal testimonies from some of the world's top nematology experts. Hopefully, the cross-fertilization resulting from this global mix and decades of nematological knowledge will lead to new ideas and insights that will enable us to meet the challenges we face today and tomorrow.

Acknowledgements

The editors want to thank Marleen Riemens and Edward Sikora for their support in reviewing and adding to the quality of the chapter.

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SECTION II:

Field Crops

2 A triumph of tolerance: Managing the threat to wheat production by the root lesion nematode *Pratylenchus thornei* in the subtropical grain region of eastern Australia

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Introduction

The root lesion nematode *Pratylenchus thornei* is a severe and widespread threat to wheat (*Triticum aestivum*) production in the subtropical grain production region of eastern Australia. Yield loss of up to 65% has been counteracted by research efforts to produce integrated nematode management (INM) through understanding the biology and host range of *P. thornei* in farming systems, plant breeding and characterization of genotypes for tolerance and resistance traits, and communication with the farming community. The widespread adoption and success of INM has been driven by the release and on-going improvement of wheat cultivars with tolerance to *P. thornei* which deliver immediate economic benefits to grain growers.

Economic importance

Pratylenchus thornei was first detected in the subtropical grain region of eastern Australia in the 1960s. Its distribution closely followed the expansion of wheat production which began in 1860 in southern Queensland (described in Thompson *et al.*, 2010). Thompson *et al.* (2010) hypothesized that the change to barley (*Hordeum vulgare*) production from wheat in the 1930s was related to crop tolerance to *P. thornei* rather than a decline in soil nitrogen fertility, as originally suggested.

In the late 1970s, Queensland grain grower, Mr Alex Gwynne, asked Dr John Thompson to determine the cause of poor yield of wheat when grown as the second crop after long fallow, compared to barley which yielded up to three times that of wheat (J.P. Thompson, Toowoomba, 2020, personal communication). Subsequently, *P. thornei* was shown to be the cause — building up on the first wheat crop to severely damage the second. Mr Gywnne's insight revealed differences in tolerance between cultivars of wheat and barley and to this day he and his family continue to support research on their farm to help counteract the economic consequences of *P. thornei.* Mr Gwynne's advice to all growers in the region is 'Assume that you have these

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nematodes and grow the most tolerant wheat cultivar available' (A. Gywnne, West Prairie, 2012, personal communication).

In 2009, the estimated annual potential cost of lost wheat yields in the region was AU$104 million (Murray and Brennan, 2009). The use of tolerant cultivars and crop rotation reduced the estimated actual cost to AU$38 million. In 2018, a new economic analysis (Brennan and Murray, unpublished) estimated this cost to be AU$31 million based on the increased use and availability of tolerant wheat cultivars. For example, in 2010, 21% of bread wheat cultivars recommended for the region were moderately tolerant whereas in 2020, 52% were moderately tolerant or more so.

There is a negative linear relationship between yield of wheat cultivars and *P. thornei* population densities measured at sowing (Thompson *et al.*, 2012; Owen *et al.*, 2014). The degree of yield loss is greatest for cultivars that are intolerant and susceptible compared to cultivars that are tolerant and moderately resistant (Whish *et al.*, 2017). Yield loss of intolerant wheat cultivars is expected in all seasons, but the extent varies with site and seasonal conditions.

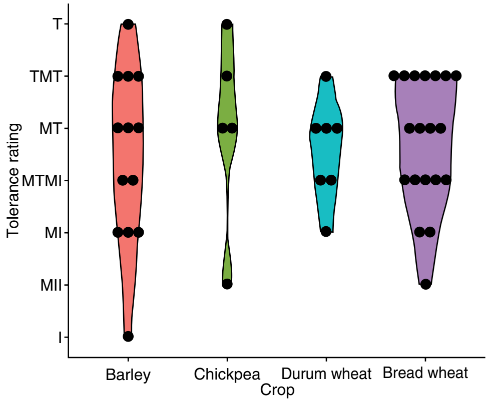

Fig. 2.1. The range and number (indicated by black circles) of cultivars within each *Pratylenchus thornei* tolerance category of commercial cultivars of barley, chickpea, durum wheat and bread wheat recommended for Queensland within the subtropical grain region of eastern Australia in 2020 (Albatross Rural Consulting (2019) 2020 Queensland winter crop sowing guide, available at www.nvtonline.com.au, accessed 13 August 2020). Categories are based on relative tolerance within a crop. T, tolerant; MT, moderately tolerant; MTMI, moderately tolerant–moderately intolerant; MI, moderately intolerant; I, intolerant. Author's own figure.

Host range

Pratylenchus thornei has a wide host range. The response of cultivars to infestation can be classified by their level of tolerance and resistance which are genetically independent traits. Each trait should be classified separately to maximize the effectiveness of INM. Tolerance is generally assessed in major crops and where there are tangible economic losses.

The diversity of both summer and winter crops grown in the subtropical grain region of eastern Australia is a double-edged sword for INM. Growers can plant non-host crops, such as grain sorghum, sunflower or oats, but susceptibility is high in most cultivars of many other profitable cereal and pulse crops, such as barley, chickpea or mungbean. Resistance and tolerance vary between cultivars within many crop species (Table 2.1 and Fig. 2.1). In response to these challenges, the Grains Research and Development Corporation funds a national programme of comparative crop variety testing called National Variety Trials. Ratings of tolerance and/or resistance to *P. thornei* of current cultivars and advanced lines of wheat, durum wheat, barley, chickpea, faba bean, field pea and oats are derived from repeated experiments and updated annually in consultation with breeding companies and researchers.

Distribution

The subtropical grain production region of eastern Australia covers an area of approximately 4 million ha between latitude 20–32°S (described in Thompson *et al.*, 2010). Typically, the soils are deep cracking clays called vertisols with a neutral to alkaline pH. In a survey of nearly 800 wheat fields in the region, *P. thornei* was more prevalent and abundant than *P. neglectus*. It was found in 67% of fields whereas *P. neglectus* was found in 32% (Thompson *et al.*, 2010).

Table 2.1. The range of resistance responses to *Pratylenchus thornei* of cultivars of grain crops grown in the subtropical grain region of eastern Australia. Author's own table.

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<tr><td></td><td></td><td></td><td colspan="4">Maize
Zea mays</td></tr>
<tr><td></td><td colspan="2">Millet[a]</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td colspan="4">Oats
Avena sativa</td><td></td><td></td></tr>
<tr><td></td><td colspan="4">Sorghum
Sorghum bicolor</td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td></td><td colspan="3">Triticale
xTriticosecale</td></tr>
<tr><td>Pulses</td><td></td><td></td><td></td><td colspan="2">Blackgram
Vigna mungo</td><td></td></tr>
<tr><td></td><td colspan="6">Chickpea
Cicer arietinum</td></tr>
<tr><td></td><td></td><td></td><td></td><td colspan="3">Faba bean
Vicia faba</td></tr>
<tr><td></td><td colspan="5">Field pea
Pisum sativum</td><td></td></tr>
<tr><td></td><td></td><td colspan="5">Mungbean
Vigna radiata</td></tr>
<tr><td>Oilseeds</td><td colspan="2">Canola, mustard
Brassica spp.</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td colspan="2">Linseed
Linum usitatissimum</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td colspan="5">Soybean
Glycine max</td></tr>
<tr><td></td><td colspan="2">Sunflower
Helianthus annuus</td><td></td><td></td><td></td><td></td></tr>
</table>

R, resistant; MR, moderately resistant; MRMS, moderately resistant–moderately susceptible; MS, moderately susceptible; S, susceptible; VS, very susceptible.

[a]*Panicum miliaceum*, *Echinochloa* spp., *Pennisetum glaucum*, *Setaria italica*.

Symptoms of damage

In wheat, *P. thornei* causes symptoms of lower leaf yellowing, decreased tillering, poor canopy closure and generally unthrifty plants (Fig. 2.2). These symptoms are associated with intolerance/tolerance responses, decreased N and P concentrations of the plant tops, decreased tillering and biomass, and grain yield loss (Thompson *et al.*, 2012). Recently, the normalized difference vegetation index of plant greenness was shown to be highly correlated with tolerance to *P. thornei* when measured at approximately 'early boot' development stage (Robinson *et al.*, 2019).

Biology and life cycle

The survival and reproduction of *P. thornei* is favoured by the soil types and cropping systems of the subtropical grain region of eastern Australia. Rainfall is summer dominant; however, both

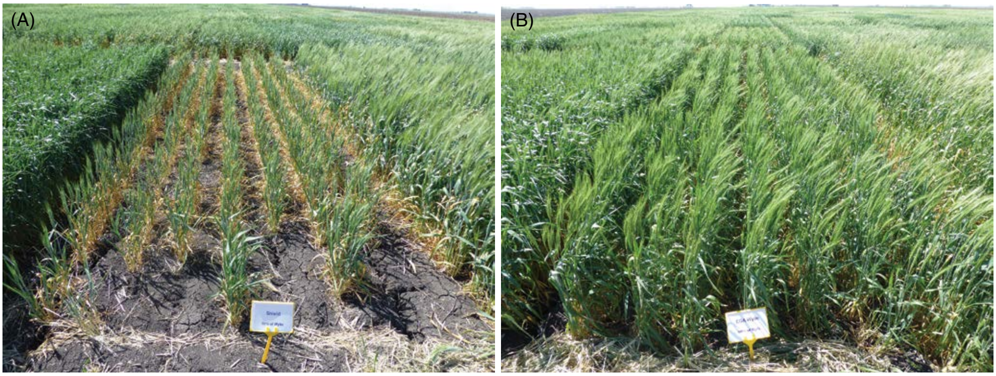

Fig. 2.2. Symptoms of *Pratylenchus thornei* infestation of **(A)** an intolerant wheat cultivar compared to **(B)** a tolerant cultivar. Author's own photographs.

summer and winter crops can be grown because the soils have a high capacity for storing water during weed-free fallow periods. *Pratylenchus thornei* passes through several generations during growth of a wheat crop. The rate of reproduction in the roots and final population densities are controlled by genotype and the environment (Thompson *et al.*, 2015). The maximal intrinsic rate of nematode population growth is genetically controlled in wheat and resistance mechanisms are likely to be produced constitutively and act post-penetration of *P. thornei*. Soil temperature strongly influences the reproduction rate of *P. thornei* with the optimum between 20–25°C, very slow reproduction at 15°C and none at 30°C (Thompson *et al.*, 2015). By modelling soil temperatures, Thompson (2015) demonstrated that sowing as early as possible, within the recommended sowing window, would allow wheat roots to establish before nematode infestation; the yield of an intolerant cultivar was predicted to increase 61% by sowing 4 weeks earlier.

Pratylenchus thornei can occur throughout the soil profile, with peak population densities found at differing depth intervals dependent on prior cropping history and seasonal conditions (Owen *et al.*, 2010; Whish *et al.*, 2017). Population densities averaged over 0–90 cm soil depth are a more reliable predictor of grain yield loss than shallower or deeper samples (Owen *et al.*, 2014). Increases in populations deep in the soil profile after fallow periods may be due to nematode movement in percolating water after substantial rainfall (Owen *et al.*, 2014; Whish *et al.*, 2017).

Populations decrease more quickly in the topsoil than deeper in the soil profile during fallows or when non-hosts are grown (Whish *et al.*, 2014). The decline of *P. thornei* is related to the rate at which soil dries and temperature (Thompson *et al.*, 2015). In soil subjected to quick drying, a greater proportion of J4 nematodes survived compared to other nematode life stages (Thompson *et al.*, 2017).

Interactions with other nematodes and pathogens

Understanding the interaction of *P. thornei* with soil-borne pathogens and arbuscular mycorrhizal fungi (AMF) is an emerging discipline in the region. Yield loss of wheat was exacerbated when crown rot (caused by *Fusarium pseudograminearum* and *F. culmorum*) was also present with *P. thornei* (J.P. Thompson, Toowoomba, 2005, personal communication; S. Simpfendorfer, Tamworth, 2012, personal communication). In contrast, the biomass and yield of wheat was dependent on AMF colonization in a very dry season despite *P. thornei* being present at damaging levels of up to 5/g soil (Owen *et al.*, 2010).

Recommended integrated nematode management

There are five strategies recommended for the management of *P. thornei* in the subtropical grain region of eastern Australia.

1. Identify genus, species and population density of plant parasitic nematodes in soil samples.

Retesting is recommended at the end of a cropping sequence if flooding has occurred or if susceptible crop species have been grown. Currently, a quantitative molecular test is available from a commercial testing service, PREDICTA®B. *Pratylenchus thornei* levels are determined from a 500 g sample consisting of soil and roots collected from approximately 15 locations in a field or production zone at 0–15 cm soil depth.
2. Grow a cultivar with the highest level of tolerance and resistance available, especially for wheat.
3. Grow two or more resistant crops consecutively to reduce population densities to <1/g soil.
4. Sow wheat as early as possible within the recommended sowing window.
5. Promote adoption of INM through regularly updated free crop growing guides providing cultivar ratings of tolerance and/or resistance, and presentations at industry symposia, workshops and field days attended by growers and agronomists.

There is high uptake of the recommended strategies for INM of *P. thornei*, particularly growing of tolerant wheat cultivars because of the immediate economic benefit, the reliability and stability of the tolerance trait and the low cost of cultivar selection. Through extension, growers and their advisers understand the importance of testing soil because of the unique responses of cultivars within each crop to each *Pratylenchus* species found in the region. Additionally, there is an openness and acceptance of a common problem that all growers face because *P. thornei* is widespread.

Disadvantages of the current INM that can decrease acceptance are:

- extended time taken to reduce nematode population densities by growing resistant crops;
- broad host range of *P. thornei* and the limited number of resistant cultivars of crops that can be profitably marketed;
- susceptibility of all major pulse crops;
- cost of the soil tests;
- sporadic publication of ratings of resistance/ tolerance of crops such as sorghum, maize and sunflower;
- subtle or no symptoms in susceptible crops and relatively minor yield loss (<10%); and
- absence of registered, effective and economical nematicides for rapid control of *P. thornei* throughout the soil profile.

Optimization of nematode management

None of the current INM strategies eliminate *P. thornei*, especially where there is a reliance on tolerant, but susceptible, cultivars. Ideally, breeding programmes should be expanded to include all crops grown in the region, particularly chickpea, faba bean and mungbean, and should mimic the success of wheat breeding to release cultivars with tolerance and resistance to *P. thornei*. Complementary research seeking novel sources of resistance, improved markers to select for these traits and optimization of phenotyping methods are needed. Additionally, ratings of tolerance/ resistance should be produced for all new and current cultivars of all crops grown in the region.

Expansion of markets to encourage diversity of crops with resistance to *P. thornei* are required. For example, crops with a limited local requirement but potential export markets, such as millet and panicum, pigeon pea (*Cajanus cajan*), linseed (*Linum usitatissimum*) and safflower (*Carthamus tinctorius*).

Agronomic approaches should also be considered. Cover crops with resistance to *P. thornei* and low water use should be incorporated into fallow periods to increase the diversity of the soil biology to take advantage of natural antagonists of *P. thornei*, greater levels of soil carbon and maintenance of AMF. Improving the frost tolerance of wheat would allow earlier sowing so that roots grow in cool soils (ideally <15°C). Additional strategies to immediately decrease *P. thornei* population densities and improve plant health are needed. This may include products which are already available for other nematode/ crop systems that prime plant defence mechanisms and protect entire root systems.

Future research requirements

Wheat breeding has been the saviour of the industry for management of *P. thornei* in the low input farming systems of the region and therefore should continue to be expanded. Effective genes for resistance to *P. thornei* in landraces, synthetic hexaploids and wild relatives of wheat have been discovered and these need to be bred into cultivars. The incorporation of other traits which offer drought tolerance may further improve tolerance and resistance to *P. thornei*, for example, selection of germplasm which favours root colonization by AMF. Understanding the mechanism of tolerance and resistance in wheat could offer new genes for selection of breeding material. Release of cultivars with tolerance and resistance to *P. thornei* and major soil-borne diseases, such as crown rot (*F. pseudograminearum* and *F. culmorum*) and common root rot (*Bipolaris sorokiniana*) will further improve wheat production.

Other relatively low-cost options to supplement current INM should include seed treatments or in-furrow application of products that are developed specifically for migratory nematodes such as *P. thornei*. These products may activate plant defences, stimulate root growth, and promote diversity of soil biology. Protection of plants throughout the season is critical in the design of future products.

Adaptation of extension material to take advantage of technology to improve ease of use is required. Linking results from soil tests to apps using models which incorporate temperature, rainfall, other diseases and the presence of abiotic stressors could be used to optimize strategies for specific farming systems within fields.

Outlook: anticipating future developments (2050+)

Climate change is likely to cause increased temperatures and drought, and fewer frost events which will impact wheat production in rain-fed farming systems of Australia. Higher temperatures, particularly for winter-grown crops, may increase the reproduction of *P. thornei* but decrease survival in hot topsoils (Thompson *et al.*, 2018). With fewer frosts, the recommended sowing window may expand to earlier in the season; however, if soil temperatures increase, sowing wheat early in the season may become a less effective strategy.

The distribution and impact of *P. thornei* in Australian wheat regions may increase in the cooler, temperate southern regions. Currently, yield loss from *P. thornei* in wheat in these regions is less severe and infrequent compared to the subtropical region despite population densities being similar, if not greater, than in the subtropical region.

Conservation agriculture, which incorporates stubble retention, zero and minimum tillage and herbicides is widely practiced in rain-fed faming systems of Australia. However, globally there is a desire to decrease chemical usage in agriculture. Without herbicides, fallow periods may become weedier, resulting in increased tillage and burning to control weeds. Adaption of INM to new farming systems may be required, particularly if weeds are susceptible to *P. thornei* and soil carbon and biology are negatively affected. Alternatively, if pasture phases were introduced, there may be changes in population dynamics of *P. thornei*, depending on the host status of pasture species, and the subsequent positive effects on soil chemistry and biology.

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3 The need for integrated management of the cereal cyst nematodes, *Heterodera* spp. in Central Western Asia and North Africa

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Introduction

Bread wheat covers over 240 million hectares globally and is considered a staple food of around 40% of the global population and contributes one-third of total world grain production. It is projected that world wheat production must increase by at least 60% to meet the estimated wheat grain demand in 2050. Among biotic factors, plant parasitic nematodes, diseases and insects are important constraints leading to substantial reductions in per unit area production. Cereal cyst nematodes (CCN), root lesion nematodes and seed gall nematodes are significant species on wheat in most regions of the world and are present in Central Western Asia and North Africa (CWANA) (Dababat and Fourie, 2018; Seid *et al.*, 2021). In the CWANA regions, wheat yield is negatively affected by a complex group of *Heterodera* species. *Heterodera avenae*, *H. filipjevi* and *H. latipons* are the most important CCN. The damage caused by CCN is not well known to those working in these countries.

This chapter emphasizes the economic importance, distribution, biology and symptoms of CCN, in addition to recommended integrated nematode management (INM) tools to control CCN in wheat.

Economic importance

Wheat produced in the rainy winter season in the semi-arid regions of CWANA is highly vulnerable to CCN and considerable yield losses of up to 50% can occur (Dababat and Fourie, 2018). *Heterodera avenae* is also the most destructive plant parasitic nematode of wheat in the climatic conditions of Northern Europe where it causes an estimated 10% loss in grain yield. Wheat in CWANA is a monoculture in most countries and CCN are serious problems with wheat grown in the cool rainy winter seasons across North Africa all the way to Northern Europe.

Although the true level of losses across all of CWANA is poorly worked out, the average yields in the region are very low when compared to the world average. This could be due to the lack of INM. Yield losses can exceed 50% under the harsh climatic condition characterized by low precipitation and high temperature in the region. Nevertheless, the reports regarding wheat grain yield losses do not accurately portray the magnitude of economic losses at the regional or national level because documentation has been mostly based on research plots located in infested areas of fields, i.e. sick plots (Smiley *et al.*, 2017).

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Further complications arise from reports initially attributed to yield reduction by *H. avenae* that are now identified as *H. filipjevi*, *H. latipons*, *H. australis*, or *H. sturhani* (Dababat and Fourie, 2018).

Host range

Heterodera avenae infects graminaceous crops especially wheat, oats, barley and rye. Other hosts include grasses from the genera *Agropyron*, *Alopecurus*, *Agrostis*, *Arrhenatherum*, *Anisantha*, *Brachypodium*, *Avena*, *Bromus*, *Dactylis*, *Echinochloa*, *Festuca*, *Hordeum*, *Koeleria*, *Lolium*, *Phalaris*, *Poa*, *Polypogon*, *Phleum*, *Setaria*, *Sorghum*, *Secale*, *Triticum*, *Trisetum*, *Vulpia*, *Zerna* and *Zea*. *Senebiera pinnatifida* belonging to the Brassicaceae family is the only non-graminaceous host recorded so far.

Major hosts of *H. filipjevi* include bread wheat, oat, false wheat, barley, rye and quack grass. However, *Triticum dicoccoides*, *T. durum*, *T. tauchi*, *T. monococcum*, *T. ovatum*, *T. turgidum*, *T. umbellatum*, and *T. ventricosum* are well characterized experimental hosts of *H. filipjevi*. Maize is a weak host of *H. filipjevi*. The 2nd stage juvenile (J2) are able to invade maize roots but females fail to reproduce and therefore the crop is used in India as a trap crop.

Distribution

Heterodera avenae is the most widely distributed CCN around the globe (Fig. 3.1). Wheat producing regions with temperate climatic conditions in Asia, Africa, North and South America, Europe and the Mediterranean are typically CCN occurrence zones (Smiley *et al*., 2017).

Symptoms of damage

CCN species establish their feeding sites called syncytia on the roots of wheat plants and this blockage of the vascular system leads to weakening of the root system. The damage caused is primarily characterized by patches of plants showing poor growth and chlorosis, which are unevenly distributed in the field. In a large field cultivated with a susceptible cultivar (monoculture is standard in the CWANA region), these patches merge and can cover the entire field within a few years. Severe infection of CCN in wheat leads to stunting, leaf chlorosis, reduction in leaf area, lower numbers of productive tillers and shorter spikes with fewer grains. Below ground symptoms caused by *H. avenae* include enhanced production of roots, i.e. root proliferation (Fig. 3.2A), and knot-like formations due to induction of syncytia containing multiple females (Fig. 3.2B). These root symptoms are usually noticeable 1 to 2 months after sowing in the CWANA region.

Biology and life cycle

Both *H. avenae* and *H. filipjevi* complete one life cycle in a year. In most cases they require a

Fig. 3.1. Distribution of *Heterodera avenae* around the globe. Figure courtesy of EPPO (2021) EPPO Global Database at https://gd.eppo.int.

Fig. 3.2. (A) Stunted wheat plants with knotted roots heavily infested with *Heterodera avenae* (right) compared with healthier plants (left). Photograph courtesy of Honglian Li, China. **(B)** White females of *Heterodera avenae* on wheat roots. Photograph courtesy of Zhenzhou, Henan, China.

diapause of up to 4 and 2 months, respectively, before the juveniles can emerge. The life cycle is illustrated in Fig. 3.3. Important is the fact that juvenile emergence only takes place when ample amounts of moisture under favourable soil temperatures are prevailing, which triggers the release of specific root exudates by the host plant. However, not all the J2 emerge at the same time, which is an important survival strategy harboured by CCN in the semi-arid regions.

Heterodera avenae infection in the root-tip region leads to growth inhibition, induction of typical branching and swelling of roots. Formation of syncytia differs between *H. avenae* and *H. latipons*; however, the impact of *H. avenae* on wheat growth and yield is more pronounced than *H. latipons*. This is due to different hatching behaviour of these two species and *H. latipons* juveniles penetrate at sites more distant from the root tip.

Race problem

CCN have considerable intraspecific diversity in the form of its pathotypes or biological races. The race system for CCN has been developed on the basis of the ability of the local populations to reproduce on barley cultivars containing different resistance genes according to ICCNTA (International Cereal Cyst Nematode Test Assortment). This system could be used to identify various races with distinct characteristics present in the CWANA region. The *H. filipjevi* pathotypes were tested for several populations in Turkey and fortunately were found to be similar. However, the race spectrum in the region for all CCN species needs to be collaboratively studied among the nematologists in the CWANA region so that different pathotypes can be identified and considered in the wheat breeding programmes. The soil-borne pathogens programme at CIMMYT annually screens thousands of wheat lines and the most resistant lines are shared with the International Winter Wheat Improvement Program (IWWIP) who distributes those materials to more than 150 collaborators representing around 50 countries in the CWANA and beyond.

Interactions with other nematodes and pathogens

Although interactions among various plant pathogens are well established, such interactions regarding CCN are not well studied. This is the reason why only a few reports concerning complex interactions of CCN with other nematodes and pathogens are available in the literature. Cook in 1970 observed the first interaction of *H. avenae* with a fungal pathogen, *Gaeumannomyces graminis* var. *tritici*, the causal organism of take-all disease in wheat, and described severe symptoms of take-all disease that are always associated with low population densities of *H. avenae* in the field. Smiley *et al*. (1994) further

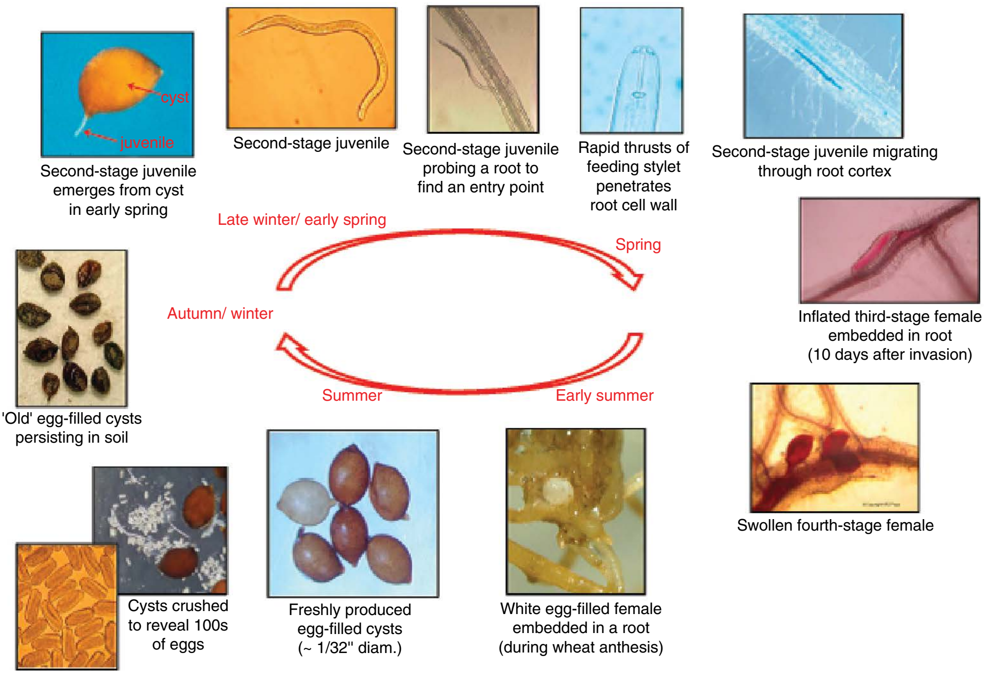

Fig. 3.3. Life cycle of *Heterodera avenae*. Figure courtesy of R. Smiley, Oregon, USA.

confirmed this interaction in winter wheat and reported substantial reduction in grain yield due to combined infestation of *H. avenae* and *G. graminis* var. *tritici* in the field.

Similar nematode–fungus interactions were reported for *H. filipjevi* and *Fusarium culmorum* pathogens in winter wheat in Iran under rainfed conditions. However, the effects of these two organisms on plant growth were additive rather than synergistic (Hajihassani *et al.*, 2013).

Recommended integrated nematode management and optimization

Wheat growers in CWANA basically do not recognize nematodes as a problem. In fact, most of them do not know which nematode species are in their fields affecting yield, which is why the term 'hidden enemy' perfectly applies to the problems in the region. INM is therefore not practiced in the entire region and nematode-induced yield losses are simply accepted.

The yield reduction in wheat due to CCN in CWANA could be lessened by improving and understanding the concept of INM in the region where the practice of winter monoculture of wheat is the norm. Management of cereal nematodes, especially CCN, could involve an integrated approach that includes crop rotation, genetic resistance, crop nutrition and appropriate water supply.

The following control measures are being used by some progressive wheat farmers in the CWANA region.

Crop rotation

Damage from CCN is greatest when monoculture practices exist especially with susceptible crops. Yield losses become very high in 2-year rotations of cereals even with the traditional summer fallow as well as in 3-year rotations such as winter wheat, spring cereal and a non-host broadleaf crop or fallow. Crop rotations that include broadleaf crops (tobacco), maize, fallow and resistant wheat, barley or oat cultivars reduce nematode density. For the most part, farmers in the CWANA region still perform summer fallow for two reasons, firstly to help the soil maintain high moisture levels and secondly to reduce diseases.

Weed control

CCN may also persist on a wide range of weed grasses. Grassy weeds such as quack grass, crabgrass, brome grass, foxtail, wild oat, rat tail fescue and others should not be allowed to grow during any phase of a crop rotation in a field that is infested with CCN. Weed control is a commonly used practice in the CWANA – not to reduce nematode densities, but instead to keep the soil moisture content at higher levels to boost the establishment and growth of the next crop with ultimately better germination.

Resistance and tolerance of wheat and barley

The use of host resistance is an effective method of controlling CCN. Resistance is defined as the ability of the host to inhibit nematode multiplication. In some cases nematodes still penetrate resistant cultivars and cause damage even if they are unable to multiply. The benefit of resistance is that it reduces the intensity of risk to the next crop of barley, oats or wheat. Ideally, resistance should be combined with tolerance to nematode penetration.

The development of cultivars with only tolerance, which is the ability of the host plant to maintain its yield potential in the presence of nematodes, could also be used in management where resistance is not known.

There are no wheat, barley or oat cultivars currently available that are fully resistant to CCN in CWANA.

Tolerance and resistance genes for CCN were recently identified by CIMMYT (Dababat *et al.*, 2016; Pariyar *et al.*, 2018). The most effective wheat resistance gene for controlling CCN and their pathotypes is Cre1 which has been crossed into local varieties. The Cre1 gene appears to suppress but not eliminate production of CCN. Sources of resistance from barley and oats have not yet been crossed into CWANA wheat varieties.

Timing of planting and trap crops

Planting winter wheat rather than a spring crop of wheat, barley or oat cultivars can favour strong, deep root development before the majority of J2 emerge from cysts during the spring. In addition, where sufficient water is available, planting a susceptible host as a trap crop during the autumn or early spring can reduce CCN densities in soil. The trap crop is invaded when J2 migrate from the cyst into the soil during early spring. The trap crop is then killed during mid-spring before new egg-bearing cysts can be developed. This strategy is particularly useful where growers plan to produce a warm-season crop such as chickpea or bean that can be planted during late spring after the trap crop has been killed in an infested field that will be planted to wheat or barley the following year.

Crop nutrition and water supply

Since the greatest crop loss occurs when nutrients or water are scarce at important growth stages, supplying optimal plant nutrition and, where possible, supplemental water during intervals of drought can minimize (mask) crop damage, particularly when the nematode damage is only slight or moderate. However, crops that are severely damaged by CCN usually do not respond well to additional applications of nutrients or water. If severe damage becomes evident early enough in the spring, it may be more profitable to destroy the crop and replace it with a non-host (broadleaf) crop.

Nematicides

Non-fumigant, in-crop nematicides especially the use of seed treatments is effective and widely used on other crops and has been used to reduce losses by CCN (Dababat *et al.*, 2014). However, the use of nematicides is not economically feasible on most grain crops.

Biological agents

Applications of currently available biological nematicides have not been effective for increasing the productivity of wheat in the region. However, in some locations, naturally occurring fungal or bacterial parasites invade and kill some of the CCN eggs that are still inside the cyst. These natural parasites of eggs reduce the density of CCN, but even in fields where they are known to be present and active, reduction in yields of wheat and barley continue to occur. Ways to amplify the benefit of these natural biological agents in commercial agriculture have not been identified.

Tillage

Tillage does not have an appreciable effect on the density of cereal nematode species. Populations are likely to be similar in both cultivated and tilled versus non-tilled fields.

Future research requirements

There is a need for more research to develop high-yielding and disease-resistant wheat cultivars adapted to a wide range of environments. In addition, improved technology for sustainable management of plant pathogens including CCN is needed (Ali *et al.*, 2015). The major requirements for the future management of CCN are:

1. Create awareness among the farmers in the developing countries especially through educating researchers at the extension services to support growers.
2. Establishment of yield losses caused by a particular CCN species in real time, which could be challenging due to uneven distribution and presence of multiple species of CCN in a single field.
3. Molecular diagnostics for reliable identification of CCN species.
4. Because different CCN species behave differently to environmental and edaphic factors, the development of reliable epidemiological models would be helpful for the management of diverse CCN species.
5. New chemistry nematicides, which are less dangerous for the environment and human health, must be developed and tested under the field conditions. Chemical companies should test any promising compound on a wide range of wheat germplasm having different levels of resistance to cereal nematodes to investigate whether chemicals have additive/synergetic effect on resistance behaviour or not?
6. Novel biological management options like seed treatment with biocontrol agents need to be examined further.
7. Cell phone apps based on the identification of the damage caused by CCN, and other IT and social media services could be developed and utilized for better understanding for the farmers.
8. Understanding the interactions of CCN with other nematodes and plant pathogens is important to devise management approaches for multiple pathogens in a particular field.
9. There is a further need for the maintenance of genetic diversity in elite lines at CIMMYT so that national programmes can breed their own resistant cultivars adapted to the wide spectrum of CWANA environmental conditions.

Outlook: anticipating future developments

The accessibility to genomic resources like genome sequences and high throughput data on genomes and transcriptomes provides a huge array of information that could be used for wheat improvement for grain yield and CCN resistance. For instance, surveys of complete genome sequences of different CCN may help to identify novel effector coding genes which could be manipulated through host-induced gene silencing technology. Likewise, recent release of the complete wheat genome offers enormous opportunities to study and understand molecular wheat–nematode interactions leading to development of CCN resistant wheat. Similarly, worldwide wheat germplasm collections of wild and cultivated wheat accessions provide unique opportunities for wheat genetic improvement. Furthermore, the availability of several genome-wide association studies could enable the wheat breeders to identify genomic regions associated with CCN resistance in wheat. Ali *et al.* (2019) recently reviewed and summarized the transgenic opportunities to enhance CCN resistance in wheat, which included employment of R-genes, host-induced gene silencing of vital effector CCN genes through RNAi, anti-feedant proteinase inhibitors, anti-invasion chemodisruptive peptides and manipulation of gene expression specifically in syncytia. One of the potential future directions to develop CCN resistance could be the pyramiding of two or more of these strategies which may lead to complete control of CCN in wheat.

Targeted mutagenesis through the application of CRISPR/Cas9 system could be deployed for non-transgenic and targeted deletion or addition of sequences to the wheat genome for induction of CCN resistance in bread wheat.

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4 Cereal cyst nematodes in the western USA

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Introduction

Two species of cereal cyst nematodes (CCN) have been reported in the western USA (Smiley, 2016). *Heterodera avenae* is detected much more frequently than *H. filipjevi*. Both species are detected in some fields (Yan and Smiley, 2010; Smiley and Yan, 2015).

Economic importance

In 2007, CCN reduced wheat production in the states of Idaho, Oregon and Washington by an estimated 21,000 tonnes, or US$3.4 million (Smiley, 2009). That estimate is now considered far too conservative.

Host range

Primary crops affected by CCN in the western USA are wheat and barley. Other potential hosts in the region include hay crops (oat and timothy) and grass-seed crops (bentgrass, bluegrass, fescue, ryegrass, brome, orchard grass and canary grass).

Distribution

Heterodera avenae is present in small, individual cereal production areas within California, Colorado, Idaho, Montana, Oregon, Utah and Washington. *Heterodera filipjevi* occurs in very small areas of Oregon, Montana and Washington (Smiley, 2016).

Symptoms

Wheat and barley roots branch excessively at locations where CCN females establish a feeding syncytium, resulting in a bushy or knotted appearance (Fig. 4.1). Invaded roots fail to grow deeply into soil and are less capable of extracting water and nutrients (Fig. 4.2). Abnormal root branching is generally not recognizable until a month or more after second-stage juveniles (J2) invade roots. On oats, *H. avenae* causes roots to become shorter and larger in diameter, without the knotted symptom.

Affected plants often appear as patches of pale green seedlings that lack vigour (Fig. 4.3). Plants may become severely stunted. Symptoms are more pronounced when affected plants are

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Fig. 4.1. Root proliferation symptom at points where females of *Heterodera avenae* or *H. filipjevi* established a feeding syncytium on spring wheat seedlings; names for this symptom include bushiness, knotting and witches' brooming. Author's own photograph.

also stressed by inadequate nutrition or water, root-rotting fungi or other nematodes.

The presence of females on roots at about the time of host anthesis is diagnostic. Most females are located at points where branch roots exhibit abnormal proliferations. They are partially embedded in roots but have a white to light-grey globose body (0.5- to 2-mm diameter) outside the root. The body glistens when wet but can be obscured by soil adhering to roots. Females are easily dislodged when roots are washed vigorously.

Damage

Economic damage by a finite density of CCN (J2 in soil, plus eggs and J2 from cysts) varies by cereal crop species and crop management. Grain yields have been reduced by 10% or more when pre-plant densities were 16, 5 or 1 egg plus J2 per gram of soil, respectively, for crops of irrigated winter wheat, rain-fed winter wheat, or irrigated and rain-fed spring wheat (Smiley *et al.*, 2017). Active management is recommended when CCN density exceeds 15, 3 or 1 egg plus J2 per gram of soil for these crops.

Biology and life cycle

CCN complete one generation each season. An illustrated life cycle is shown in Smiley (2016).

Fig. 4.2. Severe stunting of winter wheat plants caused by *H. avenae*; similar stunting can be caused by *H. filipjevi*. Author's own photograph.

The first moult occurs when eggs transform into J2 while still inside the cyst. The vermiform-shaped J2 (0.2-mm diameter × 0.5-mm length) enter the soil by emerging through semifenestrae in the cyst vulval cone. They penetrate susceptible and resistant hosts through epidermal cells near the root tip and migrate through cortical cells until a permanent feeding site is selected near the phloem. Secretions injected into the cytoplasm of cortical cells result in formation of syncytia consisting of multiple cortical cells. In susceptible hosts, J2 moult to J3, which become partially inflated and differentiate into males or females. Female J3 and J4 become further inflated and sedentary and continue to feed from the syncytium. As females enlarge, they rupture the root epidermis, exposing the vulva to the surrounding soil. Male J3 and J4 resume the vermiform shape, re-enter soil and copulate with sedentary females. Females become heavily swollen, white-coloured and filled with hundreds of fertilized eggs.

Fig. 4.3. Generalized irregularity of winter wheat plant height in a field infested with *Heterodera avenae*; both *H. avenae* and *H. filipjevi* can cause stunting of plants in patches with well-defined or diffuse peripheries. Author's own photograph.

Females die when the invaded host roots mature and die. The dead female's outer membrane hardens into a leathery, light-brown cyst that protects eggs and J2 from desiccation, cold and heat between crop cycles. Cysts darken with age, and most are dislodged into soil as roots decompose or when soil is cultivated. Eggs and J2 can remain viable within cysts for several years.

Heterodera avenae J2 emerge from cysts from mid-winter to late spring, after being exposed to a period of cold temperature lasting two or more months. Peak numbers of J2 can occur from mid-spring to early summer, depending upon the severity of cold temperatures during winter and spring. Peak numbers of *H. filipjevi* J2 occur in soil several weeks earlier than peak numbers of *H. avenae*. International literature indicates that *H. filipjevi* J2 can begin to emerge from cysts developed on a recently harvested crop, without a period of cold tempering. Emergence of *H. filipjevi* begins during autumn, decreases over winter, and peaks during early spring.

Populations of *H. avenae* and *H. filipjevi* include multiple pathotypes that vary in capacity to reproduce on individual species or cultivars of cereals. International research has shown that combinations of species and pathotypes of each species may occur within individual fields. Reproductive characteristics of *Heterodera* populations in each region must be characterized before sources of genetic resistance can be successfully deployed. Pathotypes of both species have not been adequately evaluated in the USA. However, the *Cre1*, *Rha2* and *Rha3* resistance genes provide a high level of protection against all populations of *H. avenae* tested thus far (Smiley *et al.*, 2011, 2013; Smiley and Marshall, 2016).

Interactions

Interactions between CCN and other nematodes or pathogenic fungi have not been studied in the USA. However, measurements of damage attributed to *H. avenae* and other soil-borne pathogens were made in a crop rotation experiment with 11 treatments (Smiley *et al.*, 1994). During the fifth year, half of each replicated plot was treated with aldicarb, and winter wheat was planted uniformly across the experiment. Wheat yielded equally following 1- or 2-year breaks of fallow, spring pea or weed-free lucerne. Annual winter wheat yielded 40–60% less than wheat alternated with fallow or any other rotation except 4 years of lucerne contaminated with grass weeds. Wheat yield declined in direct proportion to pre-plant density of *H. avenae*, but the greatest decline was attributed to combined damage by *H. avenae* and *Gaeumannomyces graminis* var. *tritici*. Fewest roots occurred on plants damaged by both *H. avenae* and *Pythium* species. Aldicarb reduced root damage by *H. avenae* but did not increase wheat yield because damage from *Pythium* species and *Rhizoctonia solani* were increased.

Management

Management of CCN starts with sanitation practices to prevent introduction or limit the spread of nematodes. After a field becomes infested, it is important to detect, quantify and identify the species to provide information essential for establishing multi-faceted management programmes that maintain infestations below a threshold for economic damage. Individual components of recommended management options are discussed.

Field sanitation

Common means for transporting infested soil include farm equipment, vehicles, wild and domestic animals, birds, people's boots, root and tuber crops, horticultural nursery crops, turfgrass sod, wind-borne dust and surface water moving from infested fields.

Detection

Most CCN cysts are dislodged into the top 15 cm of soil. Fields are typically sampled by collecting multiple subsamples after plant maturation, when J2 are not present in soil. Cysts are extracted from soil and crushed to quantify eggs plus J2. If samples are collected during the spring, it is important to quantify J2 in soil as well as eggs plus J2 from cysts. Sampling is most informative when focused on areas where plants of the previous crop were stunted.

Identification

It is important to distinguish which CCN species is present, and to distinguish between CCN and *Heterodera* species that occur on other crops such as pea, potato or sugar beet. Distinction of *H. avenae* and *H. filipjevi* by using a microscope is difficult, prone to error, and usually not attempted by commercial testing laboratories. Molecular procedures were therefore developed to differentiate and quantify common species of *Heterodera*, using a single DNA extract from soil or from an individual egg, J2 or cyst (Yan and Smiley, 2010; Yan *et al.*, 2013). Those methods are now used routinely in some commercial laboratories.

Crop rotation

Damage from CCN is greatest when susceptible crops are produced annually or in short rotations. Common sequences in the USA include 2-year rotations of cereals with fallow or a non-host crop, or 3-year rotations containing two susceptible crops, such as a rotation of winter wheat, a spring cereal, and a non-host crop or fallow. The density of CCN in soil are dramatically reduced by 3-year rotations which contain only one susceptible host crop.

Genetic resistance and tolerance

Production of cultivars that are both resistant and tolerant will become the most effective method for controlling CCN. Resistant and tolerance are genetically independent, and all combinations of resistance and tolerance are possible within a collection of cultivars (Marshall and Smiley, 2016; Smiley and Marshall, 2016).

Resistance is a measure of the ability of nematodes to multiply. Resistance reduces the density of nematodes remaining in soil and, therefore, the level of risk to the next cereal crop.

However, J2 invade and injure roots of resistant as well as susceptible plants, leading to reduced yields even when reproduction is prevented or greatly suppressed. Some wheat cultivars resistant to *H. avenae* are susceptible to *H. filipjevi*, and vice versa (Smiley and Yan, 2015).

The *Cre1* gene is the most effective resistance gene for controlling *H. avenae* on wheat in the western USA (Smiley *et al.*, 2013). *Cre1* has been successfully crossed into locally adapted commercial cultivars. Also of potential value are *Cre5*, *Cre7* and *Cre8*, particularly if pyramided with *Cre1* into an individual cultivar. Effective resistance of barley to *H. avenae* is expressed by cultivars containing the *Rha2* and *Rha3* genes (Smiley *et al.*, 2013). Excellent resistance of wheat to *H. filipjevi* was identified in several wheat cultivars (Smiley and Yan, 2015).

Tolerance is a measure of a plant's ability to yield acceptably well, even when roots are invaded by a nematode. Tolerance is estimated by comparing grain yields in adjacent treatments where the pre-plant nematode density is either high or low (or nil). Tolerance estimates the yielding capacity of the current crop but has no bearing on the potential for damage to the following crop, because tolerant plants can be either resistant or susceptible. When susceptible cultivars are tolerant, they produce near-normal yields but allow the nematode to multiply and pose a higher risk to a subsequent crop. Likewise, some resistant wheat cultivars are very intolerant (sensitive) to initial invasion by a CCN, resulting in reduced growth and yield.

Cereals of greatest importance in the western USA include winter wheat, spring wheat and spring barley. The goal is to identify or develop cultivars that are both resistant and tolerant to both of the *Heterodera* species in the region (Smiley, 2016). Most cultivars of spring barley in the western USA exhibit greater resistance and tolerance to *H. avenae* than cultivars of spring wheat (Marshall and Smiley, 2016; Smiley and Marshall, 2016).

Trap crop

Susceptible cereal crops can be destroyed before impregnated females mature in the spring. This is possible where climate, economics and available water permit the planting of a warm-season non-host crop after a trap crop is destroyed. If severe damage to an intended cereal crop becomes evident early enough during the spring, it may be more profitable to destroy that crop and plant a non-host crop.

Biofumigant crop

Green manure crops such as brown mustard, rapeseed or sudangrass can be used as biofumigants to reduce infestations of CCN and other pests. In the semi-arid western USA, biofumigant crops can only be used where water is not a limiting factor for growth of the cereal crop. Green tissue from the biofumigant crop must be thoroughly macerated and immediately incorporated into soil. As the macerated tissue decomposes it releases toxic products into the soil. Biofumigant products are also phytotoxic to seeds and seedlings of newly planted crops. Sufficient time must be provided for toxicity to diminish before planting a desired crop. Incorporation of mature stalks of these plants does not initiate the biofumigation process.

Planting date

Winter wheat produces a dense and deep root system before J2 emerge from cysts during the spring. Spring cereals are much more susceptible than winter wheat because roots of spring crops emerge into soil already populated by J2. The temperate semi-arid climate in the north-western USA is highly favourable for production of rain-fed winter wheat, which is therefore the principal or only crop in much of the region. CCN are most important where spring crops are more prevalent in rotations.

Weed control

Cereal cyst nematodes may persist on some weeds such as quack grass, crab grass, bromes, foxtails, wild oat, rattail fescue or others. These grasses should be controlled in all phases of crop rotations.

Crop nutrition and water supply

Reduced grain yields are exacerbated when a CCN invades plants that are also restricted by insufficient nutrients or water. When CCN damage is slight to moderate, yields can be partially maintained by supplying optimal nutrition and water. However, in heavily infested soils, crops often fail to adequately respond to application of more nutrients or water.

Nematicides

Chemical nematicides are not registered to protect commercial cereal crops against CCN in the USA. However, some fumigants applied to control nematodes in potato or sugar beet crops also reduce the density of CCN in fields where wheat or barley may be the next phase of the rotation. Biological nematicides have not been effective for increasing yields of wheat in CCN infested fields in the western USA (Smiley *et al.*, 2012).

Biological control

It is fairly common for naturally occurring but unidentified parasitic fungi to invade and kill some *Heterodera* eggs within cysts. Nevertheless, grain yields in fields where this is observed continue to be unacceptably reduced. Methods to amplify the efficiency of these natural agents have not been studied.

Optimization of nematode management

Commercial wheat and barley cultivars have been characterized for resistance and tolerance to *H. avenae* (Marshall and Smiley, 2016; Smiley and Marshall, 2016; Smiley, 2016). This strategy is still not fully developed or widely adopted and is yet to be combined with other practices such as greater diversity of crop rotations and improved control of grass weeds.

Commercial nematode identification services can now extract DNA to distinguish and enumerate common species of *Heterodera* (Smiley, 2016; Smiley *et al.*, 2017). All three populations of *H. filipjevi* in the western USA were initially misidentified as *H. avenae* and were properly identified only after the PCR-RFLP diagnostic method became available (Yan and Smiley, 2010; Smiley, 2016).

Future research and outlook

More extensive regional surveys are required and additional CCN populations must be examined molecularly to assure accurate species identifications. Farmers and their advisers must increase their ability to recognize CCN symptoms and to understand the nematode life cycle. For acceptance by farmers, cultivars with resistance plus tolerance must also exhibit desirable agronomic qualities including grain yield and quality (Smiley, 2016). Advancements in molecular sciences are needed to characterize and implement new approaches for managing CCN. Smiley *et al.* (2017) discuss effector molecules, functional analyses of genes, mechanisms of host interactions, gene silencing, marker-assisted breeding, genome editing, and transgenic procedures to enhance CCN resistance. These endeavours will require many more decades of research, with adequate funding.

Recommendations provided to farmers have been met with variable responses. Most responded by either increasing the length of rotation, emphasizing winter over spring cereals, emphasizing barley over wheat, applying more nutrients or water, or killing heavily damaged cereal crops and planting non-host crops. Responses by other farmers were unanticipated. Wheat fields were converted to a perennial grass-seed crop or were transformed into a horse pasture or a housing subdivision. Other farms continued planting wheat crops frequently and offset the economic losses by collecting crop insurance.

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5 Impact of plant parasitic nematodes on maize in mid-western USA: An unrecognized or ignored threat to production

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Introduction

Maize (*Zea mays* L.), also referred to as corn, is one of the most important crops in the United States, and globally is cultivated primarily for food, feed and biofuel. In the mid-western US, including the states of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota and Wisconsin, maize accounts for more than 75% of the total US production. These states accounted for more than 327.2 million metric tonnes produced on an estimated 30.5 million hectares of arable land during 2020. Annual production was worth over US$46.9 billion. In this chapter we will refer to corn as maize.

Maize production is affected by several factors, including diseases, which can reduce both the yield and quality of the grain. Of these diseases, plant parasitic nematodes (PPN) are economically important. These PPN are capable of causing billions of dollars in yield losses annually. Other important agronomic crops deleteriously affected by PPN in the mid-west include soybean and wheat, which are frequently rotated with maize although maize is sometimes grown continuously.

Economic importance

According to a recent report of losses due to pathogens of maize in the US and Ontario, Canada, Mueller *et al*. (2016) reported that diseases caused by soil-borne pathogens, including PPN species in the genera *Belonolaimus*, *Helicotylenchus*, *Heterodera*, *Longidorus*, *Meloidogyne*, *Pratylenchus*, and *Tylenchorhynchus,* were responsible for an estimated maize yield loss of 21.4 million tonnes in the US from 2012 to 2015. Lesion nematodes (*Pratylenchus* spp.) are often dominant in nematode assemblages in maize fields in the mid-western US and globally, especially *P. brachyurus, P. crenatus, P. neglectus, P. penetrans, P. scribneri,* and *P. thornei*. For example, lesion nematodes were detected in more than 80% and reached or exceeded moderate risk levels for injury to maize in almost 50% of the fields in Ohio (Simon *et al*., 2018a). Lesion nematodes were also detected in 84% and 51% of fields sampled in Illinois (2008) and Iowa (2011), respectively. The fact that nematodes were found in relatively high numbers in maize fields in Illinois, Iowa and Ohio was not surprising. Current crop management practices such as conservation

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tillage, continuous maize and the abandonment of soil-applied insecticides, which in the past provided the added benefit of suppressing nematodes, favour nematode population increase.

Quantifying the relationship between maize yield and the population density of a single nematode species is difficult and may be insufficient to assess the actual impact of PPN on yield (Norton, 1984). The PPN component in soil nematode assemblages are rarely monospecific; in one soil sample five to seven PPN species are common (Niblack, 2017), several of which may impact grain yield. Therefore, the relationship between grain yield and total PPN identity and population density or cumulative damage impact may provide more meaningful information. This is important because impact on yield is dependent on complex interactions among the host plant, the environment and the nematodes, including variations among species (Niblack, 2017).

Host range

Lesion nematodes are polyphagous and can damage agronomic crops including maize, soybean, and wheat. For example, *P. crenatus* was found associated with maize and soybean in Ohio (Simon *et al.*, 2018a) and was reported as responsible for severe yield loss in maize and wheat in Europe and also associated with soybean. Fields are commonly under a maize–soybean cropping sequence. PPN species reported in association with maize that may also affect soybean and wheat growth include *Helicotylenchus pseudorobustus, Hoplolaimus magnistylus, Paratrichodorus allius, Paratylenchus neoamblycephalus, Tylenchorhynchus annulatus,* and *T. claytoni* (Fig. 5.1) (Lopez-Nicora *et al.*, 2014; Ankrom *et al.*, 2017; Simon *et al.*, 2018a, 2020).

Symptoms of damage

In most cases, symptoms caused by PPN are not conspicuous on maize roots. Above-ground symptoms are typically non-specific and found in patches in a field and include stunting, wilting and chlorosis (Fig. 5.2), and in most cases may go undetected or attributed to another cause.

However, damage to the root system may be more conspicuous when PPN numbers are very high. For example, high populations of lesion nematodes can result in lateral root proliferation, pruning of fibrous roots and the presence of dark brown discrete lesions in coarse and fibrous roots (Fig. 5.3).

Fig. 5.1. Greenhouse-grown dwarf maize roots at 10 weeks after planting, inoculated with *Tylenchorhynchus annulatus* (right) and non-inoculated (left). Author's own photograph.

Another endoparasite, the lance nematode (*Hoplolaimus* spp.) can also cause extensive damage to the cortical cells of the root, resulting in the appearance of necrotic lesions. Migratory ectoparasites such as *Helicotylenchus* and *Paratylenchus* may cause numerous small light to dark brown lesions on maize roots when population densities are very high. Species of *Longidorus*, *Paratrichodorus* and *Belonolaimus* can cause distinct above- and below-ground symptoms, such as severe localized stunting, wilting, root pruning, stubby-root appearance or swollen and severely damaged root tips (Fig 5.4).

Interactions with other nematodes and pathogens

Inter-relationships between PPN and pathogens have not been well studied in maize. One that

Fig. 5.2. Damage due to sting nematode (*Belonolaimus* spp.) on maize in the mid-western US. Photograph courtesy of Tamra Jackson-Ziems, University of Nebraska-Lincoln, USA.

Fig. 5.3. Lesions caused by cumulative damage of root lesion (*Pratylenchus* spp.) and ectoparasitic nematodes on maize in the mid-western US with root-rot necrosis caused by interactions with fungal root pathogens. Photograph courtesy of Tamra Jackson-Ziems, University of Nebraska-Lincoln, USA.

Fig. 5.4. Typical stubby-root symptoms due to the presence of *Paratrichodorus* in maize. Photograph courtesy of Tamra Jackson-Ziems, University of Nebraska-Lincoln, USA.

deserves study currently is an apparent disease relationship between a lesion nematode and a root-rotting fungus observed in maize in Nebraska (Tamra Jackson, personal communication). The necrosis associated with *Pratylenchus* lesions is an indication that root-rotting fungi may also be involved in root damage (see Fig. 5.3).

Recommended integrated nematode management (INM)

Status of farm-based integrated nematode management

Maize growers do not actively practice integrated nematode management in the mid-west and therefore accept a certain PPN induced yield loss factor. Management in the past was indirectly attained through the application of carbamate and organophosphate insecticide/nematicides which are no longer available. Ironically, growers never really recognized PPN as an important yield-reducing problem but ascribed yield increases to insect control. Rotation with other crops probably led to reductions in some species of PPN, but with maize now grown in near monoculture, it has little management impact. Resistant hybrids or a nematicide that produces distinct PPN control would be highly accepted by the growers.

Sampling

For management of PPN in maize, soil sampling and processing should produce accurate identification and estimation of population density and distribution throughout the field. This is a major problem because PPN vary in numbers over time and are not uniformly distributed in the soil profile nor over the field. Currently, it is recommended to collect soil samples between growth stages V3 to V6 to a depth of 45 to 50 cm (Abendroth *et al.*, 2011). However, several studies indicate this standard sampling protocol is not appropriate for all PPN species and soil types. Hence, all aspects of appropriate sampling (e.g. number, distribution, depth, tool diameter and time of sampling) and diagnostics should be extensively investigated to improve INM.

Cultural practices

Altering practices such as planting date, crop rotation, tillage, weed control, application of organic amendments, use of cover crops and sanitation have been tested and demonstrated effective in reducing nematode populations in many cases (Cabanillas *et al.*, 1999). For example, conventional tillage was associated with decreased lance and stubby-root population densities, and cropping sequences were not associated with lesions according to Simon *et al.* (2018b). However, the use of these practices as management tools for PPN associated with maize in the mid-western US is rarely recommended because of conflicting or incomplete information.

Cover crops

The use of cover crops in the cropping sequence and its effect on PPN population densities, soil ecology, and the performance of cash crops have been studied and new research is being conducted for other approaches. The effect of mixtures of cover crops on the performance of maize as a result of reducing PPN damage should be evaluated. A caveat is that many PPN associated with maize have several hosts and many cover crops are suitable hosts to these nematodes. For example, *P. penetrans* has a host range of more than 350 plants (Castillo and Vovlas, 2007) and selecting a crop that simultaneously can reduce population density along with the other PPN must be considered and evaluated. Identifying the best combination of cover crops will ultimately enhance integrated pest management strategies. Presently, there is little knowledge of the influence of cropping practices and cover crops on PPN in the mid-west. Decision support tools that take all this into consideration are needed (see Chapter 60 in this volume).

Nematode management products

Many studies have shown the efficacy of nematicides such as carbamates and organophosphates for controlling PPN in maize, but use of these products is gradually being discontinued. Therefore, investing in the development of new nematode seed treatment products that are environmentally friendly and cost effective is urgently needed for INM in maize. Research will be needed to evaluate the efficacy of these products against the cumulative nematode damage complex as well as the different PPN species, impacting crop establishment and grain yield under field conditions.

Resistance and tolerance

Although breeding for nematode resistance and/or tolerance can be very costly in crop production systems, plant breeders should work with nematologists to make this management tool available to maize growers. There is limited research on the potential for managing PPN in maize with genetic resistance, despite the fact there are maize inbred lines and commercial hybrids available that may possess varying levels of resistance to certain PPN species. Presently, knowledge of varying susceptibility of maize cultivars is unknown; however, decades ago both commercial hybrids and maize inbred lines demonstrated differences in susceptibility to *Pratylenchus scribneri* and *P. hexincisus* (Todd, 2016). In another study, Davis and Timper (2000) reported that many maize hybrids are generally resistant to *Meloidogyne* species, including *M. incognita*, *M. arenaria* and *M. javanica*.

Though tolerance to PPN has received less attention than resistance, there are reports of tolerance in some crops to important cyst nematodes such as *Heterodera glycines*, *H. schachtii*, and *Globodera rostochiensis* and other nematodes such as *Meloidogyne* spp. (Pagan and Garcia-Arenal, 2020). Identification of maize genotypes capable of enduring PPN while performing well in fields infested with elevated PPN population densities will improve integrated management strategies.

Traditionally, a tolerance index is obtained for each genotype of the tested crop in PPN-infested fields. To do this, yield from the same genotype is compared in PPN-infested fields treated with and without nematicides. Currently, the difficulty is finding effective and ecologically friendly nematicides to use in tolerance studies. Research in developing experimental design, statistical analyses, genetic and sampling techniques should be developed to accurately identify maize genotypes tolerant to PPN.

Optimization of nematode management

Effective disease prevention relies on early pathogen detection. Therefore, continued surveillance of PPN presence, distribution and population densities in fields is critical, as is the development of reliable and cost-effective techniques, both traditional and novel. Over the past decade, there has been interest in improving non-destructive phenotyping methods through remote sensing and data acquisition technology that can be used to quantify nematode damage and help improve management. For example, the use of unmanned aerial systems, such as drones with remote multispectral sensing technology, can be used to identify areas in which PPN damage may be occurring. In addition, aerial visible imaging, thermography and/or spectrometry can be used to identify PPN hot spots, to localize and guide soil sampling and to develop management strategies to reduce and limit PPN damage in fields. Leveraging such data along with multidimensional information from physical-chemical soil analysis, georeferenced yield maps and the use of spatial statistics tools such as predictive models and interactive maps, can be developed in order to inform growers not only of current PPN problems, but to alert them to future issues as well. These technologies are discussed in other chapters in this volume (e.g. Chapter 65).

Future research requirements/ problems of the future

Relatively little has been invested in the study of PPN on maize in the US. Whether this is because both maize and the PPN associated with maize are native and have co-evolved more or less commensal relationships, or because the large increases in yield over the past 50+ years have camouflaged antagonistic relationships has not been addressed by research. Some studies have been done on the effects of single nematode isolates or field populations on a limited array of maize selections, but these should not form the basis for recommendations about future research. There are two major reasons for this assertion. Firstly, modern maize hybrids bear little resemblance to the selections actually used in breeding programmes or production. The interaction between a host and a PPN is affected by variation in the host due to imposed adaptation (breeding) and by genetic variation in the nematode population; thus, what we learned about a maize-nematode relationship 20 or 50 years ago cannot be used as a basis for recommendations today. We need current research based on modern tools to assess the apparent threat and propose means of managing it. Secondly, proceeding from the first, is that climate change will affect maize production and the relationships between maize and its root microbiome – including the nematodes found there, not to mention other maize root pathogens that may interact directly or indirectly with PPN. The currently undescribed PPN assemblages that may account for reductions in maize yield require meticulous study, along with investment in environmentally friendly tactics to manipulate the assemblages.

Outlook: anticipated future developments

As nematologists, we are committed to understanding the biology of PPN and their interaction with their hosts and environment. These hosts are the crops that are produced by farmers, processed by industry and, ultimately, purchased by consumers. The information and thoughts presented here are intended to help us better understand the damage and threat that PPN pose to maize in the mid-west and maybe elsewhere in global agriculture.

Detectable and measurable PPN damage in maize fields is, in many cases, the result of multiple events that lead to unbalanced biotic and abiotic soil factors and the lack of dynamic equilibrium in the living soil ecosystem. We understand, therefore, that only through a multidisciplinary approach can we understand the multidimensional interaction among the different components of the soil environment, including the role of nematodes under different scenarios. We anticipate the following future developments:

- Metagenomics: improve analysis of soil biotic community assemblages from soil DNA extraction, which will allow us to assess and predict which soil health conditions will have a positive impact on maize production.
- Surveillance and detection: development of portable molecular tools to fully sequence genomes will help us to rapidly and accurately survey and detect emerging or re-emerging PPN that may negatively affect maize production.
- Multidimensional data analysis: advancements in artificial intelligence and algorithm development, coupled with remote sensing data collection and proper statistical analyses, will shed light on the rhizobiome environment and will assist in localized PPN field management.
- Plant breeding and root protection: integrated management that will combine plant breeding for resistance (and/or tolerance) to PPN.
- Seed and soil treatment: biological and/or environmentally safe chemical products that will protect roots and/or induce resistance to PPN attack.

Through this chapter, we encourage researchers to turn theory into action and aid local growers in developing sustainable economic and agricultural systems. Research will allow us to provide recommendations to farmers, improve maize production in a more sustainable agricultural environment, and secure food supply to an ever-increasing population.

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6 Maize and root-knot nematodes: A problematic, deep-seated association

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Introduction

Damage caused to maize in sub-Saharan Africa (SSA) as a result of nematode infection is considered a non-critical aspect of production, mainly due to the relatively low monetary market value of the crop compared to that of a high-value cash crop such as potato. Inputs not directly linked to profit (increased yields and quality) pose a financial risk, e.g. the use of a nematicide under harsh rain-fed conditions not justifying the application costs. Although nematicide costs can be justified for commercial and seed maize produced under irrigation, nematode management is usually at the bottom of the priority list of dryland producers, whom by far produce the majority of maize in SSA.

Maize crops are mainly damaged by root-knot (RKN) and lesion nematodes (*Pratylenchus*). The omnipresence, combined occurrence and wide host range of these genera explain their high pest status. In South Africa (SA) and other SSA countries the main RKN species infecting maize are *Meloidogyne incognita* and *M. javanica*. Lesion nematodes occur together with RKN in the majority of maize fields in SSA; *Pratylenchus brachyurus* and *P. zeae* are the predominant species causing damage (Mc Donald *et al.*, 2017; Coyne *et al.*, 2018).

Economic importance of the nematode

In SA, between 12% and 60% of maize yield losses in commercial fields have been ascribed to plant parasitic nematode assemblages, generally dominated by RKN (Mc Donald *et al.*, 2017). Smallholder producers are usually unaware that their maize crops are infected by RKN, often due to the absence of distinct root galls.

Host range

Root-knot nematodes have a wide host range, e.g. bean (*Glycine max* and *Phaseolus vulgaris*), potato (*Solanum tuberosum*), sunflower (*Helianthus annuus*) and a variety of vegetable crops (including indigenous vegetables such as *Amaranthus*) that are rotated or intercropped with maize. In maize fields in SA, the presence of >1000 RKN individuals 50 g^{-1} roots is common and occurs especially where highly susceptible

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Fig. 6.1. Galled roots of *Euphorbia damarana* infected by *Meloidogyne javanica*. Author's own photograph.

crops are included in maize-based systems (personal observation). Numerous weed species also host RKN (Ntidi *et al.*, 2017); for example, in SA *Senecio consanguineus* and *Euphorbia damarana* (Fig. 6.1) occur widely in maize production areas.

Distribution

Meloidogyne incognita and *M. javanica* are the predominant RKN species infecting maize in SA (Mc Donald *et al.*, 2017). These species also occur, in single populations or mixed communities, in other SSA countries where they parasitize maize (Coyne *et al.*, 2018). *Meloidogyne arenaria* and *M. hapla* to a lesser degree also infect maize roots in SA (Mc Donald *et al.*, 2017), while *M. enterolobii* was also found infecting the crop (Pretorius, 2016).

Symptoms of damage

Despite the general absence of distinct above- and/or below-ground symptoms, RKN parasitism results in typical internal tissue gall formation (Fig. 6.2A) with pink-stained sessile juveniles (Fig. 6.2B) and females (Fig. 6.2C) visible in the vascular cylinder. Black and/or brown discolorations of RKN-infected roots indicating concomitant infection by pathogenic microbes are also evident in some cases (Fig. 6.3). The lack of root gall symptoms is probably the reason why RKN damage is not detected in SSA countries where maize is produced mainly by smallholder farmers.

Aerial symptoms of RKN-infected maize plants may be absent, subtle and/or in many cases confused with those caused by other diseases and pests, insufficient/excessive fertilizer application, drought and water logging. Typically, poor or inconsistent germination, stunting and yellowing of maize plants in small, localized areas within a field are indications of RKN infections (Fig. 6.4).

Biology and life cycle

Low baseline temperatures, 9.8°C and 10.6°C for the thermophilic *M. incognita* and *M. javanica*, respectively (Dávila-Negrón and Dickson, 2013), contribute towards their high pest status. Due to short or mild winter periods in subtropical and tropical areas, RKN reproduction occurs for the greater part of the year at temperatures exceeding these baseline values and therefore contributes towards the continuous accretion of RKN densities. Multiple life cycles are completed during a growing season with an exponential increase in RKN offspring that will infect maize/rotation crops and weeds. Sandy soils and high temperatures also contribute towards high rates of RKN development and reproduction, and resultant yield losses (Mc Donald *et al.*, 2017).

Interactions with other nematodes and pathogens

Despite the wide occurrence of soil-borne diseases in SSA maize fields, limited information exists regarding their concomitant association with RKN. Yield decline of 1.8 t ha^{-1} for every 25% increase in root rot incidence has been reported, with various *Fusarium* and *Pythium* species and

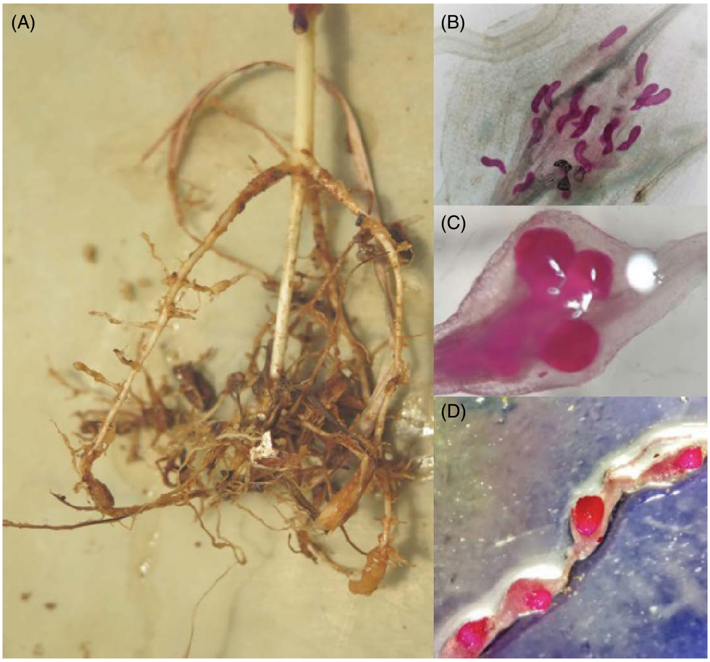

Fig. 6.2. (A) A root-knot nematode infected, galled maize root (*Meloidogyne javanica*) **(B)** with pink-stained sessile juveniles (*M. javanica*: 80× magnification); **(C)** females (*M. enterolobii*; 80× magnification) in the vascular cylinder and **(D)** females and egg masses (*M. enterolobii*; 20× magnification). Photographs courtesy of Raymond Collett, NWU.

Rhizoctonia solani (Schoeman and Craven, 2016), amongst others, being important soil-borne diseases of maize in SA.

Recommended integrated nematode management (INM)

Knowledge transfer for the unknown

A great need exists to strengthen extension systems and IT programs that can make uninformed growers (i) aware of RKN nematode problems; and (ii) INM technologies available to increase yields (outlined below). Cell phone technology and internet information services will also benefit all growers and decision support tools (see Chapter 61 of this volume) would also be supportive.

Conservation and/or regenerative agriculture, with the basic principles being (i) minimum tillage and soil disturbance; (ii) permanent soil cover with crop residues and/or live mulches; and (iii) crop rotation and intercropping (FAO, 2014) is increasingly adopted in SSA. Such soils are generally richer in organic content, contain more water, and are buffered against extreme temperatures, with crop rotation and/or intercropping further enhancing the soil biodiversity. An INM approach will benefit from such

Fig. 6.3. A white root-knot nematode female embedded in a maize root infected with pathogenic soil-borne diseases causing dark, brown/black areas. Author's own photograph.

Fig. 6.4. Patches in a maize field with yellowish and stunted maize plants infected with root-knot nematodes. Author's own photograph.

enhanced soil health conditions, but such habitats may also be optimal for the development of pathogenic microbes and other pests.

Crop rotation, ideally using crops that are non- or poor hosts of RKN, should be the main pillar of an INM approach and has a multipurpose approach since it is cost effective, environmentally friendly and readily available to producers. Non-hosts of RKN are not available for SA grain producers, while poor hosts or resistant cultivars of maize and rotation crops to a limited extent do exist in various SSA countries. Smallholder producers in particular can benefit from using the following sequence resulting in a 70% reduction in *M. javanica* densities after 4 years: soybean (resistant cv. A7119) – carrot (susceptible cv. Chantenay Karoo) – dry bean (poor-host cv. Mkuzi) – cabbage (resistant cv. 3306) and maize (resistant cv. SC701) (Venter *et al*., 2004). In SA, commercial producers that grow winter cereals in cold areas reap the benefit of reduced RKN densities in the follow-up season, mainly because of low temperatures not allowing reproduction of thermophilic species.

Resistant crop genotypes, with varying levels of resistance, are available but the information is not necessarily available to growers since screening of maize genotypes, and those of other rotation or intercrop crops, for their host suitability to RKN species are rarely done annually. Seed companies in SSA should invest in introgressing RKN resistance, especially into Bt and Round-up Ready® maize genotypes since it will protect maize against multiple biological constraints (insects, weeds and nematodes) hampering sustainable production.

Nematicides, specifically synthetically derived older generation compounds, are still used by commercial SSA producers to reduce RKN densities, especially under irrigation (commercial and seed maize) where higher yields are realized and the costs of the nematicides justified (Fourie and De Waele, 2019). Inconsistent yield benefits following nematicide application is, however, common under rain-fed conditions mainly due to low and/or erratic rainfall spells where the cost of the nematicide outweighs the monetary value of yield increases (Mc Donald *et al.*, 2017). The continuous withdrawal of old-generation nematicides and the lack of newly released, less toxic compounds pose further challenges, but the recent registration of a new-generation nematicide Velum® 1g (a.i. fluopyram) will benefit SA producers.

Biological and chemical seed-coating technology, was welcomed. It is substantially cheaper than the existing nematicides, represents a more targeted approach and greatly eliminates concerns about pollution of the environment and exposure of humans and animals to toxic nematicides. In SA, two seed-coat nematicide products are registered for use on maize: Avicta® 500FS, avermectin/macrocyclic lactones as a.i. and VotIVO® (*Bacillus firmus* as a.i.). In conservation agriculture, the use of seed-coat products in combination with RKN resistant or poor-host maize genotypes will be particularly beneficial and above all represents a realistic, integrated control measure.

Biological control, another nematode management option to be used by both large-scale commercial and subsistence smallholder producers is challenging under the harsh environmental conditions (high temperatures, low and/or erratic rainfall) prevailing in most SSA maize production areas. Biological agents have to establish and proliferate in soils under these adverse conditions. The exploitation and use of bionematicides to complement efforts aimed at optimizing soil health and protect natural resources from harmful chemicals are increasing in SSA countries (Fourie and De Waele, 2019). This approach will probably be most viable and practical under conservation agriculture where a more beneficial soil habitat exists for their establishment.

Nematode management at the farm level

Because of the absence of symptoms and/or a lack of general knowledge about the importance of RKN in maize, SA farmers do not practice any direct form of INM. The commercial growers in SA, generally aware of RKN problems, produce maize using modern agricultural inputs and technologies. This is not the case for all SSA countries, especially for smallholder growers who do not have the financial means and infrastructure.

INM in smallholder agriculture

Smallholder producers generally rely on low-input cultural control strategies to reduce RKN losses, e.g. application of organic animal- and plant-based manures, crop rotation and/or intercropping. In almost all cases they are unaware of RKN problems on maize or other crops and extension will be a valuable addition.

These producers will use host plant resistance (poor-host or resistant maize genotypes), chemical nematicides, botanically derived nematicides (e.g. derived from *Cucumis africanus* and *C. myriocarpus, Lantana camara* and *Tulbaghia violacea*) and products containing effective microbes where available (Mashela *et al.*, 2017). These technologies can all be exchanged and/or combined, depending on what is available in particular areas, to efficiently reduce RKN densities.

Optimization of nematode management

The use of only one control practice is not sufficient to effectively and sustainably keep RKN densities below threshold levels. Several tested management approaches should be combined into an INM, an approach that is gaining more momentum due to the increased withdrawal of toxic nematicides from markets (Sikora and Roberts, 2018) and the realization that we have to conserve our natural resources to enable food production and security. Pro-active thinking, planning and the implementation of practices to prevent or limit RKN infection should, however, be the first line of defence. That the majority of maize crops is grown under harsh, rain-fed conditions should always been borne in mind when nematode management is considered, and the following strategies should pursued:

- The RKN species present should be identified correctly to allow for improved recommendations regarding suitable rotation crops and other management measures. The discovery of *M. enterolobii* in a maize production area in SA is, for example, of utmost importance due to its aggressiveness and resistance-breaking abilities in some vegetable crops that may be rotated with maize.

- Strengthening of the extension and internet knowledge systems need to be supported and made available to all producers (e.g. cell phone technology or internet).
- Use of poor-host or resistant maize genotypes, also applicable for other crop genotypes (used in rotation or intercropped with maize) is the most realistic and cost-effective strategy that should form the basis of RKN management. This strategy should receive priority since resistant or poor-host susceptible genotypes can easily be identified and used without additional cost to the farmer.
- Improving soil health by increasing the biodiversity of beneficial organisms is another complementary approach that needs to be considered on a bigger scale in SSA maize-based rotation systems, e.g. the inclusion of a variety of cover and/or green manure crops. Soil densities of RKN can be reduced by manipulating the biodiversity in such a way that soils become less suitable for these pests, e.g. growing specific crops that favour the build-up of less pathogenic nematode pests to outcompete or 'replace' highly pathogenic RKN.

Future research requirements

Future research in terms of optimizing existing and/or developing novel nematode management strategies in maize should undoubtedly focus on innovative and state-of-the art genetic technologies such as the development of genetically modified genotypes that can prevent or reduce RKN life-stage development and reproduction, such as by CRISPR gene editing.

Nonetheless, basic research and available resources should not be disregarded and should focus on:

- Continuous monitoring of RKN densities and identification of dominating species, as well as those of other genera that co-exist in maize fields.
- Identification and exploitation of endemic microbe strains to be used preferably as seed-coat products should receive priority to reduce RKN densities, especially when combined with resistant or poor-host maize genotypes.
- Targeted application of nematicidal products in maize fields where RKN cause problems. In large-scale maize production fields, representing hundreds of hectares, precision application of nematicides, bionematicides and/or a combination of both can make a pronounced difference in input costs. This particularly applies to rain-fed areas where broadcast nematicide application is not cost effective and inconsistent in terms of the efficacy. The volatility of markets, furthermore, challenges producers to employ precision-based strategies to combat RKN since higher market prices will allow producers to incur input costs related to nematode management.
- Determining the status of beneficial nematodes in maize fields to employ management strategies that will enhance soil health and contribute towards reducing nematode damage.
- Dissemination of on-farm results regarding effective management strategies to inform producers so that they can minimize RKN damage.
- Closer collaboration with maize-related industries, extensionists and producers to understand what the needs, options and expectations of producers are in terms of nematode management.
- Development of online applications where results of experiments and observations by producers can be posted to reach a wider platform regarding nematode pests and their impact on maize production.

Outlook: anticipating future developments

Management of RKN and other concomitant microbe-induced diseases will become more challenging and asks for innovative approaches and wider perspectives by all stakeholders. Climate change will impact on RKN densities with the most probable scenario being an increase in their densities in shorter time periods within a cropping season, and greater damage to maize. Soil preparation (or the lack thereof, e.g. where regenerative agriculture is practiced) and covering of soil with cover- or rotation-crop residues will become more important in order to prevent large increases in soil temperatures (beneficial for RKN development and reproduction). This will contribute towards the conservation of soil moisture and benefit plant development and growth in rain-fed maize production areas, in particular where more robust and stronger plants will perform better in RKN infested fields. Incorporation of soil amendments, biological or synthetic, will further benefit soil health efforts and the optimal production of maize crops under climate change. Ultimately, higher maize prices will reduce the yield increases required to cover the cost of INM strategies.

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7 Cumulative damage impact of plant parasitic nematodes in smallholder maize cropping systems in East Africa

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Introduction

Maize (*Zea mays*) is a vital staple crop and source of livelihood to millions of families in East Africa (Shiferaw *et al.*, 2011). Demand for maize is rapidly increasing due to population growth coupled with increasing industrial and animal feed usage. Most countries in East Africa (EA) are already experiencing permanent and serious deficits in maize supply due to low yields related to small-scale subsistence production systems and intensive cultivation of land. Most of the maize farmers in EA are smallholders who practice rain-fed mixed-cropping systems where between three and seven different crops are grown simultaneously on 1–2 ha of land (Woomer *et al.*, 2016). There is also a small segment of medium-sized farm holders who grow maize on 2–10 ha and a few scattered large farms.

Increasing maize productivity in smallholder farms is constrained by a lack of farmer access to inputs, land tenure problems, climate change effects, decline in soil fertility, and build-up of pests and diseases (Coyne *et al.*, 2018). These problems expose the smallholder farmers to minimal access to the tools needed for integrated management of pests and diseases, including nematodes (Sikora *et al.*, 2020). On a yearly basis, the land is subjected to continuous 12 months of cropping pressure with no opportunity for ecosystem recharge. Due to limited financial resources, smallholder maize production is associated with minimal or no external inputs in terms of fertilizer, pesticides and poor-quality seed of low-yielding cultivars. Inevitably, this leads to further reduction in the sustainability of maize production in the region.

Economic importance of plant parasitic nematodes

Lesion (*Pratylenchus* spp.) and root-knot (*Meloidogyne* spp.) nematodes are the most widely distributed plant parasitic nematode (PPN) species associated with maize in EA (Odeyemi *et al.*, 2011). A number of different species of *Pratylenchus* are considered to be major pests responsible for significant yield loss to maize worldwide. In EA, *P. brachyurus, P. zeae and P. penetrans* as well as other species of unknown importance cause damage to the crop (Kimenju *et al.*, 1998; Kagoda *et al.*, 2015). The economic importance of lesion nematodes on maize in most parts of the world is unknown and integrated nematode management, in almost all cases, is not actively practiced. The reason for this lack of knowledge is related to the illusion that the damage caused

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by PPNs is not significant. Although the actual amount of yield lost to the lesion nematode has not been well established, experiments with nematicides have shown that losses can exceed 10% (McDonald and Nicol, 2005).

Cumulative nematode damage

The existence of a community of nematode species that simultaneously reduce maize yield exacerbates yield losses in maize. The term 'cumulative nematode damage' (CND) is used here to illustrate the complexity of the problem. CND is valid in that lesion nematodes always co-exist with other economically important species such as *Meloidogyne, Paratylenchus, Hemicycliophora, Hoplolaimus, Paratrichodorus, Tylenchorhynchus, Xiphinema, Helicotylenchus, Longidorus* and *Scutellonema* in maize fields (Kimenju *et al*., 1998; Kagoda *et al*., 2015). The simultaneous presence of these multiple nematode species leads to CND that necessitates evaluation of the additive and synergistic harm to the root system of crops grown together in smallholder farms.

Currently, there is scanty information on the impact of CND on yield in smallholder fields where mixed-cropping systems are common (Coyne *et al*., 2018). In addition, natural regulation of nematode communities is compromised due to continuous tillage and reduction in microbial biodiversity. The disruption of soil suppressiveness favours nematode build-up.

Symptoms of damage

Reduction in root mass and necrotic lesions are common symptoms in maize plants harbouring PNNs, with varying severity depending on maize variety, soil type, fertility and the predominant nematode species present (Kagoda *et al*., 2015; Coyne *et al*., 2018). Above-ground tissues display non-specific symptoms that include yellowing and stunted growth (Fig. 7.1). Root damage is present in the form of dark lesions along the entire root system. In addition, nematode infection predisposes plants to opportunistic pathogens thus triggering development of disease complexes and this is responsible for the dark colour of the root lesions (Fig. 7.2). The resultant symptoms are modified, and the nematode damage is usually masked (Coyne *et al*., 2018).

Recommended integrated nematode management programmes

Most of the maize farmers, farming on 1–2 ha and 2–10 ha, do not have access to most of the nematode management tools, compared to large-scale farmers in the region. Therefore, sustainable management of *Pratylenchus* species and other PPN complexes remain a complicated task that requires careful balancing of pertinent concerns. The options selected should blend well with the technological, environmental and economic realities in subsistence or semi-commercial maize production. In this regard, the most widely recommended strategies include host resistance, crop rotation, cover cropping, conservation agriculture, and addition of organic amendments to improve soil fertility and stimulate natural biological control.

Host resistance

Currently, no maize cultivars are being released with resistance to plant parasitic nematodes in EA. However, studies indicate that certain genotypes could be included in maize breeding programmes as donors of resistance genes against

Fig. 7.1. Non-specific symptoms showing cumulative nematode damage on maize in a smallholder farm. Photograph courtesy of Johan Desaeger, University of Florida, Florida, USA.

Fig. 7.2. Lesions caused by cumulative damage of root lesion (*Pratylenchus* spp.) and species of ectoparasitic nematodes and necrosis caused by the interaction with fungal pathogens. Photograph courtesy of Tamra Jackson-Ziems, University of Nebraska-Lincoln, Nebraska, USA.

PPNs and tolerance to drought (Kagoda *et al.*, 2015). Occurrence of multiple nematode species in maize production sites is a barrier to attainment of the full benefits of host resistance since finding suitable cultivars that confer resistance against multiple nematode species is complicated (Coyne *et al.*, 2018).

Crop rotation

The benefits of crop rotation are common knowledge to growers and the extension service. However, the practice is associated with limited adoption mainly due to scarcity of arable land coupled with the dictates of consumer preference. The real hindrance to crop rotation, away from land, is the rigid dietary behaviour of the people in EA who are dependent on maize for survival. The picture is slowly changing with the adoption of maize substitutes such as rice, potato, sorghum, millet and cassava, especially among the urban and youthful populations.

Cover cropping

Cover crops have exhibited proven potential in the management of parasitic nematodes infecting maize and they tend to have simultaneous impact on multiple species. Deployment of appropriate cover crops reduces spread of the nematodes through run-off, maintains soil fertility and restores biodiversity in the soil. For instance, a significant increase in grain yield was reported in on-farm studies where maize was intercropped with *Canavalia ensiformis* or *Mucuna pruriens* as cover crops (Arim *et al.*, 2006). In some specific maize genotype and cover crop interactions, the soil nematode infestation and disease severity was reduced by up to 50%. Cover cropping has the added advantage of suppressing weeds, which may act as alternative hosts of economically important nematode species, between the crop seasons or even in established maize fields. The fact that some cover crops may cause an increase in numbers of certain nematode species underscores the need for taxonomic expertise and comprehensive knowledge of the entire system. Lack of market value, both as food or feed, renders adoption of some of the cover crops that are suppressive to nematodes impractical in the smallholder family farms.

Conservation agriculture

The practices of minimum tillage, crop rotation and mulching, as prescribed in conservation agriculture (CA), are effective in maintaining and restoring soil health. Conservation agriculture boosts soil suppressiveness to phytonematodes and reduces their spread due to soil cover and minimal loosening of the soil. Sustainable adoption of CA requires appropriate mechanization and herbicides. In view of the prohibitive cost of machinery, innovative strategies of procurement and sharing of farm equipment needs to be developed.

Application of organic amendments

Resource-challenged farmers usually use inputs that are available on their farms and/or in adjacent neighbourhoods, for crop production. Organic amendments in the form of mulch, compost and farmyard manure are frequently applied in smallholder farms instead of synthetic fertilizers that are unaffordable.

Improvements in soil nutrients, microbial diversity and soil structure are some of the most outstanding changes that serve as a boost towards rehabilitation of intensively cultivated and degraded soils even at the smallholder level. Although large quantities of organic amendments are required to achieve optimum results, the advice is to apply any available quantity as frequently as possible. This is often done by applying organic amendments to the planting hole, which can add to protection of the seedling from early root damage by nematodes.

Improvements in current nematode management methods

Improvement of the knowledge base

Integrated nematode management is a knowledge-intensive system that aims at establishing a biological balance through application of a collection of appropriate options. This knowledge system, for the most part, does not exist at the smallholder level and the reasons for this void are manifold. The fact that most small-scale growers have experienced declining yield sets the ground for a knowledge-based approach to the problems related to both nematodes and soil health. It is important that the key stakeholders are convinced that nematodes are a real problem and that the actual cause of some of the symptoms and yield loss that is mistakenly attributed to nutrient deficiencies is nematode induced. Thereafter, nematode management options, that can be adapted to farm-specific situations, could be formulated through participatory processes. Higher investment by governments is needed to foster knowledge-based crop production.

Adapting conservation agriculture to the smallholder

Although CA has proven to be an important tool in maintaining and restoring soil health, the notion that one-size-fits-all situation is out of tune with the heterogeneous nature of smallholder agriculture. Farmers and extension staff should be equipped with knowledge to formulate variants that work under their circumstances. For instance, in situations where crop residues are removed as fodder for livestock, both farmyard manure and other forms of biomass transfer should be encouraged.

Resistance and tolerance

Damage by PPNs is not highly ranked among the biotic constraints to maize production. In addition, the diversity of agroecological zones in which maize is produced also dampens the business prospects of the seed companies, for specific pest or disease resistant cultivars. This leaves crop protectionists with the option of screening available genotypes for resistance to the predominant nematode species in the target production sites. However, it should not be overlooked that the nematode species complexes are important, and that screening should look at more than one species or group of nematodes.

Rotations

Maize yield has declined to levels that can hardly sustain household food security in most of the areas where intensive small-scale production is practiced. When this is coupled with the negative effects of climate change, the future is bleak. This reality is forcing the smallholder farmers to adopt new crops, driven by survival instincts. Horticultural crops have a strong appeal and should be recommended on the strength of having higher market value and faster growth that is consistent with the shorter rainfall spells. However, most horticultural crops are also hosts of the nematode species complexes and other soil-borne pathogens and therefore not a direct solution to the problems on maize. In order to enhance adoption, crop rotation should be holistic in promoting all components of crop and soil health. A participatory approach is required in the selection of crop sequences that are in tandem with the social economic and ecological realities of individual farmers as opposed to prescriptions made in research institutions. A major problem is changing these farmers' approaches from mixed cropping to standard rotations when the former is an approach to survival when one crop fails.

Recommendations for future research

Most African countries rely heavily on optimization of rain-fed production to advance food and nutrition security. Climate change has been and will continue scuttling the gains that have been made. As temperature rises against dwindling water resources, maize yield will decrease. Considering the vulnerability of the tropical and subtropical regions, nematode pest dynamics are likely to be influenced by rising temperatures leading to shortened life cycles and more rapid pest build-up (Coyne *et al.*, 2018). Additional work is needed on the impact of complex mixtures of PPNs on different hosts in mixed-cropping systems and how these mixtures affect nematode population dynamics (Noe and Sikora, 1990).

The risk of new species becoming established or becoming dominant as harmful pests is foreseeable, especially with the anticipated change in virulence of nematode pests and disruption of natural regulatory mechanisms. Maize cultivars are expected to be more susceptible over time due to weakening of their resistance and tolerance towards parasitic nematodes at higher temperatures. This calls for continuous development of resilient crop cultivars both to higher temperature and to multiple nematode species.

Host resistance is regarded as the bedrock of integrated nematode management packages especially where low value crops or subsistence production is involved. Breeding for nematode resistance needs government support in order to move forward. The new cultivars must be supported by a functional and responsive seed system in order to deliver the benefits to the end user. In this context, certified seed of the resistant and/or tolerant maize cultivars should be made available and affordable to the smallholder farmer. Important here would be government subsidies that are for the most part non-existent.

Seed is the most critical input for improving crop diversity and productivity. Improving the genetic base and seed quality is the most pragmatic approach of increasing productivity particularly in low-input crop production. New technologies such as seed treatment with combinations of fungicides and systemic nematicides need to be availed to the smallholder farmers (Sikora and Roberts, 2018). Biological seed treatment, as used with soybean and maize in other parts of the world, also need to be made available to the smallholder farmers. This will require comprehensive support from the governments if food security is to be attained in the future.

In addition, research on locally available substrates that foster proliferation of particular microorganisms that are antagonistic to plant parasitic nematodes are needed to support establishment of seedlings and biocontrol in the rhizosphere. Seed treatment with botanical products that are not as harmful as synthetic nematicides should be developed to offer protection against nematodes at the early and most critical stage of crop growth.

An increase in acceptance of agricultural biotechnology has been recorded in EA with remarkable steps being made towards release of genetically modified crop cultivars. It is anticipated that pro-poor and food security crops like maize will continue to attract a lot of attention as candidates for genetic modification in a bid to reduce the high losses caused by pests and diseases and to offset the problems of food security (Sikora *et al.*, 2020). In view of the looming threat from plant parasitic nematodes and climate change, root health of life-sustaining crops should be prioritized. Genetic transformations targeting vigorous root growth could impart resilience to a diverse range of factors.

The 'plant doctor' concept, developed by CABI under the Plantwise Programme, has proved to be an effective approach where a basic nematode diagnostic service is provided to the smallholder farming community (https://www.plantwise.org/poms-support/). The concept should be upgraded to facilitate sharing of global knowledge resources through ICT-based platforms (Sikora *et al.*, 2020). Additional work is needed to deliver the service closer to the farmers through cell-phone driven diagnosis and management of plant parasitic nematodes.

Outlook: anticipating future developments

According to available models, climate change will impact agriculture in Africa more greatly than elsewhere, especially in the semi-arid regions of the continent (see Chapter 64 in this volume). It will take an integrated and holistic approach, founded on a solid knowledge base, to secure freedom of smallholder maize in mixed-cropping production from CND. Apart from the requisite technologies encompassing crop improvement, seed technology and improvement of soil health, governments should create innovative escape routes for the farmers. Continued and increased investment in research, technology transfer and public support to the seed systems are minimum inputs to sustainable maize production and food security in EA.

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8 Management of root-knot nematodes in rice

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Introduction

Rice is grown on about 42 million ha in India, including the rice–wheat cropping system which is practiced on about 10 million ha in the northern states and Indo-Gangetic plains. The average productivity of milled rice is less than 3 mT ha^{-1} in India, but as high as 7 mT ha^{-1} in China and 8.38 mT ha^{-1} in the USA. The difference in yield is due to plant genetics, soil, water and climate conditions, as well as production technology and management of diseases and pests. The seasonal warm-humid weather, besides its biological characteristics, renders rice susceptible to many kinds of pests and diseases, including above- and below-ground parasitic nematodes.

The vast majority of rice farmers in India, and Asia and China as a whole, are small and marginal with 1–2 ha farm sizes who grow rice as a staple food crop. Only 1% of them are considered large farmers with 10 or more ha. The smallholder farmers are not financially strong and therefore grow crops that require limited cultivation inputs and costs and face lesser risks. This adds to the problems facing nematologists attempting to design integrated nematode management approaches. Plant parasitic nematodes, especially the root-knot nematode, *Meloidogyne graminicola* is emerging as a serious and expanding problem. Its importance and impact on yield is, for the most part, still largely unknown to most farmers and administrators, and even to agricultural extensionists. This chapter deals with this expanding root-knot problem that affects the rice and even wheat in commonly used rotations in India.

Nematode parasites of rice

The root-knot nematodes known to infect rice are *Meloidogyne graminicola, M. hainanensis, M. lini, M. incognita, M. javanica, M. oryzae, M. salasi* and *M. triticoryzae.* A number of other plant parasitic nematodes are also economically important on rice including, *Heterodera oryzae, H. oryzicola, H. sacchari,* a number of species of *Hirschmanniella* and the root lesion nematodes, *Pratylenchus indicus, P. pseudopratensis, P. zeae, Aphelenchoides besseyi and Ditylenchus angustus* (Peng *et al.*, 2018). However, the root-knot nematode, *M. graminicola* is the most important as well as most difficult to control. It is widely distributed in rice and wheat fields in the country.

Economic importance

M. graminicola can cause serious quantitative and qualitative losses in upland, lowland and

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deep-water rice. The population levels which caused 10% loss in yield of upland rice were 120, 250 and 600 eggs/plant at 10, 30 and 60 day-old plants, respectively, in direct seeded crops (Rao *et al.*, 1986). Crop losses range from 5–10% to as high as 60–90%, and even total crop failures have been reported in various experimental trials and in farmers' fields. Significant damage also occurs in the nursery beds and direct seeded rice, since young seedlings are more susceptible and even 1 second-stage juvenile (J2) per 2 cm^3 soil could cause damage.

Generally, crop damage has been measured in terms of loss of grain yield, whereas losses due to reduction in the quality of the grain to be used as food or seed have received little attention. Patil and Gaur (2014) reported that high population densities, 8 J2 per cm^3 soil of *M. graminicola* reduced grain weight by as much as 44.5% in rice cv. Pusa Sugandh-5 and 50.7% in cv. Pusa-44. More important was the loss of protein and amylose content in the grains that was significantly reduced in both cultivars.

Distribution

Meloidogyne graminicola, is widespread in the rice growing areas not only in India, but in most rice producing countries around the world (CABI, 2016). It is considered an important invasive species and is a quarantine pest in countries where it is not yet reported. In India, high frequency of occurrence and significant crop damage was reported in Orissa, Assam and Kerala (Rao *et al.*, 1986), and more recently in many other areas of India. Root-knot is emerging as a serious concern especially with its increasing severity under low water and decreased tillage-based and aerobic rice farming practices, and low soil organic matter content.

Host range

Meloidogyne graminicola prefers monocots but can also multiply on several weeds and cultivated dicots (CABI, 2016; Peng *et al.*, 2018). The nematode infects both the *indica* and *japonica* races of *O. sativa* as well as some vegetables. In India, *M. graminicola* is known to reproduce on other crops like wheat, barley, sorghum, soybean, okra, green gram, berseem (*Trifolium alexandrinum*) and some cultivars of potato, and also on common weed species like *Cyperus rotundus, Echinochloa colonum, E. crusgalli, Leptochloa coloniculus* and *Phalaris minor* (Sabir and Gaur, 2005). In some areas where rice cultivation was introduced only recently, severe damage to rice by root-knot could be attributed to the fact that the sorghum and millets traditionally grown there for fodder had supported a reserve of these nematodes.

Symptoms and damage

Above ground, the rice plants appear pale and stunted in growth in patches in the field and in nursery beds (Fig. 8.1). The size of such patches increases over the years. The leaf tips often turn whitish, giving the impression of zinc or iron deficiency, since their uptake is reduced in infected roots. Tillering is reduced, panicle emergence is poor. Panicles are small, bear fewer and lighter grains with decreased amylose content. In cases of heavy infestation, few grains are formed and spikelets remain empty. Root-knot nematode species are generally more damaging in upland and irrigated rice, but *M. graminicola* can also cause serious damage to deep-water rice. Prior to flooding, symptoms are the typical stunting and chlorosis of young plants. When flooding occurs, submerged plants with serious root galling are unable to elongate rapidly, and do not emerge above the water level and get drowned (Bridge and Page, 1982).

Below ground, the infected root tips become swollen and hooked or take a clubbed shape, a characteristic of *M. graminicola* and *M. triticoryzae* (Fig. 8.2). Galls can also be seen on other parts of the root, including on secondary roots. Thus, the root becomes excessively branched, bushy and shallow. The new galls appear whitish and turn brown or grey after 2 to 3 weeks as the females lay eggs into a gelatinous matrix mostly embedded within the aerenchymatous root cortex and rarely seen protruding outside the root. The J2 can migrate within the aerenchyma of roots to new sites to produce new galls.

Biology and life cycle

The life cycle is basically the same as for all root species and is usually completed in 3 to 4 weeks,

Fig. 8.1. Yellowing and stunting of rice infested with *Meloidogyne graminicola* in India. Author's own photograph.

Fig. 8.2. Root tip galls caused by *Meloidogyne graminicola* on rainfed rice in India. Author's own photograph.

but this can vary with host cultivars, temperature and moisture conditions. Population growth is higher in direct seeded and unpuddled soil and under intermittent flooding conditions that provide more aeration in the soil (Chandel *et al.*, 2002). Invasion is lower in rice transplanted in well-puddled soil followed immediately by flooding.

Survival and dissemination

Although *M. graminicola* is more serious in low and moderate moisture levels and light textured soils, it is well adapted to flooded conditions and can survive in waterlogged soil as eggs in egg masses or as juveniles for long periods to a depth of 1 m for at least 5 months (Bridge and Page, 1982). Reinvasion of new roots occurs quickly after the water is drained or percolated from the field. The unhatched eggs can survive in quiescent state for long periods in the slowly dried root galls remaining in soil.

Population dynamics

Depending upon the duration of the crop and prevailing temperature, three to four generations are completed in rice and one to two in wheat. Even in good monocot host crops like wheat, which is equally susceptible as rice at temperatures between 25°C and 30°C, population growth is reduced because of the lower winter season temperatures. Yet, these nematodes are able to complete at least one generation on early or late sown wheat. This is sufficient to maintain population densities to provide enough initial population densities for the following rice crops. This has been found to be one of the major reasons for the fast build-up of population densities and declining productivity of the rice crop in the popular 10 million ha rice–wheat cropping systems in parts of Nepal and the Indo-Gangetic region of India.

Interaction with other nematodes and pathogens

Generally, there is a negative correlation between the abundance of *Hirschmanniella* spp. with *M. graminicola*, since there is a relative difference in their adaptation to high water and low oxygen conditions, with *Hirschmanniella* spp. tolerating these conditions better. Most of the other nematodes like root-knot, root lesion and lance nematodes prefer aerobic conditions. Injuries on roots due to nematodes facilitate invasion of fungal and bacterial pathogens.

Recommended integrated nematode management

Extension driven knowledge transfer

Farmers are generally not aware of the infestation and seriousness of nematodes. Due to lack of trained taxonomists, correct identification of root-knot nematodes continues to be a challenge. There is a tendency toward incorrect identification of the species making management even more difficult. *Meloidogyne graminicola* is the most widespread species, but *M. triticoryzae, M. incognita* and some other species or intraspecific variants may infect rice at some places. Extension nematologists are scarce and this leads to increased nematode damage. Some effort has been made recently by ICAR-All India Coordinated Research Project (AICRP-Nematodes, 2020) on nematodes and some of the State Agricultural Universities. As a result, *M. graminicola* in rice has recently gained attention of the Central and State Governments. Transfer of this knowledge to the farmers is for the most part still insufficient.

Management systems

A number of cultural, physical, chemical, biological and genetic methods can be deployed to reduce nematode damage to rice in India. The tools available for nematode management need to be designed as logical components of an integrated nematode management (INM) package. Various approaches for root-knot and other plant parasitic nematode management have been extensively reviewed in the recent decade and are summarized in Peng *et al*. (2018).

In 2003, Gaur proposed an integrated package, elaborated below, to manage root-knot nematodes in the rice–wheat cropping system of India made up of the following management approaches (Gaur, 2003):

- Summer ploughing: Ploughing of main field two to three times in summer and exposure to solar heat and desiccation.
- Rotation:
  - including short duration leguminous crops in the rotation, for example, mung bean after wheat, as a grain and later green-manure crop;
  - incorporation of the haulm back into the soil after harvest before preparing the field for planting rice to increase natural soil suppressiveness;
  - expanding the usual rice–wheat rotation by periodically growing a non-host crop such as rape, mustard or chickpea;
  - expanding the rice–wheat rotation at spaced intervals by adding poor hosts to the rotation such as cabbage, cauliflower, fenugreek or spinach instead of wheat in the autumn–winter (*Rabi*) season; and
  - improving control of grassy weed hosts to minimize root-knot nematode population build-up.
- Nematode control in seedbeds:
  - during the pre-planting period, the area intended to be used to raise rice seedlings should be ploughed to stimulate desiccation of nematodes in the soil, then lightly irrigated and covered with 40 μm thick colourless polyethylene sheet to expose the soil to summer heat for 3-6 weeks;

- if infestation levels are still too high, apply a nematicide or de-oiled neem seed cake before sowing; and
- after 2 to 3 weeks, prepare main field soil with water (puddling) and transplant nematode-free rice seedlings
  or
- grow suitable resistant or tolerant cultivars of rice, if available.

Many rice varieties/lines have been reported to be resistant to *M. graminicol*a in India, e.g. Achhoo, Naggardhan, HPR2373 in Himachal Pradesh (Srivastava *et al.*, 2011), IR36, JR201, Kranti, Luchai, Mamaya and Shriram in Madhya Pradesh (Dhurwey *et al.*, 2019), but few have been accepted widely at farmers' level.

These recommended integrated management practices fit in very well with the agronomy and agroecology of the region under rice–wheat cropping systems. These are adjustable with, and also add to, the efficacy of weeds and other pests and diseases of rice.

Farmers' practice

As mentioned earlier, the majority of farmers grow rice on 1–2 ha farms and for the most part lack financial resources for costly inputs. This makes any integrated approach to nematode control problematical. Medium-sized farms with up to 5 ha or more have more options.

The average farmers in northern India follow raising rice seedlings and transplanting, and

- a few farmers apply neem seed cake or carbofuran to the nursery-bed before sowing;
- summer ploughing of field, puddling of the main field before transplanting; and
- submergence of field soil with water for 6 to 8 weeks or longer.

Some farmers apply insecticides, including carbofuran 3G (or in the past phorate 10G) in the main field, more for control of soil-borne insects with a positive spin-off adding control of nematodes.

Rotations with other crops in the wheat season are not popular with rice farmers who prefer the cultivation of the standard rice–wheat cropping system due to familiarity, limited resources and technical convenience. This rotation also has relatively lesser chances of crop failure and yields good minimum support prices. They avoid other crop rotations, making nematode management problematical.

In the deep-water and lowland rice areas the above practices may not be feasible. Crop rotations with *Crotalaria spectabilis*, *C. juncea*, etc. have been found to reduce root-knot nematodes in subsequent rice crops. Farmers need to be convinced not to grow multiple successive crops of rice on the same field, a common practice in eastern India, Bangladesh, Myanmar and other Asian countries.

In some areas, especially parts of eastern India, rice seed are sown/drilled directly. Young rice plants are exposed to nematode invasion and suffer heavy damage.

Outlook: anticipating future developments

Rice is generally a water-guzzling, warm-season crop and is susceptible to a plethora of pests and diseases. With decreasing availability of water for irrigation and submergence, for decreasing energy consumption, as well as to reduce damage to soil structure, the agronomists are professing reduced tillage, direct seeding and raised bed planting, etc., all of which are known to support root-knot nematodes. Nematode management will be essential if these practices are adopted. Because of the size and resources available to the small-scale rice farmer, host plant resistance is extremely important and the most advantageous method to manage root-knot nematodes. There are only a few commercially acceptable nematode resistant cultivars currently available. However, considerable variability and sources of resistance exist, especially in the African *Oryza glaberrima* germplasm. Marker assisted breeding, transgenic and CRISPR-Cas technologies show much hope for the future.

In the future, with ever increasing human populations there may be a need to increase farm size by improving farmer access to additional land. Consolidation of farm holdings and adoption of co-operative farming will be helpful. By increasing farm size, more modern methods of INM would be economically accessible to the rice farmers. This shift in farm size has been observed in Africa and elsewhere and may need consideration (Muyanga and Jayne, 2020).

Until then, and even after, farmers should rely on the above-mentioned traditional technologies.

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9 The unseen rice root nematode problem in irrigated rice

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Introduction

Several species of *Hirschmanniella* have been reported in association with irrigated rice all over the world (Peng *et al.*, 2018). In India, *H. oryzae* and *H. mucronata* are the dominant species infecting the rice crop. Rice root nematode is a problem mainly in irrigated and semi-deep-water rice.

Economic importance

It has been estimated that *Hirschmanniella* spp. infest 58% of the world's rice fields, causing 25% yield losses (Peng *et al.*, 2018). The yield losses vary depending on the rice ecosystem. *Hirschmanniella oryzae* reduced the yield by 8.3% in old lowland areas, 9.4% in new lowland areas, but no losses were noted in new upland areas (Cho-Hen *et al.*, 1994). The average single grain weight was observed to be the most affected yield component (Poussin *et al.*, 2005).

Host range

Several weeds grown in and around rice fields, for example *Cynodon dactylon, Brachiaria* spp., *Mariscus umbellatus* and *Kyllinga monocephala* have been reported to host *Hirshmanniella* spp. (Mohandas *et al.*, 1979). Other common weeds such as *Echinochloa colona, Sesbania aculeata, Cyperus rotundus, Boerhavia diffusa, Eclipta alba* and *Polygonum plebejum* also harbour *H. oryzae* (Kumar, 1990).

Distribution

Simultaneous prevalence of two or more species of *Hirschmanniella* spp. has been reported from irrigated, semi-deep-water and deep-water rice environments in most rice growing regions of India (Mathur and Prasad, 1971). Several species of rice root nematode also have been reported in association with irrigated rice all over the world. In India, *H. oryzae* and *H. mucronata* are the dominant species infecting rice in many states in the country. Both species are reported from China, India, Nepal, Pakistan, Bangladesh, Sri Lanka, Korea, Japan, the Philippines, Vietnam, Egypt, West Africa, Brazil, Portugal and Iran (Peng *et al.*, 2018).

Symptoms of damage

There are no easily identifiable above-ground symptoms of this nematode's damage in established

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rice fields. Infestation by the rice root nematode, however, does result in retardation of plant growth (Fig. 9.1), and reduced tillering in early growth stages and flowering may be delayed by 14 to 15 days (Muthukrishnan *et al.*, 1977). Infected roots first show a yellowish to brown colour which gets darker over time. Heavily infected roots eventually decay. Infected seedlings show reduced survival, delayed emergence of tillers and discolored older leaves. Rapid root regeneration, however, often results in plant recovery. Due to the practice of thorough puddling and levelling of soil prior to transplanting of irrigated rice, the rice root nematode populations get evenly distributed in the field, resulting in a non-distinguishable uniform stunting and damage to the crop in the infested fields (Prasad and Somasekhar, 2009).

Biology and life cycle

Hirschmanniella species are migratory endoparasites of roots. Eggs of *H. oryzae* are deposited in the root cortex and hatching occurs 4 to 6 days after deposition (Fig. 9.2). The nematodes, once within a rootlet, proceed through the parenchyma toward the base parallel to the stele (Fig. 9.3). The nematode usually enters seedling roots either in infested seed beds or prior to irrigation/flooding of the newly planted fields. The nematode remains in the root tissue after infection and survives in the roots during the anaerobic conditions that prevail following prolonged flood of the fields.

All the stages feed on the cortical cells and vascular region of rice roots. The life cycle is completed in 30 days. In north India, *H. oryzae* completes one generation in a cropping season (Mathur and Prasad, 1971). Four peaks of *H. mucronata* population – during the last week of September, first week of November, third week of December and second week of February – were reported in south India (Mahapatra and Rao, 1980). The nematode was active particularly in the presence of standing crop. Positive correlations were observed between the fresh weight of roots and soil temperature at 5 cm depth, and the build-up of the nematode. Maximum root populations were recorded at tillering stage of the crop (Rao, 1985).

Fig. 9.1. Yellow patch of plants in field showing *Hirschmanniella oryzae* infection in rice. Photograph courtesy of J. Bridge.

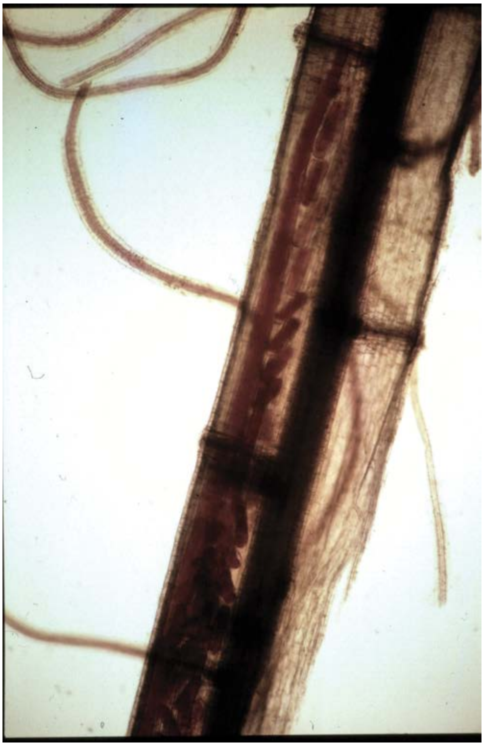

Fig. 9.2. *Hirschmanniella oryzae* eggs and female in rice root tissue. Photograph courtesy of J. Bridge.

Fig. 9.3. *Hirschmanniella oryzae* in rice root tissue. Author's own photograph.

Interactions with other nematodes and pathogens

This nematode is shown to increase the intensity of sheath blight disease in rice caused by *Rhizoctonia solani* in experimental studies (Gokulapalan and Nair, 1986).

Recommended integrated nematode management (INM)

Integrated management practices recommended for the management of this nematode include: (i) use of resistant cultivars like TKM 6; (ii) postponing the planting date from mid-June to mid-July; (iii) green manuring with legumes like *Sesbania rostrata* and *Aeschynomene afraspera*; (iv) rotation of rice with cabbage and tobacco; (v) application of organic amendments like neem cake @ 1 t/ha or sugar factory by-product pressmud @ 10 t/ha; (vi) seed treatment with biological control agents like *Pseudomonas fluorescens* Migula strain Pf-1 @ 10 g/kg seed; (vii) bare root-dip treatment of seedlings with chlorpyriphos after infestation of *Hirschmanniella* spp, or with triazophos and phenamiphos as prophylactic treatments; and (viii) carbofuran application (1 kg a.i./ha) in nursery soil at 7 days prior to transplanting and in main field 45 days after transplanting. Among these, the most effective and adoptable recommendations are incorporation of green manure crops/trap crop like *S. rostrata* or *A. afraspera* before the nematode completes its life cycle, incorporation of non-edible oil cakes in the nurseries, and application of carbofuran in nursery and main field, which give good control of this nematode.

Optimization of nematode management

The green manure crops *S. reticulata* and *S. rostrata* are susceptible to *Hirschmanniella* spp. These plants act as trap crops and incorporation of the host prior to completion of the life cycle of the nematode (i.e. at 40 days) helps to reduce the nematode population. The weed *Sphenoclea zeylanica* grows abundantly in lowlands. Its root secretions have been reported to be detrimental to *Hirschmanniella* spp. Leguminous crops *S. rostrata* and *A. afraspera* do not generate direct return and using them to control the rice root nematodes was not economical despite significant yield increase obtained with their cultivation.

Sesbania rostrata, when applied as a green manure, reduced field populations of *Hirschmanniella* spp.; however, it is a very good host for *Meloidogyne graminicola* when grown in non-flooded soils. Hence its cultivation as a green manure before rice in non-flooded soils infested by *M. graminicola* may increase their number considerably. Therefore, it is suggested that under rain-fed conditions, *S. rostrata* should not be used and other leguminous crops resistant to *M. graminicola* should be used as alternatives. The application of chemicals in standing water or placement at the base of the rice hills in mud balls was found inferior to soil incorporation in controlling the rice root nematode *H. mucronata.* Even though nitrogen amendments were able to counterbalance the negative effects of *H. oryzae*, nitrogen applied at 80 kg N/ha level was not considered a sustainable alternative because it increased nematode populations.

Future research requirements

Nematode damage often goes unnoticed due to their microscopic size and the lack of distinct above-ground symptoms. Therefore, bringing awareness among farmers and extension workers about diagnosis and management of nematode pests of rice crops is essential for minimizing yield losses caused by these nematodes. Future studies on rice nematodes should focus on the following aspects: (i) developing precise GIS based distribution maps to help target control inputs; (ii) creation of awareness of the nematode among farmers and extension workers using IT tools such as mobile applications with short video clips and pictures of nematode damage symptoms; (iii) development of locally feasible, low-cost and sustainable nematode management methods; (iv) development of sustainable rice based cropping systems with due consideration to susceptibility/tolerance/resistance of the component crops to rice nematodes; (v) exploiting the antagonistic potential of fungal and bacterial endophytes for nematode management and developing effective low-cost delivery system for these microbes such as seed treatment/nursery treatment/root dip to seedlings; and (vi) incorporation of resistance into agronomically superior cultivars using conventional breeding and biotechnological/transgenic approaches (Prasad and Somasekhar, 2009). Application of molecular techniques is required for understanding the intricate host parasite relationships.

Outlook: anticipating future developments

The yield losses due to rice root nematodes have been observed to be decreasing in recent years due to changes in cultivation practices of rice. Traditionally, long-duration rice cultivars (150 to 170 days) used to be cultivated in lowlands, particularly during the rainy season as no crop other than rice can be planted due to monsoon-generated soil water stagnation. Nurseries also used to be grown in the field soil that was often nematode infested. As a result, native *Hirschmanniella* spp. populations were transported from nursery to the field within the roots of infested seedlings, resulting in heavy population build-up and higher yield losses. However, with the introduction of short-duration rice cultivars (120 to 130 days) along with improved fertilizer use efficiency, the nematode reproduction window is only 85 to 90 days for invasion and multiplication which limits damage. Furthermore, seedlings from raised seed beds with lower infestations, following soil seed bed solarization or application of chemicals provide less chance for *Hirschmanniella* seedling infection and transfer to the field, giving the plants a growth head start.

Interestingly, the increasing insect problems like gall midge and stem borer in early stages of crop growth followed by severe build-up of plant hoppers resulting from the excessive use of nitrogen fertilizers has been offset by the application of granular systemic insecticides. Many of these insecticides (e.g. carbofuran, phorate, etc.) have been reported to have negative effects on rice root nematode population. Due to the fact that the rice crop could be harvested within 125 days, farmers started growing other high-value short-duration maize, vegetable or pulse crops which are non-hosts to *Hirschmanniella* spp. to take advantage of the residual moisture. Uniquely, it was observed that early root penetration of low densities of the root nematode stimulated the root and plant growth of these crops.

Recently, water-saving technologies such as aerobic rice, direct-seeded rice and system of rice intensification are being promoted to overcome water scarcity. With the change to these new systems there is a possibility of a decline in the population of rice root nematode species that prefer irrigated and inundated systems (Prasad and Somasekhar, 2009). This, however, will lead to an increase in populations of more pathogenic species like root-knot and root lesion nematodes that prefer upland or aerobic environment in the years ahead (Prasad and Somasekhar, 2009). However, since the rice root nematode is the only nematode pest adopted to infest rice under anaerobic conditions, this nematode has the potential to adapt to new environmental conditions and this could lead to a resurgence as a prominent pest of irrigated rice. Therefore, we should focus our research on increasing our understanding of the biology and damage potential of this nematode in newly emerging rice cultivation systems.

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10 *Pratylenchus* in sugarcane: A diminishing problem?

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Introduction

Sugarcane is a perennial crop that is grown primarily for the extraction and production of sugar and in some countries, ethanol. The worldwide pressure on sugar, however, has prompted the development of many other products, including packaging. The soil surrounding sugarcane plants hosts a large diversity of plant parasitic nematodes. Within this diversity, *Pratylenchus* is the most common plant parasitic nematode genus. It is often found in combination with *Meloidogyne* spp. and/or *Helicotylenchus* spp. at and in the roots and a host of other nematode genera in the soil. Although at least 20 species of *Pratylenchus* have been isolated from sugarcane worldwide, only four species (*P. brachyurus*, *P. neglectus*, *P. scribneri* and *P. zeae*) have been recorded in South Africa (SAPPNS). Of these, *P. zeae* is the most frequently encountered both worldwide (Ramouthar and Bhuiyan, 2018) and in South Africa (SAPPNS).

Economic importance

The last crop loss estimate due to nematodes in sugarcane in South Africa was provided by Spaull and Cadet (1995). The author estimated that the industry loses approximately 700,000 tonnes cane per annuum as a result of a complex of nematode genera. Given that *Pratylenchus* is present in more than 80% of the samples in South Africa and on average in growth-reducing numbers, it can be assumed that *Pratylenchus* is a major contributor to this figure (Ramouthar, 2014; Berry *et al.*, 2017).

Host range

Of all the plant parasitic nematodes worldwide, *Pratylenchus* spp. seems to have the broadest host range (Duncan and Moens, 2013). Management of this pest is thus particularly problematic as many different plants can increase numbers within fields. Green manure crops chosen for rotation with sugarcane must thus be carefully selected if *Pratylenchus* spp. is a problem in fields.

Distribution

Pratylenchus spp. is almost always encountered when sampling a sugarcane field in South Africa. The only true survey conducted in South African sugarcane fields was conducted in 1978

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(Spaull, 1981). One hundred and twenty-four fields were sampled throughout the sugar growing region and *Pratylenchus* was found in 96% of those fields. Two later studies analysed the database of samples received in the nematology lab at the South African Sugarcane Research Institute (SASRI) in 2004 and 2012 (Berry, 2006; Ramouthar, 2014). Based on these studies, it appears as if the frequency has decreased from the initial survey and then stabilized. Berry (2006) recorded a frequency of 83% and Ramouthar (2014) 88%. Therefore, *Pratylenchus* spp. is consistently encountered more than 80% of the time in sugarcane in South Africa. Multivariate analysis of the 1978 survey showed that *Pratylenchus* was associated with the coastal area of South Africa, which included the irrigated area of Pongola, as compared to the inland, higher altitude areas. This coastal area was characterized by higher temperatures, radiation and soil pH (Spaull *et al.*, 2003).

Symptoms of damage

Pratylenchus zeae can be identified by the presence of red, reddish purple or brown lesions on the roots of sugarcane plants (Stirling and Blair, 2000) (Fig. 10.1). The lesions become necrotic and turn purplish black, causing the root system to darken in colour (Ramouthar and Bhuiyan, 2018). It also significantly reduces the presence of fine root hairs (Blair, 2005). *Pratylenchus* has been shown to reduce root mass, shoot mass and the number of shoots, as well as causing necrosis of cells in the root cortex and distort roots (Cadet and Spaull, 2005). Infection with *Pratylenchus* is characterized by a sparse, less well-developed root system with fewer root hairs (Fig.10.2), which if present are distorted, and fewer feeder roots (Harris, 1974; Blair, 2005; Cadet and Spaull, 2005). A 28% reduction in root weight was observed in South Africa (Harris, 1974) and 9–55% in Australia, with a 58% and 47% reduction in root length and tertiary root surface area, respectively (Blair, 2005).

Biology and life cycle

Root growth in sugarcane occurs between 12°C and 30°C (Van Dillewijn, 1952). Similarly, *Pratylenchus* requires temperatures of between 17°C and 30°C for completion of its life cycle (Duncan and Moens, 2013). Given that moisture is required for both root development and nematode survival, it can be deduced that conditions ideal for root development in sugarcane are thus ideal for infection by *Pratylenchus*. The wide host range of *Pratylenchus*, its ability to survive as eggs in the soil and its parthenogenic nature coupled with the perennial nature of sugarcane (roots always available) and the short time span between removal of the old crop and planting

Fig. 10.1. *Pratylenchus* lesion and overall symptoms on a sugarcane root system. Photograph courtesy of Sugar Research Australia.

Fig. 10.2. Reduced root system and presence of fine root hairs in sugarcane due to the simultaneous presence of a combination of different genera of plant parasitic nematodes, including *Pratylenchus*. Author's own photograph.

the new crop make *Pratylenchus* nearly impossible to eliminate from sugarcane fields. Its presence within fields, that are for the most part monocultures, must thus be managed differently than in most other field crops. Given that *Pratylenchus* can complete its life cycle in 3–7 weeks, at temperatures between 17°C and 30°C, treatment should be applied when these conditions are expected. Furthermore, the endoparasitic nature of the nematode suggests that treatments with systemic activity are required.

Interactions with other nematodes and pathogens

Pratylenchus is almost never found exclusively in a sugarcane soil. As such, its effect on cane yield is usually as part of a complex containing other plant parasitic nematodes. In South Africa, however, no evidence exists for additive effects of different genera but rather dominance of one genera over another (Berry *et al.*, 2017). The combined effect of infection with a diverse community of plant parasitic nematodes (including *Pratylenchus*) and the bacteria *Leifsonia xyli* subsp. *xyli*, the causal agent of ratoon stunting disease, is additive (Spaull and Bailey, 1993). No other economically important root pathogens are present in sugarcane in South Africa, so little information on interactions is available. Pot trial work indicated that sugarcane roots infected with *Fusarium verticillioides* supported significantly higher numbers of *Pratylenchus* spp. in the early stages of plant growth when compared to the uninoculated control (McFarlane *et al.*, 2013).

Recommended integrated nematode management (INM)

Sampling and identification

In South Africa, all growers are encouraged to sample their soils first to test for the presence of specific highly damaging nematode genera. Recommendations are thus based on the presence or absence of these genera as thresholds are not reliable. It is suggested that all fields of a farm with a clay percentage of 20% or below are sampled to determine the plant parasitic nematode status. Currently this recommendation, as with all other recommendations, is done through the presentation of courses in sugarcane production, printed information generated by SASRI and one on one interaction between grower and extension specialist or scientist. In future, sampling of soil and roots for nematodes in soils with <20% clay will also be recommended when soil fertility samples are received by the Fertilizer Advisory Service, run as a subsidiary of SASRI. When growers do not break the sugarcane cycle with a green manure, repeated sampling in fields where nematodes have previously identified is not recommended. Where a green manure crop is planted before replanting of sugarcane, it is recommended that the green manure crop is sampled to assess the effect of the green manure crop on nematode populations. Depending on the green manure crop used, benefits can be either positive or negative on populations of *Pratylenchus* spp.

Nematicides

The most widely used nematode management method in South Africa is currently the use of chemical nematicides. Three active ingredients (carbofuran, furfural aldehyde and oxamyl) are registered for use in South Africa, but these are all classified as either very toxic (red band) or harmful (yellow band). Chemical treatment is only recommended once in the season, in the furrow at planting and shortly after harvesting in every successive ratoon. If the cane is harvested in winter, treatment with a nematicide is delayed until spring to coincide with extended periods of new root growth. Current research, which looks promising, is focusing on combinations of traditional nematicides and biological control products.

Green manure cropping

Sugarcane is grown as a perennial monoculture, thus allowing very little space for crop rotation. At every replant cycle, however, growers are advised to add a green manure crop before replanting of sugarcane. Common green manure crops used in sugarcane in South Africa include black oats in winter and sun hemp or forage sorghum in summer. Green manuring has numerous benefits but can significantly alter the nematode population in a field. Growers are advised of both the positive and negative effects of green manure crops on the nematode population and that it is important to assess that effect, through sampling, when growing sugarcane in a sandy soil. They are also encouraged to identify the nematode genus responsible for yield loss and choose green manure crops that will reduce numbers of that genus in the soil. Several commonly used green manure crops within the South African sugar industry hosted lower *Pratylenchus* numbers than sugarcane, in the soil. Crops tested included buck wheat (*Fagopyrum esculentum*), cabbage (*Brassica oleracea* var. *capitata*), cowpea (*Vigna unguiculata*), dolichos beans (*Lablab purpureus*), forage peanut (*Arachis glabrata*), forage sorghum (*Sorghum bicolour*), giant English rape (*Brassica* sp.), grazing vetch (*Vicia sativa*), hairy vetch (*Vicia villosa*) Japanese millet (*Echinochloa esculenta*), pearl millet (*Pennisetum glaucum*), lucerne (*Medicago sativa*), lupin (*Lupinus* sp.), oat (*Avena sativa*), marigold (*Tagetes* sp.), red clover (*Trifolium pratense*), Rhodes grass (*Chloris gayana*), seradella (*Ornithopus sativus*), sunn hemp (*Crotalaria juncea*), velvet bean (*Mucuna pruriens*) and wheat (*Triticum aestivum*). Cowpeas, marigold and sunn hemp were also found to be non-hosts for *P. zeae* as no *Pratylenchus* was found in the roots. The authors also showed that the effects of planting a 3-month green manure lasted in the cane crop for up to 15 months (Berry *et al.*, 2011). This suggests that green manuring is an effective option in the plant crop but in the ratoon, a follow-up with a nematicide is usually required.

Resistant varieties

No resistant sugarcane varieties are available and consistent tolerance against yield depression of a particular variety across a wide range of environments has yet to be proven. A differential response to nematicide, however, has been demonstrated across varieties. This is also variable and makes it very difficult to provide guidelines across the wide range of environments under which sugarcane is grown in South Africa. The growers, however, rely on this information and find it very useful. To get the information out to growers and account for the variability, on farm demonstration trials with different varieties either left untreated or treated with a nematicide are planted on grower cooperator farms. Trials are planted in conjunction with the grower, extension specialist and the scientist and include varieties relevant to the grower and the region. These trials also serve as knowledge-exchange tools in raising awareness of the importance of nematodes in sugarcane.

Planting and harvesting dates

In irrigated areas, planning the planting and harvesting date to coincide with reduced nematode activity (early season) will minimize the impact of plant parasitic nematodes on the growing crop (Berry *et al.*, 2017).

Farmer acceptance of INM

Reasons for non-adoption of integrated nematode management is complex. Through a large focus on knowledge exchange in recent years, the awareness of nematodes and the impact of plant parasitic nematodes on sugarcane yield has been raised and this is evidenced by the increase in the number of samples sent into the laboratory as well as the increased requests via extension for information on nematode management. However, many still fail to effectively manage their nematode problem. Even though it is recognized that plant parasitic nematodes are a production constraint, growers have a long list of pests and diseases, not to mention practical considerations, and as such nematodes are somewhere at the bottom of that list. It is still a pest that cannot be seen nor are the symptoms obvious and it is therefore easy to ignore. In addition, for too long, growers were able to rely on aldicarb as an effective solution. It thus became easy to treat sandy fields with a nematicide and ‘solve’ the problem. One would assume that the removal of aldicarb would allow for the increase in the adoption of integrated management but in fact it has led to the abandonment of management. A suitable replacement which is a combination of a nematicide (oxamyl) and insecticide (imidacloprid) has since been registered in South Africa but the perceived high cost of this option is limiting its use. The use of effective green manure crops is gaining momentum and many growers are exploring this option. However, it is still being used for the general benefits of green manuring and needs to be targeted towards nematodes in order to be an effective nematode management option. The other limiting factor to effective nematode management is the current state of the South African sugarcane industry. Many growers are switching to other crops and as such are disinvesting in sugarcane. Money is being diverted into alternate high-value crops such as macadamias, avocados, kiwis and other fruit and tree crops. What is encouraging, however, is that the awareness of nematodes in sugarcane is being transferred into the production of these other crops and nematode management is set up if required.

Optimization of nematode management

The key to long-term sustainable nematode management in sugarcane in South Africa lies in the development of a completely integrated management strategy. The first step would be reintroducing nematode resistance genes into modern sugarcane varieties using ancestral sugarcane as parents. Modern sugarcane varieties show no resistance to nematodes and introduction of parent material that shows resistance to the most important nematode genera could improve the resistance of modern varieties. Suitable parents are currently being screened and show potential.

An effective safer chemical option is also required. This could be developed either through a completely new and safer active ingredient, optimization of application methods or both. Furthermore, identifying and exploiting interactions between nematicidal and fungicidal active ingredients show potential for reducing active ingredient loads without compromising on efficacy. Combining different active ingredients with different targets (even without interactions) into one product will also add value. Even low levels of nematode–fungal interactions in the root can be damaging but are not easily detected.

There exists real potential to further exploit the use of green manure crops. This will involve focusing on both the correct crop and the length of growth of the green manure crop before sugarcane or in the ratoon. Crops that have maximum effect in the shortest space of time as well as additional benefits will be the best choice. During the ratooning crop cycle, the addition of organic matter to the crop is crucial. This can be achieved through mulching at harvest or intercropping of alternate crops in the interrow of sugarcane. The latter has greater potential within small-scale farming. The use of biological products in combination with traditional chemical products also has potential. Failing to develop such a strategy will result in non-management of plant parasitic nematodes and associated yield loss.

Future research requirements

Future research into nematology in sugarcane in South Africa, and elsewhere where sugarcane is grown under similar conditions, should focus on development of new, routine methods for nematode identification, the impact of the introduction of other crops into old sugarcane fields and understanding the impact of good soil health on the ecosystem and its role in plant parasitic nematode management.

The techniques used to communicate to growers must also be critically evaluated. In addition, the interaction between *Fusarium* and other similar soil organisms with plant parasitic nematodes warrants further investigation. The provision of management solutions that provide benefits that transcend beyond just nematode management in a single crop and provide benefits of more than one pest in more than one crop will be critical in alleviating damage due to plant parasitic nematodes in sugarcane in future.

Outlook: anticipating future developments

It is highly likely that the yield loss due to plant parasitic nematodes in sugarcane in South Africa in the future will decrease due to the need for reducing the size of the sugar industry in South Africa. Many growers are removing sugarcane and planting mainly tree crops, particularly macadamia. These crops prefer well-drained sandy soils in which sugarcane productivity is limited and growth is significantly hampered by plant parasitic nematodes. As such, sugarcane in these soils is being replaced by alternate crops. Furthermore, the switch to alternate crops that require more intensive management has improved the quality of the sugarcane produced and thus improved the plants' ability to withstand the damage caused by nematodes. The closure of the Nematology Department from the SASRI in August 2020 will, however, influence nematode management in the short and long term. The lack of expertise may also further prompt growers to remove sugarcane production from sandy soils due to ineffective management and as a result, poor yields. *Pratylenchus* in sugarcane is thus described as a diminishing problem due to the high likelihood of sugarcane no longer being grown in the soils in which it is currently a problem.

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11 Problems and solutions to integrated nematode management of root-knot, reniform and lesion nematodes in cotton in Brazil

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Introduction

Cotton is one of the major high-value crops in Brazil, with a cultivation area of 1,671,000 hectares and production of more than 4,000,000 tonnes. The main producers are the states of Mato Grosso and Bahia, which are responsible for almost 90% of the total Brazilian yield.

Plant parasitic nematodes are major problems for cotton growers in Brazil, with *Meloidogyne incognita*, *Rotylenchulus reniformis* and *Pratylenchus brachyurus* considered to be the most important. The root lesion nematode, *P. brachyurus*, is the most widely distributed in cotton growing areas in Brazil, although losses due to the attack of this nematode are not commonly reported. Although the reniform nematode, *R. reniformis*, has a limited distribution in cotton fields in Brazil, it has been reported by growers from Mato Grosso State as a concern for the crop because of management difficulties (Santos, 2017). The root-knot nematode (RKN) *M. incognita* is considered to be the most damaging to cotton and is also widely distributed in the main Brazilian cotton growing regions.

Economic importance

Under favourable conditions, RKN can cause severe damage, including extensive abortion of flowers that lead to losses of about 10–20% of the yield when only ten juveniles are present in 200 cm^3 of soil (Inomoto and Asmus, 2006). Considering that yield in Mato Grosso is about 4290 kg per hectare, these losses correspond to 429–858 kg per hectare. Damage caused by reniform and lesion nematodes are, for the most part, not recognized by the growers at this time. Reniform nematode causes damage to cotton at high population densities (Inomoto and Asmus, 2006). Nowadays, the common crop rotation with soybean in Brazilian cotton fields – both species are susceptible to the reniform nematode – means that the population densities in soil are increasing rapidly, according to the Association of Seed Producers of Mato Grosso (Aprosmat). In this succession scheme, soybean is cropped for 100–110 days, from mid-September to mid-January or early February, with cotton cropped in the second season, for 170 to 220 days, from early February to mid-September.

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Some soybean cultivars (e.g. M 8644 IPRO, NS 7901 RR and TMG 2183 IPRO) greatly increase reniform nematode populations, with reproduction factors from 146 to 192 under greenhouse conditions (R. Silva, Rondonópolis, 2020, personal communication).

Host range

Cotton monoculture during the summer season is the primary production system in Brazilian cotton growing, which is adopted in about 83% of cotton fields in Mato Grosso State (Lamas *et al.*, 2016). In this scheme, there is an intense soil disturbance and commonly pearl millet (*Pennisetum glaucum*) is cropped as cover crop in the mid-season, from September to October, with cotton sown from early December, 15–20 days after pearl millet desiccation. Pearl millet is not a host for *R. reniformis*, but can increase *M. incognita* and *P. brachyurus* populations, depending on the cultivar. Generally, the top 10–20 cm of soil is highly compacted in fields under cotton monoculture in Mato Grosso State, which leads to poor cotton development; subsoiling before cropping is a common practice in these areas and this procedure distributes nematodes across the area, independently of the soil texture.

On the other hand, in the west of Bahia State, where cotton is mostly cropped under irrigated systems, common plant species used in succession to cotton are common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*), both of which are highly susceptible to the three nematode species important to cotton (Inomoto, 2016). As expected, soil nematode populations are also increasing greatly in this important cotton-growing region.

Distribution

Meloidogyne incognita is widely distributed in the states of Mato Grosso and Bahia (Santos, 2017), which corresponds to 93% of the total Brazilian cotton growing area. *Rotylenchulus reniformis* has a limited distribution in cotton fields in Brazil, and information from western Bahia revealed that this nematode is present in 15% of the sampled fields, while RKN is present in 25% and the lesion nematode in 70% of the sampled fields. In Mato Grosso, the reniform nematode and RKN are present in 15% and 25% of the sampled fields, respectively (R. Galbieri, Primavera do Leste, 2020, personal communication).

Symptoms of damage

RKN can cause severe damage, including extensive abortion of flowers and reduced and galled root systems with few lateral roots. It is common to observe mottled yellow-reddish leaves, a symptom known as 'carijó'. Symptoms of damage caused by the reniform nematodes are not easily visualized in field conditions and losses can occur without any specific symptoms. Under severe infestations, plants are stunted with reduced root systems, but the 'carijó' sign is rarely seen. In relation to *P. brachyurus*, the root lesions are non-specific and can be confused with those caused by other soil pathogens, nutritional deficiencies or hydric stress (Galbieri and Asmus, 2016).

Biology and life cycle

In Brazil, race 3 of *M. incognita* is the most disseminated in cotton fields and its occurrence is associated with hot climate and sandy to medium-clay soils. It is a sedentary endoparasitic, obligate mitotic parthenogenetic species in which the second-stage juvenile is the infecting stage, and the subsequent juveniles complete their development in the roots, after the formation of the nurse cells, until the adult phase. Males, when present, are not parasites and females deposit eggs in a gelatinous matrix external to the root system. The reniform nematode is a sedentary semi-endoparasite and the immature female is the infecting stage, inciting the formation of nurse cells to complete its development until the mature phase, when it lays eggs in a gelatinous matrix. Its reproduction is amphimictic and males are present in the field populations. *Pratylenchus brachyurus* is also an obligate mitotic parthenogenetic species, but all the developmental stages are vermiform and penetrate roots as a migratory endoparasite. The lesion nematode female can lay up to 30 eggs during its life cycle, whereas *R. reniformis* females lay about

100 eggs and RKN lay up to 1000 eggs (Belot and Galbieri, 2016).

The life cycle of nematodes is influenced by several factors, including temperature, humidity, and host, but in general complete the cycle in 3 to 4 weeks. Therefore, when cotton is cropped in succession to soybean, there is about 250 to 280 days with a susceptible host in the field, allowing a great number of generations per crop season, even with the dry conditions found at the end of the cotton season (Lamas *et al.*, 2016).

Interactions with other nematodes or pathogens

Damage caused by the RKN favours the incidence of fusarium wilt in cotton fields, caused by *Fusarium oxysporum* f.sp. *vasinfectum*, since root damage caused by the RKN facilitates the entrance of the fungus, even in wilt resistant cultivars. *Verticillium dahliae* wilt is also favoured by the presence of nematodes, but this disease is more commonly observed in soils with high organic matter content and in colder climates and it was not detected in Mato Grosso and Bahia States.

Recommended integrated nematode management (INM)

The management of nematodes, in particular RKNs, in cotton in Brazil is based on three principal practices: the use of resistant or tolerant cultivars, crop rotation with poor or resistant hosts, and the use of chemical or biological nematicides. Despite the wide distribution of *P. brachyurus* in Brazilian cotton fields, its management is not justified due to its low capacity to damage cotton plants. However, there are no cotton or soybean cultivars resistant to this root lesion nematode and most plant species used for crop rotation, cover crops or green manures are good hosts for this nematode.

Resistance

Resistant cultivars to *M. incognita* are recommended when low population densities are present in the soil (<50 juveniles/200 cm^3 of soil), but there are few options available for growers. Recently, the cultivar IMA 5801 B2RF, with resistance and tolerance to *M. incognita* (Galbieri *et al.*, 2019a,b), has been rapidly adopted by cotton growers in Brazil, with about 10% of the total Brazilian cotton area planted with this cultivar in only 1 year since its market launch (Rafael Galbieri, Primavera do Leste, 2020, personal communication). Unfortunately, this cultivar is classified as moderately intolerant to the reniform nematode (Galbieri *et al.*, 2019a,b). IMA 5801 B2RF can reduce by about 98% the *M. incognita* population observed in the susceptible check, FM 975 WS, with a gall index of 0.5 (Galbieri *et al.*, 2019b) (Fig. 11.1A,B).

Unfortunately, resistant cultivars are not yet available to reduce *R. reniformis* populations and only tolerant cultivars are used to limit yield loss in infested areas (Galbieri *et al.*, 2019a). Cultivars DP 1746B2RF, FM 983 GLT and IMA 8001 WS are reported to be tolerant to reniform nematode under field conditions in studies conducted in the municipality of Campo Verde, Mato Grosso State (Fig. 11.1C).

A typical situation in Mato Grosso is the adoption of few cultivars in the cotton fields. Lamas *et al.* (2016) reported that more than 50% of the cotton growing area is cropped with only one cultivar. If this cultivar has no resistance genes to nematodes, the consequences to cotton yield are disastrous and other management practices must be adopted.

Management of root-knot and reniform nematodes using resistant cultivars should also comprise soybean resistant cultivars when soybean is cropped as a rotational crop. Two soybean cultivars, TMG 4182 and NS 7497 RR, can be classified as resistant to *R. reniformis*, while many cultivars are available that are resistant to *M. incognita*, generally with the resistance source derived from cv. Bragg.

It is recommended to implement at least two management strategies to obtain better results. Inomoto and Asmus (2006) reported that under severe infestations of *M. incognita*, with losses reaching 750–1500 kg/ha, nematicides have the potential to recover only 225–375 kg/ha. Higher cotton yields can only be achieved by the concomitant adoption of crop rotation and resistant cultivars.

Fig. 11.1. (A) Comparison between root symptoms in susceptible and resistant cultivars to *Meloidogyne incognita*, evidencing the root galls in the susceptible (left) and the absence of root galls in the resistant IMA 5801 B2RF (right). **(B)** Different cultivars in a field infested with *M. incognita*, showing intolerant (left) and tolerant (right) genotypes. **(C)** Different cultivars in a field infested with *Rotylenchulus reniformis*, showing tolerant (left) and intolerant (right) genotypes. Photographs courtesy of Rafael Galbieri.

Crop rotation

Significant reductions in *R. reniformis* soil and root populations in soybean–cotton fields were observed when cotton was substituted by maize intercropped with *Urochloa ruziziensis* (=*Brachiaria ruziziensis*) for one season or by *U. ruziziensis* for 18 months. Soil populations were reduced by more than 80% in both situations compared to soybean–fallow without soil disturbance according to Fundação MT. However, in areas with historical occurrence of *Scaptocoris castanea*, the brown burrower bug, planting maize or brachiaria before cotton can increase the incidence of this pest and its damage to cotton.

Fallow is a practice that has been used in many areas under cotton monoculture, especially in Mato Grosso State. However, due to the occurrence of weeds, fallow is a practice only acceptable if it is dry enough to avoid the development of weeds and remaining cotton plants.

Green manure crops

Although brachiaria, especially *U. ruziziensis*, can reduce populations of the reniform nematode, the introduction of *Crotalaria spectabilis* for one cropping season can improve the management of this nematode. In a study conducted in a naturally infested field in Mato Grosso State, a significant increase in the productivity of cotton cultivar TMG 47B2RF was observed when it was cropped after the succession of *U. ruziziensis* associated with biological control agents (*Trichoderma asperellum* + *Bacillus subtilis*) cropped in February and *C. spectabilis* cropped in October, as compared to the sequence of cotton TMG 47B2RF (February), followed by *U. ruziziensis* (October), and cotton TMG 47B2RF (January), where a reduction of 1620 kg/ha in cotton yield was observed (R. Silva, Rondonópolis, 2020, personal communication).

Crotalaria spectabilis and *C. ochroleuca* are also recommended to reduce *M. incognita* populations, although the efficiency of *C. ochroleuca* is variable under field conditions. High reductions in *M. incognita* populations are observed and cotton yield is improved after only 1 year of crop rotation with *C. spectabilis*. *Crotalaria breviflora* has similar effects to that of *C. spectabilis* in the reduction of nematode population densities, but *C. juncea* must be avoided since it is susceptible to *M. incognita*.

In the case of *M. incognita* under field conditions in Mato Grosso, *U. ruziziensis*, *C. ochroleuca* and pearl millet (*P. glaucum*) cv. ADR300 significantly reduced the RKN population both in soil and roots of cotton cropped in succession. However, maize hybrid 2B688 PW cropped alone or intercropped with *U. ruziziensis* did not reduce the nematode population. According to Aprosmat, most of the maize hybrids available to Brazilian growers are susceptible to *M. incognita*. Other options to reduce *M. incognita* and *R. reniformis* populations in cotton fields are *U. decumbens, U. brizantha* and *Panicum maximum*.

Nematicides

In Brazil, chemical and biological nematicides are applied mainly through seed treatment and, to a lesser extent, in furrow and via drench (Machado, 2016; Machado *et al*., 2016). Nowadays, the main strategy adopted by cotton growers in Mato Grosso is biological control. Bacterial agents are preferred as seed treatments, especially those from the genus *Bacillus*, while fungi are applied in furrow. Although very few data about the use of bacteria to manage nematodes in cotton are available, cotton growers in Mato Grosso have chosen nematicides that combine more than one *Bacillus* species, such as the biological nematicide Presence®, composed of *B. subtilis* lineage FMCH002 and *B. licheniformis* lineage FMCH001, commercialized by FMC. According to the Brazilian Ministry of Agriculture, Presence® is recommended as a seed treatment for *M. incognita* and *P. brachyurus* management at dosages of 100–150 g per 100 kg of seeds. *Bacillus firmus* (Votivo®) and *B. amyloliquefaciens* also proved to be efficient agents to reduce *M. incognita* populations when used as seed treatment in cotton under experimental conditions.

Biological nematicides composed by fungi that have been used by cotton growers in Mato Grosso include *Pochonia chlamydosporia* (Rizotec®, isolate PC10 from Stoller do Brasil S.A.) and *Purpureocillium lilacinum* (Nemat®, isolate UEL PAE 10 from Ballagro AgroTecnologia). Both are applied in furrow at sowing of cotton, or in the debris following soybean desiccation prior to cotton, when humidity and temperature control inside the mulch provide optimal conditions for fungi establishment and development. However, better results are obtained when these fungi are applied in the sowing of cover crops, as seed treatment or in furrow, cultivated in the interseason, before cotton, as pearl millet, *Urochloa* spp. and sorghum (Machado *et al*., 2016).

Future research requirements and an outlook for INM in Brazil

Although resistant cultivars are important to nematode management in cotton, for a long time the development of these genotypes ran into the difficulties imposed by phenotyping a high number of genotypes to this trait. With the advance of molecular markers and technologies such as gene introgression, CRISPR and genetically modified organism development, cotton breeding programmes have been placing greater emphasis on this issue. For reniform nematodes, to which resistance in upland cotton (*G. hirsutum*) is reported to be not complete, the impact of the molecular approaches can be crucial. Resistance genes from *G. longicalyx* or *G. barbadense* have been introgressed into *G. hirsutum* cultivars, but until now no resistant cultivar to *R. reniformis* is available to cotton growers in Brazil. It is expected that Brazilian cotton breeding programmes may have resistant genotypes in the medium term. Regarding *M. incognita,* the cultivar IMA 5801 B2RF mentioned earlier is expected to become increasingly adopted over the coming years.

In relation to nematicides, it is expected that adoption of biological control by growers in Brazil, like much of the rest of the world, will increase over the coming years. However, novel biological products that have an extended shelf-life and, more importantly, with formulations that avoid easy degradation of the organism by the extreme temperature and humidity conditions found in the fields, should be made available for growers. In addition, especially with regard to chemical nematicides, molecules with lower phytotoxicity and affordable prices are necessary to improve market competition with the biological nematicides.

Cotton growers are facing new challenges with the emergence of nematode species that, until now, were not reported as potential problems, such as *Helicotylenchus dihystera*, *M. enterolobii* and *Aphelenchoides besseyi*. The spiral nematode, *H. dihystera*, has been reported as an emergent potential pathogen for soybean in Brazil and its wide distribution also in cotton fields is of concern (Fig. 11.2A). *Meloidogyne enterolobii*, an aggressive nematode species of several crops, especially guava and legumes, was recently described infecting cotton in Minas Gerais State in Brazil (Galbieri *et al*., 2020), causing symptoms of stunted and chlorotic plants with root galls. The cultivar IMA 5801 B2RF, resistant to *M. incognita*, was found in this field showing root galls larger than those typically associated with *M. incognita* in cotton (Fig. 11.2B).

In Brazil, *A. besseyi*, the white tip nematode, is the causal agent of the 'soybean green stem and foliar retention syndrome', or 'Soja Louca II', which leads to losses of about 60% in soybean yield under field conditions, due to the high level of flower and pod abortion (L. Favoreto, Londrina, 2019, personal communication). The concern about this nematode is that Favoreto *et al.* (2018) reported similar symptoms in cotton in Brazil, i.e. stunted plants, flower abortion and foliar distortion (Fig. 11.2C), caused by *A. besseyi*. In Mato Grosso, under favourable conditions, which include high humidity during the cotton season, some growers have reported symptoms caused by *A. besseyi*, and verification of its presence in the aerial top parts of cotton was done by expert nematologists. Yield losses

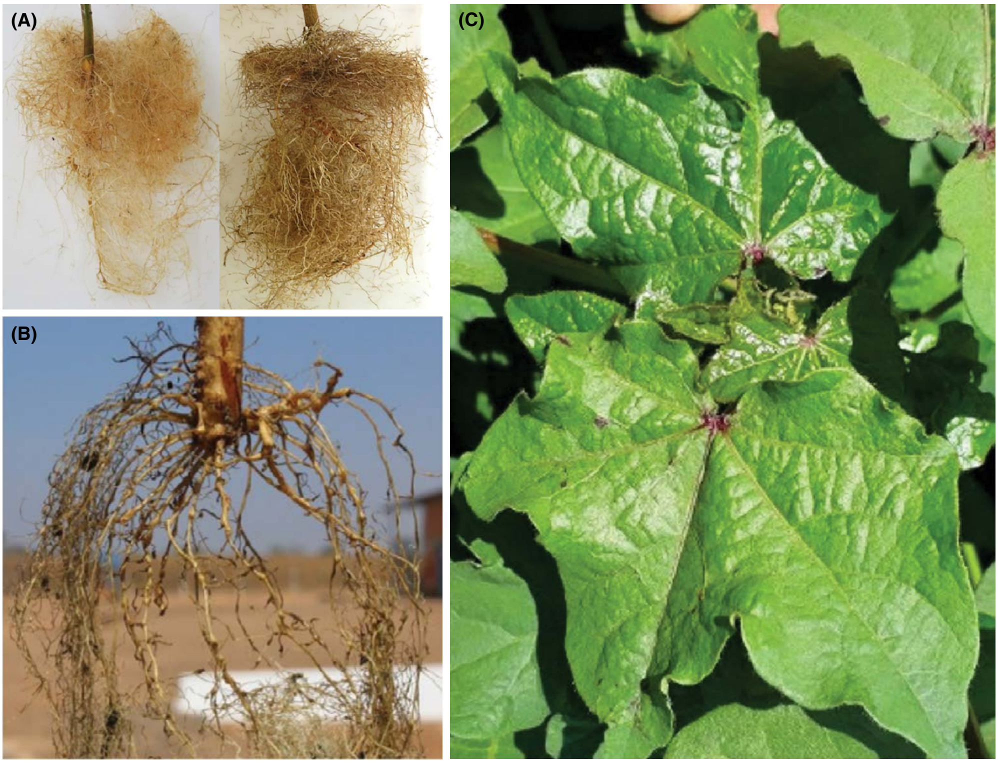

Fig. 11.2. **(A)** Root lesions caused by *Helicotylenchus dihystera* in cotton cultivar FM 975WS in a non-inoculated plant (left) and infected with the spiral nematode (right). Photograph courtesy of Santino Aleandro da Silva. **(B)** Root galls caused by *Meloidogyne enterolobii* in cotton line M-120 RNR. Photograph courtesy of Rafael Galbieri. **(C)** Foliar distortion caused by *Aphelenchoides besseyi* in cotton. Photograph courtesy of Rafael Galbieri.

can be significant and there are reports of symptoms occurring in the total area of farms of more than 400 hectares in Mato Grosso (L. Favoreto, Londrina, 2019, personal communication). In these situations, cotton growers are testing several tools to manage this nematode, such as fungi-based biological nematicides, abamectin and other chemical nematicides and insecticides, but no promising results have been obtained to date (R. Silva, Rondonópolis, 2020, personal communication).

In addition, the tropical conditions in which cotton is grown in Brazil, together with the intensive cropping systems adopted in the main cotton growing areas, allow a series of pests, diseases and other disorders to damage cotton plants and lead to high yield losses. Faced with both old and new emerging nematological problems, Brazilian researchers have a long road ahead with the constant challenge to discover novel tools and practices to avoid crop losses.

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12 *Hoplolaimus columbus*: A prime candidate for site-specific management in cotton and soybean production

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Introduction

Several species of *Hoplolaimus,* all referred to as 'lance' nematodes, occur on agronomic crops in the United States. *Hoplolaimus galeatus* is the most widely distributed, occurring throughout the southern and mid-western United States where it is a common and significant pathogen of maize. It is also commonly recovered from row crops in North Carolina, but rarely is it recovered from cotton or soybean in South Carolina. A recent survey showed that *H. stephanus* is commonly found in soybean fields in North Carolina. However, only *H. columbus* has been found in South Carolina soybean fields (Holguin *et al.*, 2011). A third species, *H. magnistylus*, is found in limited areas of Alabama, Arkansas and Mississippi but has not been found in South Carolina. It appears to be much less damaging to cotton or soybean than *H. columbus* or *H. galeatus*.

Economic importance

Yield losses due to Columbia lance nematode (CLN, *H. columbus*) have been reported only from Georgia, North Carolina and South Carolina. Losses in individual cotton and soybean fields may be as high as 50%, but probably average between 10% and 25% in severely infested fields. State-wide in South Carolina, losses to due CLN are estimated annually at 3% in cotton and 2% in soybean. In comparison, losses in cotton to southern root-knot (SRK) and reniform nematodes averaged 4% and 3%, respectively. Yield losses due to CLN in maize are significantly lower, generally less than 10%.

Approximately 200,000 hectares are planted collectively to maize, cotton and soybean in South Carolina annually. Over 70,000 of these hectares are infested with CLN, resulting in a loss of US$100 per hectare, or an annual loss of US$7 million to the state's economy.

Host range

The CLN has a wide host range that includes maize, cotton, grain sorghum, millet, soybean, wheat, and many grass crops and forage legumes. Peanut is the only significant agronomic crop that is not a host. Vegetable hosts include lima bean, common bean, cow pea, sweet corn, cantaloupe, cucumber, okra and watermelon. Many winter cover crops, including crimson clover, oats, rye and vetch, are hosts, but infection

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and subsequent damage and yield losses are low if soil temperatures at planting in the autumn are below 18°C, and if the crops are destroyed prior to soil temperatures increasing in the early spring. Many common weeds are hosts for CLN.

Distribution

The CLN is found in approximately ten counties in the coastal plain of east-central Georgia (R. Kemerait, pers. comm.) and nine counties in North Carolina, primarily along the South Carolina border. Martin *et al.* (1994) recovered CLN from 61% of 1219 cotton and soybean fields surveyed in South Carolina with densities exceeding the damage threshold in 37% of those fields. This was more than twice the percentage of fields with SRK or reniform nematodes over their respective thresholds. In all three states CLN is restricted to the coastal plain, where most of the soils are classified as sands, loamy sands or sandy loams. These are typically highly eroded soils and contain high percentages of coarse textured sand and very low percentages of soil organic matter. Columbia lance nematode is not found in the sandy clay loam soils of the Piedmont region of these states, presumably because they lack the coarse textured sands that CLN requires.

The widespread distribution of CLN in South Carolina may be the result of the extensive history of monocropping cotton. Many fields in the Pee Dee region of South Carolina had been continuously planted to cotton annually for 25+ years. This led to severe soil erosion in some fields, promoted a build-up of fusarium wilt and was highly conducive to the development of SRK and CLN populations.

Symptoms of damage

Columbia lance nematodes are migratory ecto/endoparasites. They do not create the galls, egg masses or cysts typical of root-knot, reniform or soybean cyst nematodes. In cotton and soybean, CLN often feed on or just behind the growing point of the root. This feeding can destroy apical dominance in the root, leading to a loss of the original tap root. This root 'forking' due to excessive branching (Fig. 12.1) can result in severe above-ground stunting, especially in young cotton or soybeans. Infection of young maize roots can lead to a 'stubby root' or a 'bottle brush' symptom similar to, but usually less pronounced, than those caused by *Belonolaimus*, *Pratylenchus* and *Trichodorus* species. Yield losses in cotton due to CLN are more severe when early season damage to the tap root is severe, since the

Fig. 12.1. Healthy cotton root (on left) compared to root 'forking' and excessive branching due to Columbia lance nematode feeding causing destruction of apical dominance and loss of the original tap root (on right). Author's own photograph.

plant must produce compensatory root growth causing a delay in above-ground growth. In many cases, above-ground symptoms in cotton can be subtle with mild stunting and leaf discoloration resembling nutrient deficiencies. Low to moderate levels of root damage may delay flowering, causing plants to produce bolls that will not mature in time for harvest (Bond and Mueller, 2007). Symptoms of CLN on soybean are more pronounced, and development of mid-season chlorosis and stunting is common (Fig.12.2). Chlorosis is a direct result of nitrogen deficiency that occurs because of poor nodule formation and efficiency. Additional effects of CLN infection include reduced photosynthesis, stunting, poor pod formation and low yields. Some older soybean cultivars such as ‘Braxton’ and ‘Perrin’ are very susceptible to CLN and exhibit high levels of chlorosis and yield loss, whereas other cultivars such as ‘Centennial’ and ‘Foster’ appear to be tolerant, sustaining yields even while supporting infection and reproduction by CLN.

Biology and life cycle

While males are common in populations of *H. stephanus* and *H. galeatus,* in most field populations of CLN males are absent or rare. Therefore, reproduction in CLN is by mitotic parthenogenesis. Population densities commonly range from 25 to 250 CLN juveniles and adults per 100 cm^3 soil in row crop fields in South Carolina. All stages from second-stage juveniles to adults are infective. They appear to prefer feeding on young emerging roots with multiple nematodes feeding adjacent to each other. Columbia lance nematode has a very thick cuticle, four to five times thicker than the cuticle of soybean cyst nematode (Lewis and Fasssuliotis, 1982). This allows it to survive desiccation and rapid changes and extremes in temperature. A high survival rate is important since CLN has a relatively low fecundity level (Appel and Lewis, 1984). Unlike root-knot, reniform or soybean cyst nematodes whose populations may increase 60 times over one growing season, CLN populations increase at most five times. During a growing season CLN is more likely to maintain or perhaps double its initial population density. However, overwintering populations usually maintain at least 75% of their autumn density. The fact that females may live for more than a year and continue to produce eggs during that time frame helps sustain the population. The wide host range of CLN contributes to its high rate of survival because even in fallow fields CLN can feed on its many weed hosts.

Columbia lance nematode has a higher optimal temperature for reproduction than most nematodes. In greenhouse experiments on soybean, it had higher levels of root penetration and reproduction at 30°C than at either 25°C or 20°C (Nyczepir and Lewis, 1979). At optimal temperatures CLN takes 17 to 23 days to complete a life cycle, although in the field the time to complete its life cycle is closer to 30 days.

Interactions with other nematodes and pathogens

Columbia lance nematode is reported to suppress *Meloidogyne incognita* on cotton to the extent that it can replace *M. incognita* as the predominant plant parasitic nematode in a field. Information on interactions of CLN with fungal pathogens is limited. It does seem to increase populations of *Rhizoctonia* spp. in soybean (Lewis and Fassuliotis, 1982).

Recommended integrated nematode management (INM)

Nematode control programmes are based on pre-plant damage thresholds. Damage thresholds for CLN for cotton and soybean are between 50 and 100 per 100 cm^3 soil depending upon the soil texture and time of year when the samples were taken (Dickerson *et al.*, 2000). Thresholds decrease as the percentage of coarse textured sand particles increases. Autumn damage thresholds per 100 cm^3 soil for CLN in sandy/sandy loam soils are maize 56/150, cotton 50/100, grain sorghum 50/150 and soybean 50/80. Nematode samples should be taken approximately 20 cm deep within 10 cm of the plant stems. Soil samples can be run using standard wet sieving and sugar flotation. During mid-summer, nematodes may be concentrated in roots. A root extraction using a modified Baermann funnel may be helpful in getting an

Fig. 12.2. Mid-season chlorosis and stunting resulting from CLN feeding and damage to soybean roots. Author's own photograph.

accurate estimate of the number of nematodes present.

Cultivars resistant to CLN are not available in cotton, soybean or maize. Tolerance has been reported in soybean (Nyczepir and Lewis, 1979) but not in cotton (Koenning, 2003). A seed treatment or low rate of an in-furrow nematicide is currently the most commonly used control strategy for CLN in cotton or soybean. Rotation options are limited due to the nematode's broad host range. Peanut is often included in cropping sequences to reduce CLN and SRK nematode populations. One year of peanut is usually sufficient to reduce CLN populations below the damage threshold for cotton. If peanut is eliminated from the cropping sequence and maize, cotton or soybean are grown in any sequence, significant yield losses will occur. Attempts to utilize early planting dates to avoid higher soil temperatures that favour CLN have been unsuccessful in cotton and soybean (Koenning *et al.*, 2003).

Cotton and soybean growers in the southeastern United States normally budget US$74 per hectare or less for nematode control. This will cover the cost of chemical seed treatments. However, seed treatment nematicides have an inconsistent record for improving yields. Low rates of in-furrow nematicides such as aldicarb or fluopyram are more reliable and are still within this budget. The only current fumigant nematicide available is 1,3-dichloropropene. In some irrigated, high-yielding fields, producers will budget for 28 litres/hectare of 1,3-dichloropropene at almost US$148 per hectare, although the target nematode for this treatment is usually SRK nematode rather than CLN.

Optimization of nematode management

Given the narrow profit margins for cotton and soybean in the US, uniform applications of chemical nematicides or biological control agents across an entire field is often too expensive to be implemented by growers. Variable-rate, site-specific nematicide application technologies and strategies have been developed to lower the costs and environmental impacts of nematode management programmes but are not being fully utilized (Overstreet *et al.*, 2014). Equipment is now available for GIS-coordinated 'on the go' changes in application rates for both granular and liquid pesticides, including nematicides.

Field distribution of species such as CLN is highly correlated with the distribution of coarse textured sands within a field. Columbia lance nematodes are found most frequently in soils with greater than 70% coarse sand whereas species such as reniform nematode are found in areas with less than 65% sand (Mueller *et al.* 2010; Holguin *et al.*, 2011). Technology is available using mobile soil electrical conductivity carts to define the textural relationships within fields so that 'high-risk' soils that are the most conducive to high nematode population densities can be defined and linked to GIS-referenced maps as described in Chapter 60 in this volume. These maps, in turn, provide targets for sampling and ground-truthing nematode problem areas for site-specific delivery of nematicides. Currently, simple systems utilizing soil texture maps based on soil electrical conductivity to predict areas of nematode-induced damage and simple on/off switches for 1,3-dichoropropene or aldicarb application are being used by some growers in South Carolina and Georgia. Typically, they maintain cotton yields while applying nematicide to less than 50% of the field. This provides significant economic and environmental benefits.

Because these technologies are relatively complex, it is likely that growers and consultants will need in-person and online training programmes, particularly with respect to operating and maintaining the hardware and software. In addition, multiple species of nematodes often occur in different zones within the field, so interpretation of soil sample data by a nematologist will also be necessary in determining nematicides and application rates (Holguin *et al.*, 2015). Cultivar resistance may also be utilized in these zones. Planters are currently available that can change cultivars while planting. High-risk zones also allow for the use of SRK or reniform nematode resistant cultivars on a site-specific basis according to the resident nematode population within each zone. Similarly, these planters might plant nematicide-treated seed or nontreated seed to match the nematode threat level present in different areas of the field thus improving yields, saving money and lessening environmental impacts.

Future research requirements

Nematode management on cotton is currently undergoing a very substantial change. Resistance to SRK is now available that prevents yield losses and suppresses populations into the subsequent year. Reniform nematode resistant cultivars will be available in agronomically acceptable cotton cultivars starting in 2021. Currently, only a few of these cultivars are resistant to both root-knot and reniform nematodes. Consequently, sampling for nematodes to identify the resident population will be necessary so that growers can choose the proper type of resistance. This increased need for nematode testing should also make growers more aware of other nematode species such as CLN.

A second aspect of the utilization of SRK and reniform nematode resistance may be a reduction in the competition for feeding sites on roots due to reductions in population densities of SRK or reniform nematodes. This could allow species such as CLN and lesion nematodes to become more prevalent. A greater understanding of the relationships among nematode species in the soil as well as more detailed knowledge of the impact of nematodes other than SRK and reniform will be necessary. In the future, control programmes will need to be focused on the community of nematodes including CLN and lesion nematodes rather than on one species such as root-knot nematode. More information is needed on the population dynamics of polyspecific nematode communities and how they impact economic crops. Additionally, nematode sampling programmes and strategies as well as existing damage thresholds for individual species and combinations of species will likely need to be modified.

The greatest need currently for CLN control would be the development of a resistant or tolerant cultivar. In soybean there is strong evidence that tolerance exists, but there are no current efforts to develop tolerant cultivars for any nematode species. In cotton tolerance seems to be tied to the growth habit of the cultivar (Koenning *et al.*, 2003). Indeterminate varieties appear to have the potential to compensate for the delays in flowering and boll development caused by nematode infection. Increasing the speed with which a plant can compensate for damage with new root growth may be a trait that can be exploited.

In the past nematicide development and deployment for cotton has focused on controlling SRK and reniform nematodes. With resistance to these species now available, the focus on nematicides can turn to developing products specifically for other species such as CLN. These species may respond more favourably to active ingredients or biological control agents that were not effective against SRK or reniform nematodes. Similarly, the shift in focus from controlling SRK and reniform nematodes will require that growers select their cover and green manure crops with CLN, lesion or sting nematodes in mind.

Outlook: anticipating future developments

Cotton and soybean are both desirable products for a multitude of commercial uses and will be viable crops for the foreseeable future. With the onset of climate change many agricultural production areas may become more arid and temperatures are increasing year round. Conventional farming practices, with only limited use of conservation tillage and routine cover crop planting continue to lead to depletion in soil organic matter content and erosion. Columbia lance nematode is a species which thrives under hot, arid conditions. It will continue to be a serious pathogen of maize, cotton and soybeans wherever it is found, and may become increasingly prominent as an economic concern. Cultivars that are resistant or at least tolerant to CLN are vital to decrease reliance on nematicides. Both resistant cultivars and nematicides will be more efficiently deployed if site-specific application systems and strategies are utilized.

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13 Integrated management of *Meloidogyne incognita*, the most economically damaging pathogen of cotton in the south-eastern United States

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Introduction

The southern root-knot nematode, *Meloidogyne incognita*, was first described in cotton (*Gossypium hirsutum*) in the south-eastern US nearly 130 years ago. Despite more than a century of research, the nematode causes greater economic damage to cotton in the southern US than any other single pathogen. *Meloidogyne incognita* causes additional losses through involvement in the fusarium wilt (FW) disease complex. The south-eastern states that produce cotton include Georgia (558,466 harvested hectares), Alabama (215,292 ha), North Carolina (202,342 ha), South Carolina (119,382 ha) and Florida (44,515 ha) (USDA NASS, n.d.). Among the US states, Georgia is second in total cotton production behind Texas, Alabama is fifth, North Carolina is sixth, South Carolina is twelfth, and Florida is fifteenth. Cotton growers in the south-eastern US are typically aware of the potential for *M. incognita* and other nematodes to cause losses, but farmers vary greatly in their diligence in monitoring and managing the nematode.

Economic importance

Cotton is a major crop across the south-eastern US with the 2019 crop producing a value of US$807,720,000 in Georgia, US$331,776,000 in Alabama, US$303,245,000 in North Carolina, US$141,360,000 in South Carolina and US$52,622,000 in Florida (USDA NASS, n.d.). In 2019, *M. incognita* was estimated to reduce cotton yield by 7.0% in Georgia, 5.0% in Florida, 4.0% in South Carolina, 3.0% in Alabama and 2.0% in North Carolina (Lawrence *et al.*, 2020). Losses to *M. incognita* accounted for 62.5% of the total losses to all diseases and nematodes in Georgia, 30.9% in Florida, 37.7% in South Carolina, 30.9% in Alabama and 30.1% in North Carolina. If *M. incognita* is left unmanaged, losses of up to 30% are common with much greater losses possible. Another root-knot nematode, *M. enterolobii*, was recently found on cotton in North Carolina; however, it has an extremely limited distribution and has not been found in cotton in the other south-eastern states. Fusarium wilt can be devastating when it occurs in a field, and because FW is typically a synergistic

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interaction involving nematodes, losses to FW should be attributed in part to the nematodes. However, the incidence of FW is typically low resulting in losses of a few tenths of a per cent or less in most south-eastern states, although Alabama often has losses around 1%.

Host range

Meloidogyne incognita has a wide host range that includes many potential rotation crops, common weeds and cover crops. Common crops that are hosts for *M. incognita* include maize, melons and most vegetables. Tobacco, soybean and sorghum also are hosts, although highly resistant varieties are available. Of the common row crops in the south-eastern US, only peanut is a non-host. Winter cover crops are common in the south-eastern US, and many of them are good hosts for *M. incognita*, including leguminous cover crops such as clovers and vetches. Rye is by far the most commonly grown cover crop, and it is a moderate host for *M. incognita*. Winter soil temperatures limit *M. incognita* reproduction such that population levels do not increase on rye, although levels can increase on many of the legumes. Many summer and winter weeds are moderate to good hosts for *M. incognita* and can increase nematode levels in a field and reduce the nematode-suppressive effects of non-host or poor-host rotations and fallow periods. Among the important weeds of cotton in Georgia, prickly sida (*Sida spinosa*) is an excellent host for *M. incognita*, smallflower and ivyleaf morning glories (*Jacquemontia tamnifolia* and *Ipomoea hederaceae*, respectively) are moderate to good hosts, whereas yellow and purple nutsedge (*Cyperus esculentus* and *C. rotundus*, respectively), Florida beggarweed (*Desmodium tortuosum*), sicklepod (*Senna obtustifolia*), and common cocklebur (*Xanthium strumarium*) are moderate to non-hosts (Rich *et al.*, 2009).

Distribution

Meloidogyne incognita is the most common and widespread root-knot nematode species in most of the south-eastern states and it can potentially be found anywhere cotton is grown in those states. It is the dominant plant parasitic nematode of cotton in the sandy soils of the coastal plain where most cotton is grown in the southern states. Where cotton is grown outside the coastal plain, soils typically have more silt and clay, and the reniform nematode (*Rotylenchulus reniformis*) causes greater losses than *M. incognita*, except on river flood plains where soils have a higher sand content favouring *M. incognita* over *R. reniformis.*

Symptoms of damage

Symptoms of *M. incognita* damage in cotton can be subtle, especially at low to moderate nematode levels where yield losses up to 10% can occur with few or no above-ground symptoms. As damage increases, reduced plant growth may be visible and infected plants typically wilt more readily than healthy plants; however, the effects of drought and *M. incognita* are independent and additive, so increased irrigation will not reduce the yield loss caused by the nematode (Davis *et al.*, 2014). Severely damaged plants may display interveinal chlorosis on leaves (Fig. 13.1). Above-ground symptoms are often misattributed to drought stress, poor soil fertility or other causes. Root galling is a diagnostic symptom that occurs on all infected plants and is the only symptom caused solely by root-knot nematodes (Fig. 13.2). Compared to many crops, the galls caused by *M. incognita* on cotton are relatively small. The extent of galling may be underestimated on

Fig. 13.1. Leaf symptom of cotton caused by severe *Meloidogyne incognita* damage. Photograph courtesy of R. Galbieri.

moderately infected plants if plants are pulled from the soil, which can break off smaller roots where many of the galls are located, rather than dug from the soil. Symptoms and yield loss will be most severe when young plants are infected (Fig. 13.3); however, plants are rarely killed by *M. incognita* in the absence of FW.

Biology and life cycle

The *M. incognita* life cycle is typical of parthenogenetic root-knot nematodes (Moens *et al.*, 2009). Males are occasionally produced, but they are not common and are not known to mate or feed. Only eggs, J2 and males are found outside of cotton roots, rendering those stages the most susceptible to chemical and biological controls. Egg production usually begins around 25 days after infection and may continue for up to 2 weeks. The nematode does not develop or mature at soil temperatures below 10°C and does not move or penetrate roots below 18°C (Roberts *et al.*, 1981). In the south-eastern coastal plain, up to five generations of *M. incognita* may be produced on cotton, and up to two generations may be produced on susceptible plants growing between cotton harvest and planting the following spring. The nematode survives the winter as eggs or J2 in the soil or in roots of susceptible plants. Cotton roots are still living following harvest and *M. incognita* can continue to develop and reproduce; however, mowing cotton stalks immediately after harvest interrupts post-harvest development and reduces nematode levels in the field. Pulling roots from the ground may have a greater suppressive effect than mowing.

Fig. 13.2. Severe root galling of cotton caused by *Meloidogyne incognita*. Photograph courtesy of P. Kumar.

Interactions with other nematodes and pathogens

Fusarium wilt, caused by the fungus *Fusarium oxysporum* f.sp. *vasinfectum* (Fov), in the south-eastern US is typically a disease complex caused by an interaction between Fov and a nematode, most often *M. incognita* (Fig. 13.4). The reniform nematode, *R. reniformis*, and the sting nematode, *Belonolaimus longicaudatus*, can also interact with

Fig. 13.3. Undamaged cotton (left) compared with cotton stunted by *Meloidogyne incognita*. Photograph courtesy of R. Davis.

Fig. 13.4. Symptoms of fusarium wilt–nematode disease complex of cotton. Photograph courtesy of M.B. da Silva.

Fov to cause FW. Fusarium wilt can occur in the absence of nematodes when the Fov inoculum level is very high, but severe FW can occur with much lower Fov inoculum levels when certain nematodes are present, and most instances in the south-eastern states involve nematodes. The FW disease complex is a synergistic interaction between *M. incognita* and Fov in which disease severity is greater than additive when both pathogens infect the plant. Because nematodes, primarily *M. incognita*, are involved in most instances of FW, effectively controlling the nematodes is an effective control of FW. Many fields have multiple nematodes that can damage cotton, and it is assumed that the effects of the different nematode species are additive.

Recommended integrated nematode management

Sampling and thresholds

The basis for nematode management decisions is predictive sampling for comparison to action threshold levels, which are estimates based on data and experience. Nematode management is likely to be beneficial when nematode levels are above a threshold. Although many factors can influence the accuracy of soil samples and threshold levels, there is not yet a better option. The greatest number of nematodes will typically be found in samples collected at the end of the season (October), and the numbers will begin to decline as roots begin to die and soil temperature drops. Nematodes may be nearly undetectable in soil samples collected in mid-winter or spring. The action threshold in Georgia for *M. incognita* is 100 J2/100 cm^3 soil. Some states recommend lowering the threshold to 50 J2/100 cm^3 soil for samples collected in mid-winter (January) and raising it to 130 J2/100 cm^3 soil for samples from non-sandy soils (e.g. clay loam). Additionally, the level of galling observed the previous year can help predict the damage potential in a field.

Crop rotation and cover crops

Crop rotation is effective in managing *M. incognita* if poor or non-host crops are used. Peanut is the best rotation option because it is very profitable and a non-host for *M. incognita* and the other major nematodes damaging cotton. Unfortunately, fewer hectares of peanut than cotton are grown, and many cotton growers are unable to grow peanut. Rotation with resistant varieties of soybean, tobacco or sorghum can effectively manage *M. incognita*; however, these rotations are rarely used in Georgia. Although a single year of growing a non- or poor-host crop effectively reduces *M. incognita* levels and increases subsequent cotton yield, nematode levels quickly rebound when cotton is again grown. Two years of a poor host is slightly more effective than a single year. About half of the cotton hectarage in Georgia is rotated to some other crop after 1 year of cotton, about 40% is rotated after 2 years of cotton, and about 10% is never rotated out of cotton. The two most commonly used rotation crops in Georgia are peanut (a non-host for *M. incognita*) and maize (a good host). Winter cover crops should be chosen carefully to avoid crops that can increase *M. incognita* levels. Neither poor nor non-host cover crops increase *M. incognita* levels relative to fallow. A winter cover crop, mostly rye, is planted on up to 30% of the cotton hectarage in Georgia.

Nematicides

Nematicides are the primary method of nematode management in cotton production. They suppress nematode parasitism of young plants, allowing them to grow with minimal damage and crop loss. However, nematicides do not provide season-long control and nematode levels at the end of the growing season are typically unaffected by nematicide application. Up to 75% of the cotton hectarage in Georgia is estimated to have one or more potentially damaging species of plant parasitic nematode; however, less than 40% is treated with a nematicide either because nematode levels are below action thresholds or farmers do not realize that nematodes are reducing yield. Many hectares that would benefit from treatment are not treated. The major nematicides available include seed treatments, in-furrow treatments, and pre-plant fumigation treatments. Seed-treatment options are abamectin, fluopyram, heat-killed *Burkholderia* spp. or thiodicarb; in-furrow options are aldicarb and fluopyram; and a fumigant option is 1,3-dichloropropene (1,3-D). Additionally, oxamyl can be applied to growing cotton seedlings as a supplement to a seed-treatment or in-furrow nematicide. Estimates for Georgia are that seed treatments are applied to 20% of the total cotton hectarage, aldicarb is applied to 7%, fluopyram is applied to 9% and 1,3-D is applied to 1%.

Seed treatments are the least costly nematicide option and are the easiest to use since they do not require separate equipment or calibration. Seed treatments are typically recommended for fields with low to moderate nematode damage potential (at or slightly above threshold levels). In-furrow aldicarb or fluopyram are often recommended for fields with moderately high damage potential that may not be adequately controlled by seed treatments. Fumigation with 1,3-D is the costliest nematicide option, but it is the most effective option for fields with high or very high damage potential. Post-plant applications of oxamyl may be used to supplement seed treatments or in-furrow nematicides if 1,3-D cannot be used. Equipment calibration is critical to apply the correct rates of nematicides.

Resistance

Cotton cultivars with a high level of resistance and tolerance to *M. incognita* have become available in the last few years. Resistant cultivars are very effective in suppressing *M. incognita* and they are also very tolerant and suffer far less damage than susceptible cultivars even under very high nematode pressure. In contrast to nematicides, resistance provides season-long suppression of *M. incognita* thereby providing benefit for a following crop. Unfortunately, the resistance is only effective against *M. incognita* and will not reduce damage from other nematode species that may still need to be controlled through nematicide use. Up to 10% of the cotton hectarage in Georgia is planted with a resistant cultivar, and the percentage has increased each year. Some farmers choose to plant a susceptible cultivar and use a nematicide instead of planting a resistant cultivar believing that it will maximize their profit, but that belief has not been rigorously tested.

Optimization of nematode management

Economic return can be reduced by failing to manage nematode problems or by applying nematicides to areas in which they are not needed. An optimal nematode management plan for cotton would include improved sampling that utilizes nematode management zones (MZ). MZ divide fields based on soil texture, elevation and other factors into sections that are relatively uniform within a zone but different among zones. Sampling by MZ can increase the likelihood of identifying an area that would benefit from nematode management. Nematode population levels and their damage potential can differ among zones, so sampling and managing each zone separately can more accurately target nematode management, especially with nematicides, thereby increasing profitability. The greatest limitations to adoption of MZ are that farmers need to hire an expert to identify and map the MZ for them and that utilizing MZ maps requires developing some technical expertise (or hiring a consultant). MZ are a relatively new development and additional research on the effects of the soil environment on nematode levels and damage potential is likely to increase their benefit. Increased economic analysis of various control tactics under a range of conditions should improve recommendations.

Wider adoption of resistant cultivars is recommended and would help minimize damage from *M. incognita* and could restrict the use of nematicides to areas where other nematodes require management. Use of resistant varieties has increased as their yield potential has increased. Incorporating resistance into a wider selection of high-yielding cultivars should further their adoption. Additionally, newer varieties are being released that have both resistance and competitive yield potential.

Outlook and future research requirements

All cotton cultivars with resistance to *M. incognita* derive resistance from the same original source, Auburn 623 RNR, which has two major resistance quantitative trait loci (QTLs) with different modes of action. Both QTLs are necessary for a high level of resistance. Reliance on a single source of resistance puts significant selection pressure on the nematode to overcome the resistance. Populations of *M. incognita* virulent on plants with the Auburn 623 RNR resistance QTLs have not been observed, but such populations could develop over time. Identifying unique sources of resistance with different modes of action would allow pyramiding resistance genes to prolong their durability. Incorporating resistance into rotation crops (e.g. maize) would also be beneficial.

Nematicides will continue to be necessary in many fields for the foreseeable future. Development of new nematicides that improve levels and length of control as well as reducing risk to applicators and non-target effects should continue. Similarly, development of biological control products should continue. Research and extension efforts to optimize selection and cost-effective use of these products is crucial.

Additional research on how soil edaphic and biological factors affect *M. incognita* population levels, survival and damage to crops would allow refinement of damage predictions and management recommendations. The ability to better predict winter attrition rates for *M. incognita* would improve damage prediction. Monitoring the distribution of *M. enterolobii* will be necessary.

Outlook: anticipating future developments

Increasing the accuracy and precision of determining nematode population levels and distribution could greatly improve management decisions. Time and labour-saving technologies that reduce the reliance on soil sampling (e.g. remote sensing) and provide results more quickly may be developed. Improved modelling should include many more factors than are currently considered resulting in more accurate predictions and more targeted management. Knowledge and abilities in molecular biology may increase sufficiently to allow development of engineered resistance. Our ability to manage and enhance soil suppressiveness may allow it to become a routine part of nematode management. Climate change may alter temperature, rainfall, and groundwater thereby affecting crop production and nematode management.

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14 Reniform nematode (*Rotylenchulus reniformis*) and its interactions with cotton (*Gossypium hirsutum*)

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Introduction

The reniform nematode, *Rotylenchulus reniformis* (Linford and Oliveira, 1940), is a major economic factor limiting cotton (*Gossypium hirsutum*) production in the USA. Across the United States cotton belt, 0.1–5.0% of the cotton crop is lost to the reniform nematode (RN) annually (Lawrence and Lawrence, 2020). *Rotylenchulus reniformis* has a worldwide distribution and is reported in at least 38 countries. In the United States, it occurs extensively in the mid-south and south-eastern region in 11 of 17 cotton producing states. The reniform nematode is considered to have surpassed the root-knot nematode, *Meloidogyne incognita* Chitwood, as the major nematode affecting cotton in Alabama, Louisiana and Mississippi (Robinson, 2007).

Economic importance

The 0.1–5% loss in cotton caused by RN equals approximately 190 million bales of cotton and US$50 million due to the loss of fibre alone. Specifically, cotton yields over 5 years were shown to have averaged 50% less in a reniform nematode infested field with an at planting population density averaging 1000 reniform/100 cm^3 of soil, compared to an identical field that had no detectable reniform nematodes (Dyer *et al.*, 2020). Economically, the uninfected field without reniform yielded 1597 kg/ha valued at US$1950/ha while the infested field's average yield was 803 kg/ha valued at US$992/ha. The reniform nematode is an economic tragedy reducing the grower's profitability by half. NASS (2019) indicates the average farm size in Alabama is 86 hectares. The average cotton farm without RN infestation has a projected gross income of US$167,000 in a season. Unfortunately, reniform would reduce the gross income to US$85,312. This is an estimated loss of US$82,388 for the average farm. The use of a nematicide increased yields to 1223 kg/ha valued at $1511/ha (NASS, 2019). The nematicide improved cotton yields, increasing the average farm gross income to $129,946 but still $44,634 below the farm potential without the reniform nematode.

Host range

The reniform nematode has a host range of more than 314 plant species, damaging a diversity of crops from bromeliads to dicots. Hosts include the worldwide important agronomic crops cotton,

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cowpea, pineapple, soybean, sweet potato, tea, tobacco and tomato (Robinson *et al.*, 1997), although reniform is primarily an economic problem in cotton.

Many winter cover crops are hosts to the reniform nematode. Crimson clover, subterranean clover and hairy vetch are hosts and have the potential to increase reniform numbers if spring is warm (Jones *et al.*, 2006). The nematode did not reproduce on cultivars of radish, black mustard, white mustard, canola, lupin, ryegrass, wheat, oats and rye, indicating these would be good choices for a winter cover crop.

Many common weeds are hosts for reniform. Forty-three weed species from the southeast region of the US were confirmed hosts (Lawrence *et al.*, 2008). Ragweed, coffee senna, water hemp and prickly sida were excellent hosts equivalent to cotton. Field tests confirmed that a maize rotation is effective for reducing reniform if weed management is effective. However, the field must be weed free. Non-controlled weed species sustained reniform populations in maize plots compared to weed-free maize plots. Season-long weed management during the maize rotation is necessary to suppress reniform populations.

Distribution

The reniform nematode is present in most of the cotton growing areas in the US including Alabama, Arkansas, Georgia, Florida, Louisiana, Mississippi, the Carolinas, Tennessee and Texas. In these states, the premier cotton production areas are often those infested with the nematode. The reniform nematode can cause serious damage in many soil types, but the highest population levels are reported on silty soils. A survey of Alabama cotton fields in 1989–1990 found 6.5% of the fields were infested with the reniform nematode. In 2002, 12 years later, the reniform nematode was found in 46% of the cotton fields and half of the infested fields had populations above the economic threshold (Lawrence and Lawrence, 2020). In 2007, the reniform nematode was monitored from an initial infestation point in a silt loam soil, no-till irrigated cotton production system. The nematodes moved 200 cm horizontally and 91 cm vertically from the initial point of inoculation in one growing season (Moore *et al.*, 2010). The reniform population increased steadily, exceeding the at plant nematode densities of 1000 nematodes/100 cm^3 soil by the second season. Keeping this nematode out of a clean field is extremely important for growers. All production equipment should be washed to remove all soil particles when moving from a reniform infested field to a clean field to contain the spread of this pathogen.

Symptoms of damage

The reniform nematode has a devastating effect on the growth and yield of the cotton plant. Fields infested with reniform display areas of stunted and uneven plant growth, referred to as a wave effect (Fig. 14.1A), giving the field an irregular appearance. Population densities of reniform are higher bordering the areas of poor growth. After a field has been infested for several years, the population density becomes more evenly distributed and stunting may become uniform. The damage to cotton is dependent on the population density of reniform nematodes present at planting and the amount of moisture the crop receives. The higher the nematode numbers at planting, the greater the losses that can be expected. Continuous monoculture cotton production has increased reniform populations to 100,000 vermiform life stages in 500 cm^3 of soil in some fields. Across the geographic area of this nematode, variations in reniform populations, soil types and cropping rotations will affect nematode damage potential. Warm temperatures, abundant sunshine and timely rainfall all support reniform populations as well as cotton plant growth.

Foliar plant symptoms associated with reniform are rather nondescript, resembling symptoms associated with nutrient deficiencies and hardpans. Nematode damage may appear in seedlings and young plants as light green or chlorotic foliage. Interveinal chlorosis of the lower leaves, commonly referred to as tiger striping (Fig. 14.1B) may occur on some soil types with high levels of reniform. The foliar symptom is similar to a potassium deficiency and observed as early as pinhead square or initial flower formation. The cotton root systems exposed to reniform are often fragile, with limited secondary root development. The reniform kidney-shaped

Fig. 14.1. Plant symptoms of infection by *Rotylenchulus reniformis* including **(A)** the 'wave effect' of the uneven canopy; **(B)** 'Tiger striping' or interveinal chlorosis of lower leaves; and **(C)** egg masses with soil particles. Author's own photographs.

mature females and small egg masses they produce may be visible on the root surface with the aid of a hand lens or dissecting microscope (Fig. 14.1C). A characteristic of roots infected with reniform nematodes is the presence of soil particles adhering to the nematode egg masses. The reniform colonized root systems appear dirty compared to a clean cotton root without reniform present.

Biology and life cycle

Rotylenchulus reniformis is tropical to semi-tropical preferring soil temperatures of 27–30°C (81–86°F). At these temperatures, the life cycle of the nematode on cotton requires three to four weeks. In the US cotton belt, five to seven generations are completed depending on the length of the particular growing season. Seventy per cent of the reniform nematode population in the top 15 cm of soil at harvest is not detectable after the winter season leaving approximately 30% present at planting.

All vermiform life stages of the reniform are found in the soil, which is the reason the population levels of this nematode are higher than most plant parasitic nematodes. The development from egg through the juvenile stages to the vermiform adult typically occurs in 10 to 14 days, at which time the soil population is commonly 50/50 vermiform males and females. Only vermiform adult females infect cotton roots (Fig. 14.2A). The infective female locates a root, inserts the anterior one-third of her body and begins feeding in the endodermis region. The female induces the formation of a multi-celled feeding site, where she will feed for the remainder of her life. Within days of entering the root, the posterior portion of the female's body outside the cotton root begins to enlarge, assuming the characteristic kidney shape (Fig. 14.2B). After mating, the female secretes a gelatinous matrix that surrounds her exposed body and provides protection for her eggs. Soil particles adhere to the egg mass giving the roots a bumpy, irregular, dirty appearance (Fig. 14.2C).

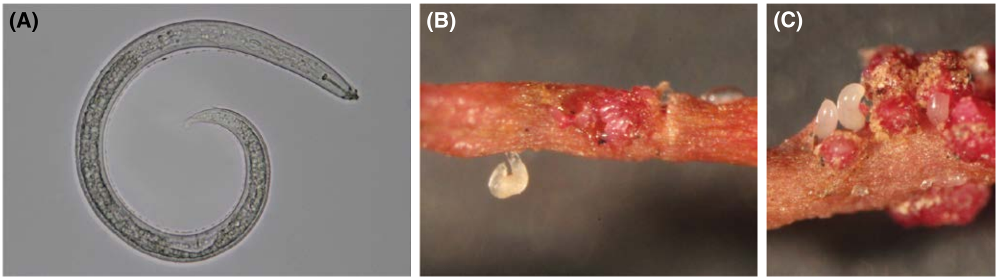

Fig. 14.2. **(A)** Infective vermiform female *R. reniformis*. **(B)** Kidney-shaped mature female on the root. **(C)** Mature female in the root with her posterior visible with red stained egg masses. Author's own photographs.

After harvest, if cotton stalks are allowed to remain in the field and soil temperature and moisture remain favourable for plant growth, populations of reniform nematodes may remain at high levels. If stalks are removed after harvest, nematode populations decline during winter months. Adult males and infective females have often been observed within the cuticle of the previous moult, which possibly represents a means of survival under adverse conditions.

Interactions with other nematodes and pathogens

Reniform can increase cotton seedling disease severity caused by several *Fusarium* species, *Rhizoctonia solani* and *Thielaviopsis basicola* (Palmateer *et al.*, 2004). The additional stress to the cotton seedling due to nematode feeding with the colonization of the seedling disease fungi can reduce cotton stands and plant vigour, although reniform populations tend to be reduced when seedling disease fungi are present. Reniform also will compete with root-knot in cotton fields and these two nematodes do not often occur at damage thresholds together in the same field.

Recommended integrated nematode management (INM)

Rotation, cultivar selection and nematicides are the principal means of reniform management in cotton. Crop rotation is an important tactic for the management of reniform. Crop rotations to non-hosts, such as maize, peanuts, grain sorghum or resistant varieties of soybean, are an effective strategy for the management of reniform. These crops all reduced initial reniform population densities compared to the continuous cotton cultivation (Moore *et al.*, 2010). Cotton yield following a 1-year rotation of maize, soybean or peanut yielded 20% higher on average than continuous cotton. Two years of maize, peanuts or soybeans increased cotton yield by 40% compared with continuous cotton. All rotations resulted in a net profit over costs compared to continuous cotton both with and without a nematicide. Winter grains, as well as bermudagrass and bahiagrass, are effective for the suppression of reniform nematode populations and may be considered in a rotation scheme. Many weed species are to some extent host to reniform and can reduce the positive effects of crop rotation if not controlled (Jones *et al.*, 2006; Lawrence *et al.*, 2008).

Cotton cultivars resistant to reniform have promised to alleviate yield loss; however, none are presently available to the growers. Reniform nematode resistant cultivars are expected to be marketed in the near future. Field trials conducted in Alabama established that reniform populations were 50% lower in potential resistant lines compared with the susceptible cotton lines. However, the addition of nematicides did increase yields of both the resistant and susceptible cotton lines (Schrimsher *et al.*, 2014).

Winter cover crops compete with weeds, decrease soil erosion, enhance soil health and provide a niche for nematode antagonistic microflora. Crimson clover, subterranean clover and hairy vetch are hosts of reniform in greenhouse tests, although field populations did not increase on these cover crops under field conditions (Jones *et al.*, 2006). Cultivars of radish, black mustard, white mustard, canola, lupin, ryegrass, wheat, oats and rye were poor hosts for reniform and did not sustain reniform populations, making them or mixtures a good option for winter cover crops.

Chemical control

Nematicides are an important management tactic for reniform in cotton and are often the first control option considered. The oldest nematicides include Telone II (1,3-dichloropropene), a fumigant applied in the autumn or up to 2 weeks prior to planting, and AgLogic 15G (aldicarb), a granular applied at planting in the seed furrow. Aldicarb was the most widely used nematicide in cotton production, although continued use resulted in enhanced degradation, decreasing its efficacy (Lawrence *et al.*, 2005). Seed-applied nematicides thiodicarb, abamectin and fluopyram are the primary nematicide option in cotton production as a part of AERIS Seed-Applied System, AVICTA Complete Cotton and COPeO Prime, and are reported to provide satisfactory management of reniform. Biological seed treatment nematicides include BIOST Nematicide 100 (heat-killed *Burkholderia rinojensis* and its spent fermentation media), N-Hibit HX 209 (Harpinαβ protein), Trunemco (*Bacillus amyloliquefaciens* strain MBI 600 plus cis-Jasmone), VOTiVO (*Bacillus firmus* I-1582) and *B. amyloliquefaciens* strain PTA-4838. Currently, AVICTA is available only on NexGen and Armor cotton cultivars, COPeO is specifically paired with Stoneville cultivars and VOTiVO is combined with the insecticide Poncho (clothianidin) and available on all Deltapine cotton varieties. BIOST Nematicide 100, N-Hibit HX 209, Trunemco and VOTiVO seed treatments can be added as an overtreatment by the retailer on any cotton cultivar at an additional cost. In-furrow spray nematicides are the most recent additions to the nematicide arsenal. Fluopyram combined with imidacloprid (Velum Total) is applied as an in-furrow spray at planting on cotton. The application of Velum Total resulted in a 90% decrease in reniform eggs/g of root over ten cotton cultivars and increased yield by 23% or 903 kg/ha (Dyer *et al.*, 2020). Oxamyl (Vydate C-LV) is a foliar applied nematicide with insecticidal properties that also provides management of RN, often in conjunction with seed treatment nematicides (Lawrence and Lawrence, 2000). Fluopyram plus prothioconazole (Propulse) is also being tested as a foliar application following a seed treatment nematicide. Oxamyl and fluopyram plus prothioconazole are the only nematicides that can be applied post-emergence to further protect cotton from subsequent generations of reniform. These two foliar sprays may be considered as a rescue application if no nematicide was applied at planting. Overall, the number of pesticides for the management of reniform is increasing, although the pairing of seed treatment nematicides with specific cotton cultivar brands has reduced nematicide choices for growers.

Optimization of nematode management

Preventing the introduction of damaging nematodes eliminates the need to manage them. Once nematodes are present, they cannot be eradicated. Washing equipment to remove soil residue which harbours nematodes when moving from a nematode infested field to a nematode-free field is necessary.

A holistic sustainable system will provide the best opportunity to manage nematodes in cotton. A stand-alone option for control of *R. reniformis* is not sufficient and a combination of management practices is needed to keep nematode population densities below damage thresholds. Primarily, if the reniform population density in the soil at planting is well above damage thresholds, the best option is to rotate to a non-host crop in that field. Cultivar nematicide combinations are not available that can produce optimum yields in a field with high numbers of reniform. If the reniform populations are in the mid to low range, cotton can be successfully grown. Selecting the cotton cultivar to be grown is the most important decision and all other management inputs will revolve around the cultivar selected. Cotton seed treatment nematicides available are determined by the cotton cultivar selected. Nematicides do not provide total nematode control, nor will they eliminate all yield loss due to the nematode. Planting a high-yielding cotton cultivar with a seed treatment nematicide or an in-furrow nematicide applied at planting will help reduce reniform populations and yield losses.

Foliar broadcast or side-dressed nematicides are an additional option for reducing reniform plant stress when the cotton plant is beginning to flower and fruit. Well-timed additional foliar applied nutrients through irrigation systems to optimize cotton health during boll set reduces nematode yield losses. Timely irrigation is also essential, particularly during flowering and boll set. All these practices eliminate stress to the cotton plant and will maximize yield. A winter cover crop is ideal for improving soil health by increasing soil organic matter. Cover crop options are broad and can be a single crop or blends of grasses, legumes and brassicas. Maximum cover crop biomass ensures the full benefit of the cover crop. The optimum INM programme includes cotton cultivar selection, nematicides, plant fertility systems with foliar feeding along with optimum weed, insect and disease management preventatives, cotton plant removal in the autumn, winter cover crops, and crop rotation sequences in a total crop management programme.

Outlook and future research requirements

Advances in IT with precision applications, remote sensing and predictive modelling combined with yield mapping will help define and predict areas where nematode management will be economically important. Field prescriptions may include nematicide and fertilizer applications balanced with insect, weed and disease management. Resistant varieties will be an additional management tool for plant parasitic nematodes yet will need to be incorporated with genetically modified insect and herbicide resistance to be effective. Reniform resistant cotton cultivars must be rotated to maintain the resistance genes efficacy. Continued assessment of alternative approaches for environmentally friendly, yet sustainable and effective nematode treatment options is essential.

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SECTION III:

Legume Crops

15 Integrated nematode management of root lesion and root-knot nematodes in soybean in Brazil

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Introduction

Soybean, after pasture, occupies the largest area of cultivation in Brazil. Brazil is the largest producer of soybean, and its cultivation is distributed throughout the national territory. Nematodes, in order of frequency and importance, are root lesion nematode (RLN), *Pratylenchus brachyurus*; root-knot nematodes (RKN), *Meloidogyne javanica* and *M. incognita*; soybean cyst nematode (SCN), *Heterodera glycines*; leaf nematode, *Aphelenchoides besseyi*; and reniform nematode, *Rotylenchulus reniformis*. Other species can occur and cause losses, but they are less frequent. The most common and productive annual system is soybean followed by maize. In approximately half of the area cultivated with soybean, maize is also grown. This system favours proliferation of polyphagous nematodes, as is the case with *P. brachyurus*, *M. javanica* and *M. incognita*, which will be the focus of this chapter.

Economic importance

In Brazil, average soybean losses of 21–50% have been reported with *P. brachyurus*, reaching up to 85% in sandy soil areas (Lima *et al.*, 2015). Studies point to a loss of up to 91 kg ha^{-1}, for each 1000 nematodes/10 g of soybean roots when sampled in the flowering phase (Ferrari *et al.*, 2015). A similar scenario is also observed for root-knot nematodes, for which losses are estimated between 10–30% of production, reaching up to 90% in situations of high infestation and in years with water deficit (Asmus, 2001).

Host range

Soybean cultivation in Brazil is complex, involving a series of crops and/or cover crops that can be rotated with soybean. The vast majority are also hosts to RLN and RKN. In general, maize, sugarcane, bean, rice, cassava, wheat, oats, sunflower, sorghum, millet, some rattlepods (*Crotalaria* spp.), brachiarias (*Urochloa* spp.), among others, are hosts to

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M. javanica; besides these, cotton, turnip, among others, are hosts to *M. incognita*. All previous crops, except for some rattlepods and turnip, are host to *P. brachyurus* and, in addition, common weeds can be hosts.

Distribution

RLN and RKN are currently present in all soybean producing regions in the country. They are very frequent in the central-west and north–north-east regions, in addition to the Brazilian cerrado, the largest cultivated areas with soybean in the country. RLN is native to the cerrado (Lima *et al*., 2015), in addition to other biomes in Brazil, and may be present in over 80% of soybean cultivation areas in the country. RKN, while also native, were distributed as soybean cultivation expanded in Brazil. They can be found in more than 30% of the area. Among root-knot nematodes, *M. javanica* is more frequent (64.1%) in soybean areas than *M. incognita* (23.1%) (Castro *et al*., 2003).

Symptoms of damage

RLN symptoms are irregular spots, distributed throughout the area, with smaller and uneven plants, with lighter green leaves and abundant secondary roots and predominant necrotic lesions (Fig. 15.1).

Uneven plots, smaller and highly chlorotic plants and abundant root knots (Fig. 15.2) are evidence of RKN symptoms. For nematodes, in general, in areas of high fertility and absence of biotic/abiotic stress, plants may not present symptoms in the aerial parts, only in the roots. Therefore, sampling is essential for an accurate diagnosis. In addition, simultaneous occurrence of more than one species can overlap the symptoms of another.

Biology, life cycles and interactions

The life cycle of the RKN takes around 4–7 weeks and of *P. brachyurus* 3–4 weeks, which have previously been described for these species under Brazilian conditions. However, it should be noted that in Brazil, a continental country, life cycles of these nematodes might vary. The higher the temperature, the shorter the cycle and the greater the multiplication and vice versa. Nematodes cause injuries to the roots, which facilitate the entry of phytopathogenic (*Fusarium* spp., *Macrophomina phaseolina*, etc.) or opportunistic fungi, increasing symptoms, damage and losses in crops (Castillo and Vovlas, 2007; Sikora *et al*., 2018).

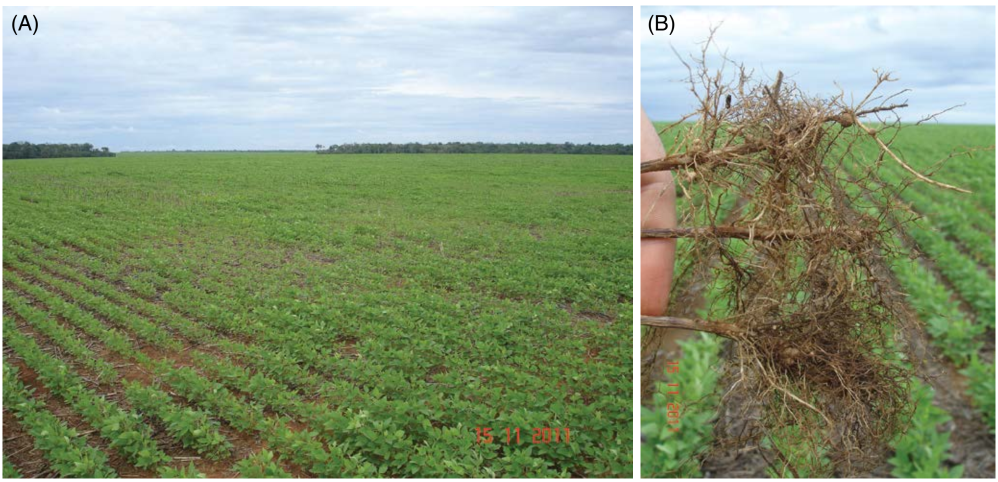

Fig. 15.1. **(A)** Soybean crop showing the characteristic field symptoms and **(B)** severe root lesion and necrosis caused by *Pratylenchus brachyurus*. Author's own photographs.

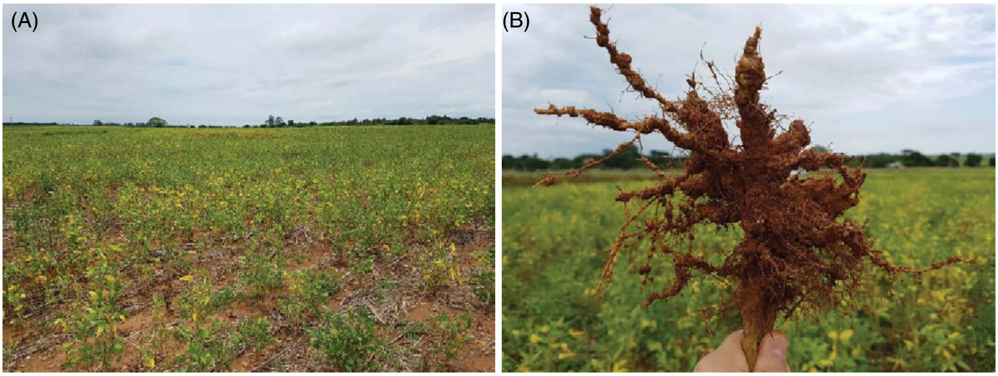

Fig. 15.2. **(A)** Soybean with characteristic above ground symptoms and **(B)** severe root galling caused by *Meloidogyne javanica*. Author's own photographs.

Recommended integrated nematode management (INM)

Survey of soil densities

Prior knowledge of the predominant species' population density, such as their occurrence and distribution in the area, is the main information needed for planning the practices for INM that must be adopted to reduce the infestation and increase crop yield.

Prevent nematodes entering and spreading

Nematodes do not spread by their own resources – it is usually human and mechanical transfer that takes them from one plot or farm to another. The entry of machines, implements and vehicles on the farm, coming from areas with a history of nematode infestation, must be preceded by washing with strong jets of water to remove the soil adhered to the tyres, gears and external parts of the machine. In addition, measures such as the establishment of contour farming, priority preparation of the exempt area, and the posterior-most infested area, are simple operations that can prevent the entry and spread of nematodes. The destruction of plants in the infested area as soon as they are detected is also a way of delaying/preventing the spread of nematodes.

Fallowing, root destruction and soil management

Fallowing soil means keeping it clean by any method in the driest and/or warmest periods of the year. However, in an area with high infestation, it is essential to mechanically destroy roots of the previous crop. This method aims to interrupt the nematode life cycle by turning the soil at least twice. This method destroys the remaining roots, exposes the nematodes to solar radiation and provides a drop in moisture in the soil, leading to a drastic reduction of the population due to starvation and desiccation in a short period of time.

These practices help to break up the soil and incorporate the correctives (limestone and plaster) that favour the development of the plants. As long as there are live roots, nematodes will multiply and infest the soil, since they are mandatory parasites which depend on living cells to feed. At the same time, the roots also serve as shelter, for long periods, mainly for endoparasitic or semi-endoparasitic nematodes.

The efficiency of the method was observed in a field with a soybean crop where fallow was used for the management of a high infestation of RLN, and soil turning was done in half of the area and not in the other, before planting the crop. Better development of soybean was evident where soil turning was done (Fig. 15.2A). Gains of at least 10–30% in productivity have been observed. Soil turning can also be useful in the management of the RKN and other species.

On the other hand, it is a technique that favours the spread of nematodes. There are also situations where nematodes occur in the total area and so there is no need to worry about the spread. Also, it can be a practice used in a part of the area instead of the whole area. Therefore, it should not be one of the preferred management measures, nor should it be adopted frequently, since it is contrary to the practices of direct seeding, resulting in harmful effects to the physicochemical and biological characteristics of the soil.

Succession or crop rotation

The most used crop in succession or rotation to soybean in Brazil is maize. Currently, there are no maize hybrids resistant to *M. incognita*, *M. javanica* or *P. brachyurus*, the species of economic importance for the soybean–maize production system. Maize is a better host for *M. incognita* than *M. javanica*, and a better host for RKNs as compared to RLNs. Although there are cultivar differences, all of them have a reproductive factor (RF) > 1.0 and are susceptible. Therefore, if maize is planted, choose a hybrid with a low RF whenever possible. Even with low RF, depending on the level of infestation and nematode species, the increase in nematodes can be a problem for the next crop. In this case, to avoid the situation mentioned, it is better not to use a maize crop. All of the previous situations apply to sorghum crops.

The effectiveness of the succession/crop rotation increases the longer the rotation period is and decreases with the presence of plants remaining from the previous crop and host weeds of nematodes. This practice has some limitations, the main one being the presence of more than one nematode species in the same cultivation area.

Inspection and sampling of soil and roots at the end of the crop cycle will indicate whether one or more cycles will be necessary and whether other practices should be adopted to complement the management and reduce the risk of losses in the subsequent crop.

Cover crops and/or antagonist

In Brazil, species of *Crotalaria* are the best-known cover crops that are used in areas with high nematode infestation. Currently, *Crotalaria spectabilis* is the most effective in reducing the population of the five most common nematodes in the soybean, grains and fibre production areas mentioned above. *Crotalaria ochroleuca* hosts the SCN (*H. glycines*), as well as *M. javanica*, while *C. juncea* multiplies *M. incognita*, *M. javanica*, *H. glycines* and *P. brachyurus*. *Crotalaria spectabilis* produces less vegetative mass and is a host of among others white mould (*Sclerotinia sclerotiorum*) and target spot (*Corynespora cassiicola*). Meanwhile, *C. ochroleuca* produces much more biomass, fixes more nitrogen and is a less favourable host for white mould. The longer it stays in the area, the greater the reduction of the nematode. However, to avoid problems with other diseases, it is recommended that crotalaria has its cycle interrupted at the beginning of flowering.

The sowing of *C. spectabilis* in the first rains that start in September and the maintenance of it for about 30 days before the soybean is planted brought a significant improvement in the development of the soybean crop in comparison to the neighbouring plot where this practice was not adopted. In addition, planting the cover crop increased soybean productivity 30% in areas with high simultaneous infestations of *P. brachyurus* and *H. glycines* (Fig. 15.3).

Species of *Urochloa* (brachiaria, Fig. 15.4B) and *Panicum* (Fig. 15.4A) are not hosts of RKN and other nematodes that affect soybean in Brazil. However, some multiplication of *P. brachyurus* and high multiplication of *P. zeae*, the most frequent species of the genus, occurs. In general, brachiaria and other forages are crops that form a lot of straw that persists on the soil in tropical conditions, allowing direct seeding into the straw.

This approach gave preference to crops that do not multiply nematodes or multiply less, in addition to providing other benefits on the soil surface and along the depth profile. The abundant straw, covering the whole soil, protects it against excessive heating and water loss, reducing the soil temperature by between 5–15°C. It also helps to reduce the spread of

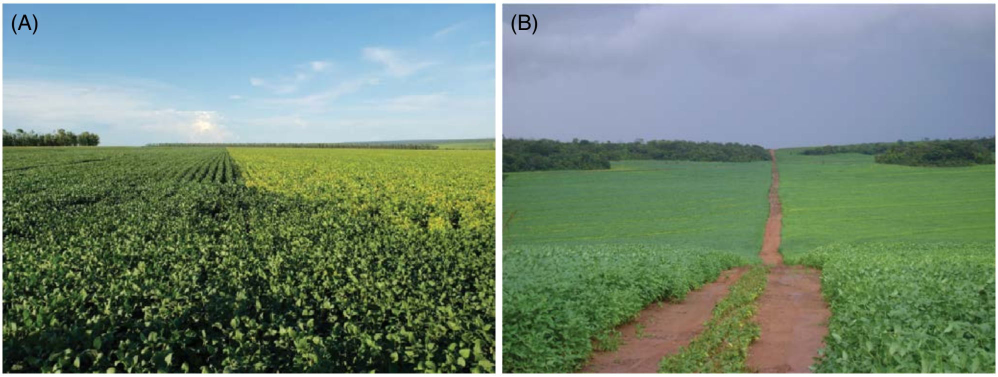

Fig. 15.3. **(A)** Effect of mechanical clean fallow for root destruction and soil desiccation in a field with a high infestation of *Pratylenchus brachyurus* on soybean growth. **(B)** Improved soybean foliage colouration following the planting of *Crotalaria spectabilis* 30 days prior to soybean and incorporation into the soil in a field with a high infestation of *Pratylenchus brachyurus* and *Heterodera glycines*. Author's own photographs.

Fig. 15.4. **(A)** Improved soybean crop on left-hand side of the road compared to the right-hand side, due to the intercrop *Panicum maximum* before soybean in a field with high infestation of *Heterodera glycines* and *Meloidogyne javanica*. **(B)** Nematode management and good growth on the right-hand side in relation to no management in the left-hand field following cropping of *Urochloa ruziziensis* before soybean in a field with a high infestation of *Heterodera glycines* and *Meloidogyne javanica*. Author's own photographs.

nematodes through machinery, rain and the wind. The straw significantly increases the organic matter of the soil, promoting the development of microorganisms that are natural enemies or competitors for space or food that disadvantages nematodes. In short, it improves the chemical, physical and biological characteristics of the soil, in addition to bringing other important benefits to the production system.

In a study carried out at Unesp in Jaboticabal, different production systems were evaluated with (i) maize (cv. DKB 390 PRO2) only; (ii) maize + *U. ruziziensis* (brachiaria); and (iii) *U. ruziziensis* only (summer 2011/2012), in between two soybean harvests (spring 2011 and 2012) in an area with high infestation of *M. incognita*, *P. brachyurus* and *R. reniformis*. The authors noted that the system '*U. ruziziensis* only' was the one that most significantly favoured bean productivity (+17 bags/ha in relation to exclusive

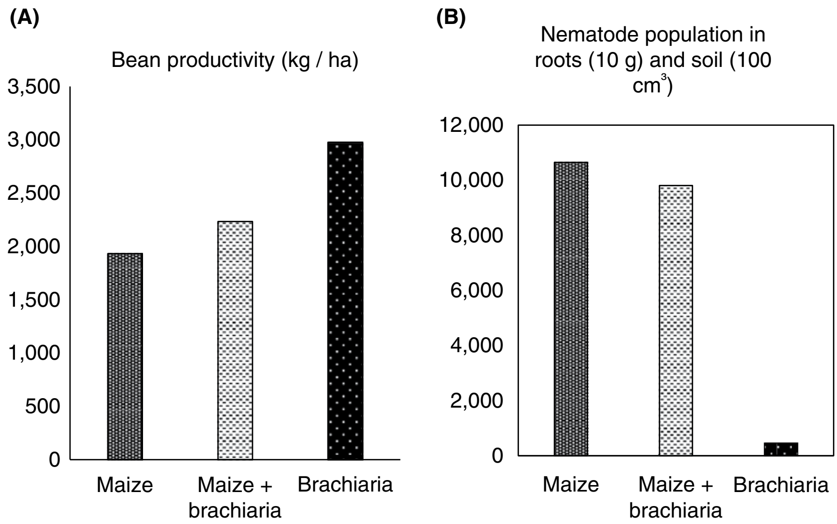

Fig. 15.5. **(A)** Bean productivity and **(B)** reduction of composite nematode populations of *Meloidogyne incognita*, *Pratylenchus brachyurus* and *Rotylenchulus reniformis*, with predominance of the first species, depending on the production system. Author's own figures.

maize), followed by 'maize + *U. ruziziensis*' with intermediate productivity (+5 bags/ha in relation to exclusive maize) and close to that obtained with the previous system mentioned. The 'maize only' system was the one that provided the lowest productivity (Fig. 15.5). Similar results can be seen in soybeans since nematodes are common to both crops. As for nematodes, the '*U. ruziziensis* only' system was also the one that most reduced nematode populations, followed by the 'maize + *U. ruziziensis*' system and the 'maize only' system. In an area with high nematode infestation, the best option is to use only the cover crop (e.g. brachiaria) or intercrop the main crop with some non-host to the nematode species present in the area.

A period of 1–6 months without a susceptible crop, alternating between a resistant/non-host cover crop, reduces the nematode population, and the longer the period, the better the results in areas of high infestation. Significant gains in productivity of soybean and other crops have also been obtained with this practice, even in areas with low nematode infestation.

Recently, there was a proposal to use mixtures of cover or antagonist plants which seems to be a great management option since, among the plant species to be used, none can be host/susceptible to any of the nematode species to be controlled. Also, proper sowing and management must be carried out to ensure good development of plants to complete closing of rows and in order to suppress weeds that can be hosts to nematodes.

Resistant crops

When available, resistant crops are one of the most effective control measures available, as long as the harvested product satisfies the market requirement, the production is satisfactory and the crop is not susceptible to other limiting problems. Currently, in Brazil, there are resistant soybean cultivars for *M. javanica* and *M. incognita*, while for *P. brachyurus* there are only tolerant cultivars.

In areas with high nematode infestation, the use of resistant cultivars enables satisfactory yields. In addition, they contribute to the restoration of the balance of the soil biota, as they promote the reduction of damaging nematode populations. In an area with high root-knot nematode infestation (*M. javanica*), the resistant crop produced 70% more than the susceptible one, in addition to dramatically reducing root infection and infestation in the area.

In an area cultivated previously with beans, and high infestation of *M. javanica*, a soybean resistant cultivar was planted together with a phosphate-based fertilizer (05-35-00) with seaweed extracts (NP Plus – Timac AGRO). It is an unprecedented technology developed in Brazil in order to have double action, nutrition and control of nematodes. This fertilizer was compared with another 07-37-06 standard fertilizer, and at harvest produced an increase of 18 bags/ha over the latter.

The resistant cultivars to *M. incognita* and/or *M. javanica* can multiply about 10–30% of the population present in the area in general, so they are not immune and they are partially resistant. The vast majority do not present resistance to all nematode species and races, therefore, additional management practices should always be adopted in combination with resistant and tolerant varieties. Also, in an area that is not infested or with low infestation, the productive potential of a susceptible crop in general is greater than that of a resistant one.

Nematicides and biological control

Chemical products with contact, and/or systemic action against nematodes can be applied as a seed treatment (ST) or via application in the furrow. The first option is one of the latest technological innovations that have arrived on the market to assist management, and in general promotes reasonable protection of the seedling roots until about 20–40 days after sowing and can increase productivity by up to 10%. After this period nematodes reappear, causing damage and visual differences between treated and untreated plots. This is common in areas with high infestation where the previous crop showed significant damage caused by nematodes and chemical control was used as an isolated measure in an attempt to obtain control.

Several naturally occurring organisms in the soil are considered natural enemies of nematodes, such as nematophagous fungi and rhizobacteria. There is a tendency to use the ST with a chemical and biological nematicide product, when compatible; if not, the chemical is applied in the seed and the biological in the seeding furrow. This strategy is very interesting, since the chemical nematicide will give that initial shock/protection effect by reducing the initial root infection. However, it has a short action period. The biological one is initially slower as it is a living micro-organism. Therefore it will act throughout the crop cycle and may even remain in the soil, with successive and continuous use, and make the soil rebalanced again regarding problems with nematodes.

Optimization of integrated nematode management

The INM techniques that are presented in this chapter are usually recommended and adopted by Brazilian soybean producers when the problem with nematodes in the area is known. Unfortunately, the knowledge about the damage caused by these organisms is still not widespread among producers, leaving control to the more technical producers. This shows a deficiency in the knowledge and sampling of the areas. Using better survey techniques, including precision agriculture, can assist in solving this problem.

Future research and outlook

Once an area has been infested with nematodes, systematic adoption of INM is necessary. The great challenge is to control nematodes in the soil and in the root tissue. Therefore, developing resistant cultivars that can be incorporated into the rotation/succession system are highly encouraged, especially for *P. brachyurus*. Also, the search for nematicides with greater control efficiency is needed. New registrations for safer nematicides have increased in recent years, and this trend will increase in view of the worldwide pressure for less toxic pesticides. In addition, the use of biocontrol fits perfectly in INM, which further favours this scenario for the coming years.

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16 Status of soybean cyst nematodes and integrated management in China

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Introduction

Soybean is one of the most important food crops in China. The country is the world's fourth largest producer with 1,750,000 tonnes in 2020. The average yield is 1.78 tonnes per ha, which is lower than the three largest producers, Brazil, USA and Argentina, with 3.39 and 3.47 and 2.32 tonnes per ha, respectively.

China is the largest soybean consumer and the largest soybean importer in the world. China's soybean production at present does not meet domestic demand. In 2019, China's total soybean production was 18.1 million tonnes, and actual soybean import was 88.51 million tonnes. More than 80% of domestic soybean needs relied heavily on import from USA, Brazil and Argentina. Soybean is a high-quality vegetable oil and protein resource in the Chinese diet. It is used for processed products such as bean curd, soybean milk and Yuzhu. Soybean meal is also an extremely important protein animal feed supplement for pigs and chickens.

In China, the soybean cyst nematode (SCN) *Heterodera glycines* is the most important pest on the crop causing huge yield losses every year. SCN has been reported to be responsible for annual economic losses of more than US$120 million in China (Ou *et al*., 2008) which may be, to some extent, responsible for overall low yields of the crop in the country.

Host range

Heterodera glycines has a relatively narrow host range in China with regards to food crops. The main cultivated crop hosts are leguminous plants, including soybean, mung bean, green pea and numerous types of edible beans. It can also damage non-leguminous plants such as *Rehmannia glutinosa*, *Paulownia fortunei*, sesame (*Sesamum indicum*), tobacco (*Nicotiana tabacum*) and tomato in China (Peng, 1999). The weed hosts that can support SCN have not been studied in detail.

Heterodera sojae was shown to infect ten crops (soybean, jequirity, cowpea, pea, lentil, mung bean, adzuki bean, sword bean, green beans and lucerne), but the species could only complete its life cycle on soybean. This could limit its spread (Zhen *et al*., 2018).

Distribution and economic importance

The SCN was first found in Korea in 1936 and then in China in 1938 where it was only found

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in a few soybean fields. In the 1940s, the distribution of SCN was mainly restricted to the north-east and the Huang-Huai River Valley, the two principal soybean production areas in China. SCN has now been detected in 22 provinces of China: Heilongjiang, Liaoning, Jilin, Inner Mongolia, Hebei, Henan, Shandong, Shanxi, Anhui, Beijing, Shaanxi, Jiangsu, Hubei, Shanghai, Zhejiang, Guangxi, Guizhou, Yunnan, Jiangxi, Gansu, Ningxia and the Xinjiang Uygur Autonomous Region (Peng *et al.*, 2016).

SCN infection disrupts host metabolism, water uptake and acts as a nutrient sink, thereby resulting in the yellowing of the above-ground parts, insufficient flower formation, and ultimately yield loss. A recent investigation showed that the level of infestation in the first nine provinces listed above were over 4 million ha, with yield losses between 20% and 30%, and in some cases 70% and 90% in severely infested soybean fields (Peng, 1999).

Symptoms of damage

Soybean symptoms of damage caused by *H. glycines* are those typically seen around the world on the crop (see Chapter 17). Common above-ground symptoms are plant growth retardation, stunting, cotyledon and true leaf yellowing and chlorosis, and stunting and death of seedlings (Fig. 16.1).

In addition, flower buds cluster, internodes shorten, flowering time is delayed and pods often do not (or only in small numbers) actually set. Sometimes, large areas in soybean fields become infected and exhibit severe levels of yellow and drying that resembles fire damage, so it is also called 'fire dragon yangzi' in China. Below-ground symptoms include poor development of main roots and lateral roots and increased fibrous roots. Often the whole root system has hair-like fibrous roots and shows a reduction in the number of nitrogen-fixing nodules, Also, swollen, white to cream-coloured SCN females can be observed on infected lateral roots four to six weeks after planting (Fig. 16.2).

Biology and life cycle

The development of the SCN does not vary from that described for the nematode in other reviews (Sikora *et al.*, 2018). There does not seem to be any unusual biological differences between the populations in China and the rest of the world.

Fig. 16.1. Stunting, yellowing and chlorosis of soybean caused by *Heterodera glycines* in China. Author's own photograph.

Fig. 16.2. White adult females of *Heterodera glycines* attached to the outside of a soybean root with their egg sac containing newly laid eggs visible. Author's own photograph.

New species

Of great interest to us in China is the fact that we now have two species of cyst nematodes: *H. glycines* and *H. sojae* damaging soybean in the country. A new species, *H. sojae* was described on soybean in Korea and was recently found on the roots and in the rhizosphere soil of soybean crops in Wuyuan, Jianxi province (Zhen *et al.*, 2018). The distribution, biology and infection process has not been studied in detail in China to date.

Races

Heterodera glycines is represented by eleven races and nine HG virulence types in China. An HG type test determines how a resistant soybean cultivar will react to *H glycines*. Races 1, 2, 3, 4, 5, 6, 7, 9, 13, 14 and X12 have been identified with races 1, 3 and 4 the most common. Race 3 occurs mainly in north-eastern China, race 4 primarily in the Huang-Huai River Valley and race 1 in the Jilin and Shandong provinces (Peng, 1999; Lian *et al.*, 2021).

ZDD2315 (Feipizhiheidou) and PI437654 are two of the most promising elite resistant germplasms in China. They are resistant to all SCN populations identified thus far, except for the newly identified race X12 (Lian *et al.*, 2017). The new race (X12) of the SCN was detected in a heavily infected field in Xinjiashe, Gujiao City, Shanxi province, China (Lian *et al.*, 2021). This new race with high levels of virulence is more aggressive than race 4. Race X12 constitutes a potentially serious threat to soybean production, especially in China, but also elsewhere should it spread.

Increases in virulence of SCN has been observed and the dominant races appear to be shifting toward greater virulence in China (Peng, 1999; Lian *et al.*, 2021), which is another serious threat to soybean production in the country.

Diagnosis of soybean cyst nematode

Based on the sequences of rDNA-ITS in the Chinese populations of *H. glycines*, a duplex PCR was developed for diagnosis of *H. glycines.* The species-specific primer GlyF1 was designed for *H. glycines* and a combination of a universal primer rDNA2 as a primer set (a specific 181 base-pair fragment) and the universal D3A and D3B primer set was used to confirm the success of DNA extraction and amplification (Subottin *et al.*, 2001).

A single randomly amplified polymorphic DNA (RAPD) marker, OPA06477, species-specific to the *H. glycines* was identified. The SCAR primer sets (SCNFI, SCNR1) of 24 nucleotides have been designed and used in straightforward, fast and reliable PCR assays to diagnose *H. glycines* (Ou *et al.*, 2008).

Recommended integrated nematode management (INM) approaches in China

Currently, application of resistant soybean cultivars, non-host crop rotation and the use of nematode-protectant seed treatments are the three primary means of managing SCN *H. glycines*. The primary goal of nematologists in China is to ensure that the management of *H. glycines* in soybean fields of the north-east and the Huang-Huai River Valley is integrated, complementary and diversified for long-term effectiveness.

Farm size and inputs

In China, the average family farmer cultivates <2 ha of land. There are, of course, large government farms and cooperatives planting larger areas of land of up to 200 ha. Integrated nematode management is therefore very different between the two farm types, due to their ability to purchase inputs such as resistant cultivars and nematicides. Due to the lack of financial inputs, small farmer management of SCN is mainly dependent on crop rotation with non-host crops and the use of resistant and tolerant cultivars. Conversely, larger cooperatives and government farms use rotation, resistant cultivars and in some cases chemical control in the form of seed treatments for INM.

Extension and knowledge transfer

China has a very good agriculture extension system. There is a National Agricultural Technology Extension and Service Center governed by the central government that maintains plant protection stations (PPS) in each province, with local PPS in each county of China. Therefore, a country-wide agricultural technology extension service network is available to the farmers for transferring knowledge on INM.

In many cases the farmers are aware of the existence and danger of the SCN due to good access to extension information from their PPS. Both small and large farms and cooperative farms have access to knowledge via 5G intelligent and social networks.

Resistance evaluation

In tests with 40 soybean cultivars for resistance to *H. sojae,* 9 cultivars were highly susceptible, 11 cultivars were moderately susceptible, 5 cultivars were moderately resistant and 5 cultivars had high levels of resistance.

The use of *H. glycines* resistant soybean cultivars to control SCN nematode is the most cost effective and environmentally friendly tool used for INM. From 1986 to 1992, a Coordinative Group of Evaluation in 1993 (CGE) of SCN supported Chinese scientists in the supervision of highly standardized tests for evaluation of soybean germplasm for SCN resistance. More than 10,000 accessions of soybean germplasm in China were evaluated for their resistance to race 1, 3 and 4 of SCN. Among them, 128 soybean accessions were resistant to race 1, and 16 accessions expressed immunity; 288 accessions were resistant to race 3 and 30 accessions had immunity; 11 accessions were resistant to race 4. Four accessions (Wuzaiheidou, Feipizhiheidou, Longraodaheidou and Wupuheiheidou) were resistant to all three of the above races of SCN (CGE, 1993).

Resistant soybean cultivars

The best method for SCN management is through the development of resistant cultivars. Currently, planting SCN-resistant cultivars is the primary method used in integrated management of the SCN in China. In China, major efforts were made to develop SCN cultivars resistant to HG types 1, 2 and 3 present in the Huang-Huai Valley and to HG Type 0 in the north-east of China (Lian *et al.*, 2021).

A number of resistant cultivars, including Kangxian1, Kangxian2, Kangxian3, Kangxian4, Kangxian5, Kangxian6, Kangxian7, Kangxian8, Kangxian9, Kangxian10, Kangxian11, Kangxian12 and Kangxian13 resistant to HG type 0, were released by the Heilongjiang Academy of Agricultural Sciences in north-east China. At present, more than 30 cultivars, including the Kangxian series, with high yield and resistance have been released into the commercial market in China between 1988 and 2020. The cultivars include Lengfeng14, Lengfeng15, Lengfeng18, Lengfeng19, Lengfeng20, Qingfeng1, Fengdou3, Qinong1, Qinong2, Qinong5, Shundou1, Pengdou1, Fudou6, Nongqingdou20, Nongqingdou24, Andou162 and Heinong531.

Non-host crop rotation

Crop rotation with non-host crops is an effective method for SCN management. Soybean rotated with non-host graminaceous crops such as wheat, maize, millet or peanut is effective in SCN control in China. In China, a 2-year rotation with maize is common in the Huang-Huai River Valley, whereas a 1-year rotation with maize or rotation with a resistant and susceptible soybean cultivar are normal rotation systems in north-east China (Peng, 1999).

Biological control

More than 2000 isolates from different species of nematophagous fungi were isolated from cysts of *Heterodera glycines* in China. Many experiments have been conducted on the biology and efficacy in greenhouse and field trials of the following: *Microbacterium maritypicum*, *Pochonia chlamydosporium*, *Purpureocillium lilacinus*, *Verticillium lecanii* and *Hirsutella rhossiliensis*. Several biological agents, including *M. maritypicum* strain Sneb159 and *Bacillus megaterium* strain Sneb207, were tested with seed-coating technology for control of SCN in greenhouse trials (Yuan *et al.*, 2020). Although these biocontrol organisms in some cases gave adequate control of SCN, they are not yet on the market for use in soybean for nematode management.

Nematode-protectant seed treatments

Nematicides have been used for the management of SCN in the past, but they are being reappraised due to environmental hazards and food safety. However, where serious infections in the field occur, nematicide seed coating has been considered an alternative option for effective management of SCN. Conversely, nematicides are expensive and, if improperly used, hazardous for small farmers. At present, a number of nematicides including fosthiazate, avermectin and fluopyram are available for managing SCN. Recently, the new Junxianke series biological seed-coating formulations (SN100, SN101 and SN102) containing *Helisu brassinosteroid* plus *B. amyloliquefaciens*, as well as *B. thuringiensis* suspensions were tested to control SCN in the field. These biological seed coatings are considered to have potential application for SCN management in the future (Yuan *et al.*, 2020).

Optimization of nematode management

On 15 March 2019, China issued the ‘Soybean Revitalization Plan’ and the Ministry of Agriculture and Rural Development established detailed implementation schemes for improvement of soybean production. In 2020, the areas in China planted to soybean expanded to 9.33 million ha compared to 8.67 million ha in 2019. The average soybean yield increased from 1.7 to 2.0 tonnes per ha. With the need to expand soybean production and improve yield even more, there will be expansion in the northern soybean growing areas where SCN occurrence and damage is high. Therefore, measures need to be taken ahead of time to improve INM in this SCN infested region. There are a number of things that need to be done to make the revitalization plan a success and one of them is INM.

1. China needs better support for survey work and modern nematological technologies.
2. There is a need for more advanced use of remote sensing technology in the survey work on SCN distribution and monitoring occurrence and damage in farm fields.
3. There is a need for more support for resistance breeding in order to produce high-yielding and SCN resistant cultivars.
4. Research on seed treatment and coating with both biological and chemical nematicides needs development in order to protect seedlings from SCN early root infection.
5. Rotations need to be optimized with more effective non-host crops.
6. Resistance management needs to be introduced where resistant with susceptible cultivars are rotated to prevent development of resistance breaking races.
7. Extension needs to be improved through IT systems to make knowledge of these SCN management options available to the small and large farming operations.

Outlook: anticipating future developments

China's soybean imports are expected to exceed 100 million tonnes in 2021. Therefore, proper pest and disease management for SCN is important to help improve soybean production in the country as affected by future higher rates of domestic consumption. Demand will continue to increase rapidly, and this will widen the gap between domestic production and imports.

In order to implement the National Food Security Strategy under increasing domestic demand, China needs to actively respond to the complex international trade environment by promoting the recovery and development of China's soybean production to international levels to reduce dependence on imports. This shift to more production will be associated with alterations in infrastructure and production management up until 2050. This change in direction will require a response by nematologists in China.

1. Expansion will probably mean that the number of small family farms will decline and merge into larger more efficient cooperative and commercial farms with increased need for advice and knowledge transfer mechanisms.
2. With climate change and climate variability (see Chapter 64) there will be an increase in the spread of both the SCN and the new species *H. sojae* in the northern soybean growing areas as soybean production expands, which will need monitoring.
3. Because of the lack of resistant germplasm and cultivars there is a need to improve research into the use of resistant germplasm that has been cloned but is still of limited use to the farmer.
4. With the rapid development of modern biotechnology and the wide application of next-generation sequencing and metagenomics technology, the identification of trait genes and the cloning of nematode-resistance genes for nematode-resistant resources will need advancement.
5. Advanced research needs to be supported and CRISPR genome editing technology has brought a revolution to the development of biotechnology and could accelerate the functional identification of nematode-resistance genes and the study of their mechanism for SCN management.
6. There are many challenges facing nematology in China and the production of soybean. SCN management is one of the most important and it will be a challenge to nematologists in the future.

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17 The soybean cyst nematode: Pervasive and destructive to soybean production in the mid-western United States

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Introduction

Most of the soybeans (*Glycine max*) produced in the US are grown in the mid-west, and the soybean cyst nematode (*Heterodera glycines*) is the most damaging pathogen of soybean throughout the region (Allen *et al.*, 2017). The status of *H. glycines* as the crop's greatest yield-reducing pathogen is a function of its biology, distribution, persistence and adaptability. There are other nematode species that cause damage and yield loss, such as the root-knot nematode, *Meloidogyne* spp., and the reniform nematode, *Rotylenchulus reniformis* (Allen *et al.*, 2017), but *H. glycines* is the primary nematode threat to soybeans throughout the region.

Economic importance

The seriousness of *H. glycines* as a pathogen of soybeans is underscored by the very large yield reductions attributed to this nematode. In the US and Ontario, Canada, the estimated yield loss due to *H. glycines* for 2010 through 2014 was more than twice the loss estimated for any other soybean pathogen. Individual annual yield loss estimates for *H. glycines* ranged from 90 to nearly 120 million bushels or 2.45 to 3.27 million metric tonnes for 2010 through 2014 (Allen *et al.*, 2017). Estimated yield losses from *H. glycines* specifically in the mid-west and Ontario were 25–37% of total yield losses from all soybean diseases during those years, and the damage totalled an estimated US$1.24 to 1.69 billion.

Host range

The host range of *H. glycines* is not limited to soybean. A large compilation of *H. glycines* host plants published by Riggs (1992) included 63 species from 50 genera. Among the hosts are the crop plants mung bean (*Phaseolus aureus*), green pea (*Pisum sativum*) and numerous types of edible beans (*Phaseolus vulgaris*). Also, several winter annual weeds support *H. glycines* reproduction, namely henbit (*Lamium amplexicaule*), purple deadnettle (*Lamium purpureum*), field pennycress (*Thlaspi arvense*), common chickweed (*Stellaria media*), shepherd's purse (*Capsella bursa-pastoris*) and smallflowered bittercress (*Cardamine parviflora*) (Johnson *et al.*, 2008).

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Distribution

Heterodera glycines has been discovered in all but one soybean producing state in the US (Fig. 17.1) and has been identified in many, most or all of the counties in the mid-western states (Tylka and Marett, 2021). The nematode continues to spread throughout the US and Canada. A map of the known distribution of *H. glycines* in North America was updated in 2020 and included 55 new counties in the US, 5 new counties in Ontario, 4 new rural municipalities in Manitoba and 15 new counties in Quebec, Canada since 2017 (Tylka and Marett, 2020).

Symptoms of damage

Symptoms of damage to soybean from *H. glycines* are those typically seen with injury from other plant-parasitic nematodes that parasitize soybean and other crops. Common above-ground symptoms are stunting of plants and yellowing of foliage (Fig. 17.2). The foliar chlorosis is generalized throughout the leaflets of the leaf and not restricted to the areas between the veins. Below-ground symptoms include browning and stunting of root systems and reduction in the number of nitrogen-fixing nodules. Also, swollen, white to cream-coloured *H. glycines* females (Fig. 17.3)

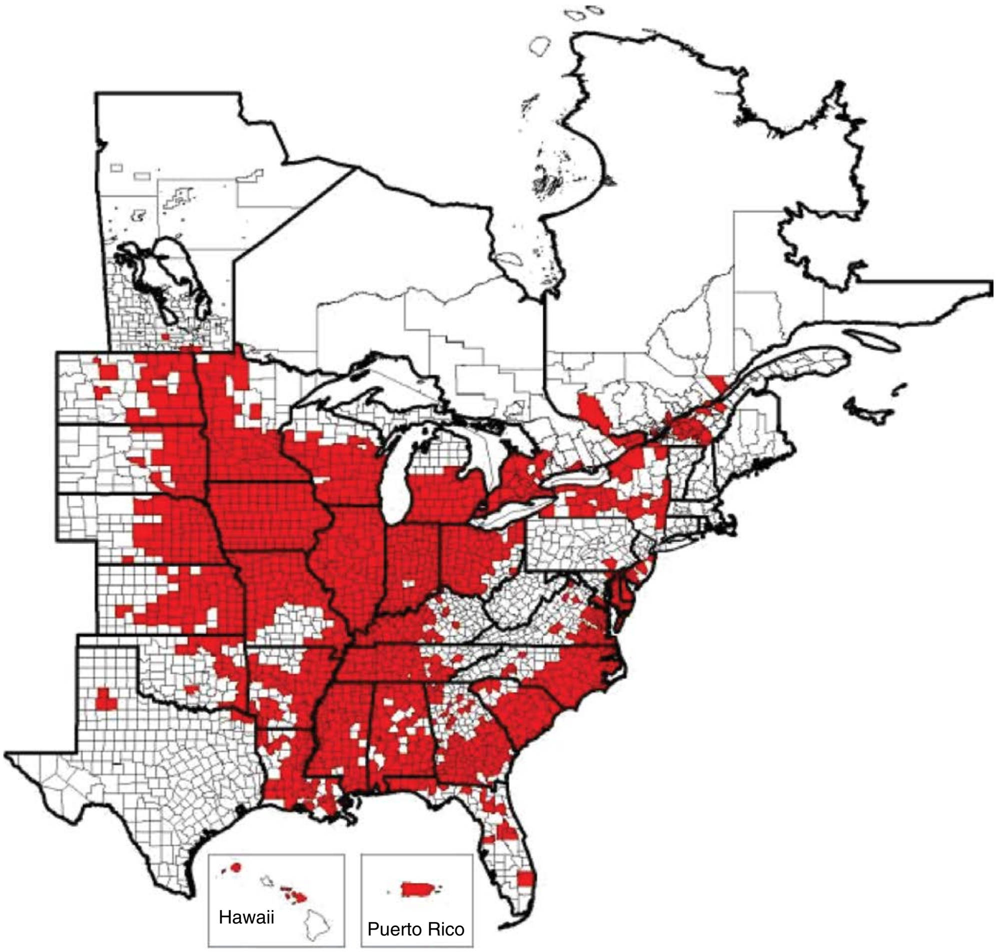

Fig. 17.1. Known distribution of the soybean cyst nematode, *Heterodera glycines*, in the US and Canada through 2020 (Tylka and Marett, 2021). Author's own figure.

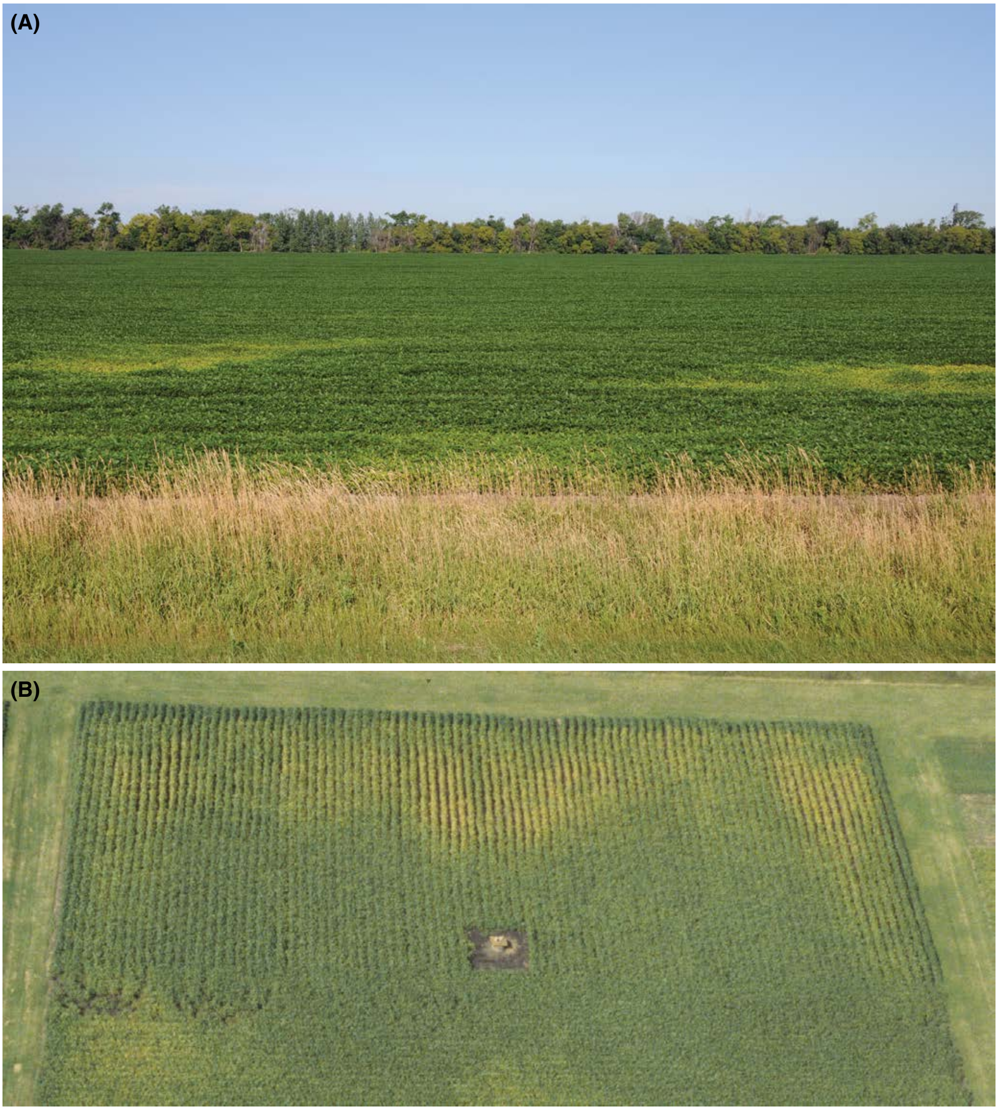

Fig. 17.2. (A) Foliar chlorosis of soybean caused by *Heterodera glycines* at ground level. **(B)** An aerial field view of a heavily infested field. Photographs courtesy of S. Markell and the author, respectively.

can be observed on infected roots beginning 4 to 6 weeks after planting.

A unique aspect of *H. glycines* in the midwest is that yield loss can occur with no above-ground symptoms appearing (Niblack *et al.*, 2006). The absence of obvious symptoms makes it difficult to convince farmers to sample fields for the presence of the nematode. Near the end of the growing season, areas of fields with high *H. glycines* population densities often will senesce earlier than areas where nematode numbers are lower. This is a little recognized but relatively consistent symptom of *H. glycines* damage.

Biology and life cycle

The life cycle of *H. glycines* is typical of most cyst nematodes. Eggs of *H. glycines* are formed following the mating of males with females, and

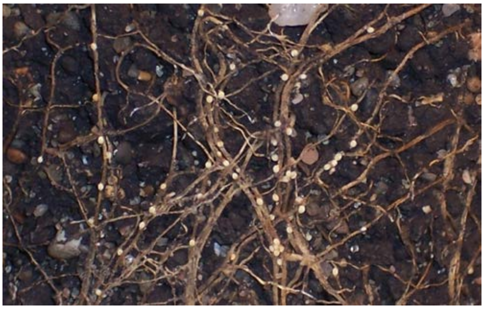

Fig. 17.3. Adult females of *Heterodera glycines* (small, cream-coloured objects) on infected soybean roots. Author's own photograph.

the resultant embryos develop into vermiform first-stage juveniles that moult once within the eggs, forming second-stage juveniles that hatch from the eggs. There are believed to be three hatching behaviours with *H. glycines*: constitutive hatching that occurs when soil temperature is adequate, inducible hatching that takes place in response to compounds emanating from host roots, and dormancy in which hatching does not occur for years regardless of conditions (Niblack *et al.*, 2006). Following hatching, development and maturation of vermiform second-stage juveniles will occur only if they penetrate a host root, migrate intracellularly through the root cortex to the periphery of the vascular stele and initiate the formation of permanent feeding sites, called syncytia. Juvenile development progresses through third and fourth stages and eventually adult males and females form.

Adult males and females of *H. glycines* have greatly different morphologies. Juvenile males and females both enlarge in later juvenile stages, but males eventually revert to a vermiform shape and exit the root as adults. Juveniles that develop into females stay attached to and feeding on the syncytia and the females swell throughout their life, becoming so large that the posterior portion of their bodies rupture out of the root and are exposed on the root surface. The adult females appear as round, white to cream-coloured objects visible to the unaided eye and are much smaller than nitrogen-fixing nodules that normally occur on soybean roots. After males and females mate, some eggs are deposited in an egg mass outside of the female body, but a majority of eggs are retained internally within the female body cavity. The egg-filled *H. glycines* females eventually die, and the body walls of the females undergo chemical changes resulting in formation of hardened cysts that encase the eggs that were retained within the females. Under optimum temperatures, the life cycle can be completed in 21 to 24 days, allowing for multiple generations to occur in a single growing season (Niblack *et al.*, 2006). Eggs can survive dormant for many years within cysts in soil.

Interactions with other pathogens

Heterodera glycines has significant interactions with several other pathogens, the most prominent being *Fusarium virguliforme*, the fungus that causes soybean sudden death syndrome (SDS). Symptoms of SDS occur earlier in the growing season and develop to more severe levels in soil that is infested with *F. virguliforme* and *H. glycines* compared to soil infested only with *F. virguliforme* (Niblack *et al.*, 2006). A similar relationship exists between *H. glycines* and *Cadophora gregata*, the fungus that is the causal agent of soybean brown stem rot (BSR). Plants infected with *H. glycines* have greater incidence and more severe internal stem symptoms of BSR than plants not infected with the nematode. Furthermore, the severity of infection or colonization of soybean stems by *C. gregata* is increased when plants are infected with *H. glycines*. Infection of BSR-resistant soybean cultivars by *C. gregata* occurs at levels typically seen in non-resistant (susceptible) cultivars. Genetic resistance to BSR disease and to infection and colonization by the fungal pathogen in soybeans is somehow nullified by *H. glycines* infection (Niblack *et al.*, 2006).

Recommended integrated nematode management

Currently, there are three primary means of managing *H. glycines*: growing resistant soybean cultivars and non-host crops and using nematode-protectant seed treatments. It is essential that efforts to manage *H. glycines* in soybean fields of the mid-west are coordinated, diversified and complementary to be effective in the long term.

Resistant soybean cultivars

Soybean cultivars resistant to *H. glycines* were first used in the US in 1978 (Blok *et al.*, 2018). Their use has allowed for profitable production of soybeans in fields infested with the nematode for several decades. Effective resistant soybean cultivars yield well and also prevent large increases in population densities of the nematode (McCarville *et al.*, 2017).

Numerous soybean lines with resistance to *H. glycines* have been identified for use in soybean breeding programmes (Blok *et al.*, 2018). The genetic basis of resistance to *H. glycines* in these soybean breeding lines is complex and specific details of the current state of knowledge are beyond the scope of this chapter. Soybean resistance to *H. glycines* was recently reviewed by Mitchum (2016) and is described as involving several disparate genes and variation in the number of copies of certain genes in the soybean genome. The complete details of *H. glycines* resistance in soybeans have not yet been fully elucidated.

Soybean cultivars available in the mid-western US since the early 1990s were developed primarily using the breeding line PI 88788 (Fig. 17.4). Throughout the years there were cultivars available that were developed with the Peking source of *H. glycines* resistance, but during that time cultivars with Peking resistance had consistently lower yield potential than those developed with PI 88788. Therefore, Peking was not widely used in soybean breeding programmes.

Continual use of soybean cultivars with resistance from PI 88788 resulted in development of, or selection for, *H. glycines* populations with elevated reproduction on the source of resistance as well as on cultivars possessing resistance genes from the breeding line (Mitchum, 2016; McCarville *et al.*, 2017). Given the widespread geographic distribution of *H. glycines*, the prolonged and predominant use of soybean cultivars with PI 88788 resistance and the resultant elevated reproduction of *H. glycines* populations on cultivars with resistance from PI 88788, profitable soybean production in the mid-west in future decades is in jeopardy.

Resistance to *H. glycines* in soybeans is not race- or HG type-specific. However, the virulence or ability of *H. glycines* populations to reproduce on the breeding lines used to develop resistant soybean cultivars is measured by the HG type test, an updated version of the original race test (Niblack *et al.*, 2006). These test results provide an indication of how well a soybean cultivar with a specific source of resistance such as PI 88788 or Peking will control reproduction of the *H. glycines* population present in the field in which the cultivar is grown.

McCarville *et al.* (2017) predicted that yields of resistant soybean cultivars developed with PI 88788 would be surpassed by yields of cultivars with resistance derived from Peking as the virulence of *H. glycines* populations on PI

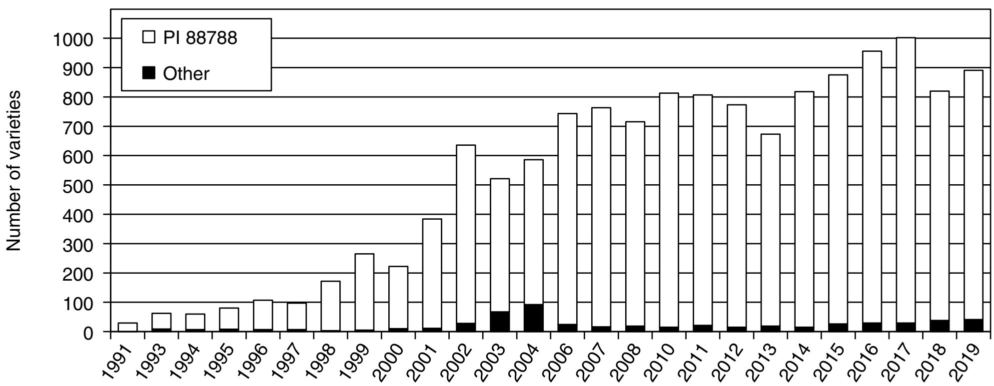

Fig. 17.4. Number of soybean cultivars resistant to *Heterodera glycines* available to farmers in Iowa from 1991 to 2019, and the proportion with resistance from PI 88788 versus all other sources of resistance. Most cultivars in the 'other' category possessed the Peking source of resistance. Data from Tylka and Mullaney (2020).

88788 continued to increase. Data from a field experiment conducted in Iowa in 2019 were consistent with this prediction. Soybean cultivars with *H. glycines* resistance from Peking yielded 12 to 15 bushels per acre (807 to 1009 kg per hectare) more than the top-yielding resistant cultivars developed from PI 88788 in the same experiment, conducted in a field with an *H. glycines* population with 71% reproduction on PI 88788 and 0% reproduction on Peking (Tylka *et al.*, 2019). Furthermore, end-of-season *H. glycines* soil population densities were significantly lower where cultivars with Peking resistance were grown compared to where cultivars with PI 88788 resistance were grown. These results illustrate what likely will commonly occur throughout the mid-west in future years unless more cultivars with resistance from sources other than PI 88788 become available for farmers to grow.

Non-host crops

Growing non-host crops is an effective means to reduce *H. glycines* population densities, and many row crops grown in the US are non-hosts. Nematode juveniles that hatch from eggs during a season in which a non-host crop is grown will perish from starvation or parasitism or predation. However, many *H. glycines* eggs in the soil will be dormant and juveniles will not hatch and die in a single season of a non-host crop. An alternating 2-year rotation of soybeans and maize (*Zea mays*) is common in the mid-west. Rotations including two or three consecutive years of non-host crops often are needed to considerably reduce *H. glycines* population densities.

Nematode-protectant seed treatments

In the mid-2000s, nematode-protectant seed treatments were introduced as new management tools for *H. glycines*, pioneered by Avicta®, from Syngenta. Thereafter, numerous other seed treatments were brought to market with a diverse range of active ingredients and reported modes of action (Table 17.1). The active ingredients of some seed treatments are biological organisms or their by-products and others are synthetic chemicals. Some are reported to affect the biology of *H. glycines* directly whereas others directly or indirectly protect soybean roots from infection by the nematode. Results of field experiments with nematode-protectant seed treatments have been variable. It is not uncommon for yield increases to be reported, but significant decreases in end-of-season *H. glycines* population densities are infrequently observed. Nonetheless, nematode-protectant seed treatments represent an important new management tool for *H. glycines* in a time when resistant cultivars continue to lose effectiveness.

Optimization of integrated nematode management

None of the current management strategies are adequate on their own; each has one or more shortcomings. For example, soybean cultivars with resistance from either PI 88788 or Peking can vary significantly in yield and nematode control. Optimizing use of available resistance requires seeking out data on performance of cultivars and selecting those with both high yields and effective nematode control. Yield and nematode control data are available from a few universities and some agribusinesses who conduct cultivar comparison plots. As explained above, soybean cultivars with the PI 88788 source of *H. glycines* resistance are losing effectiveness. Consequently, maximizing the benefits of resistance also requires seeking out and growing cultivars with the uncommon Peking resistance. There are a few such cultivars currently available (Tylka and Mullaney, 2020). Hopefully more will be brought to market in the future.

Another opportunity to optimize current *H. glycines* management relates to use of nematode-protectant seed treatments that have a wide range of active ingredients and reported modes of action. It is reasonable to anticipate that their effects on yields and on *H. glycines* population densities may vary among products and also from field to field and year to year. No university or agribusiness can conduct field experiments in a wide enough range of conditions or locations to provide sufficient guidance to farmers about which seed treatments will work best in their specific fields. Consequently, farmers and those who advise them should carefully

Table 17.1. Information on nematode-protectant seed treatments for soybeans and *H. glycines* in 2020. Treatments are listed from the first released at the top to the most recent at the bottom.

Brand name	Company	Active ingredient	Reported mode of action
Avicta®	Syngenta	Abamectin	Inhibits nerve transmission
N-Hibit®	Direct Enterprises	Harpin protein	Induces plant defences
Votivo®	BASF	*Bacillus firmus*	Blocks infection, degrades eggs
Clariva®	Syngenta	*Pasteuria nishizawae*	Nematode parasite
Ilevo®	BASF	Fluopyram	Inhibits nematode cellular respiration
Aveo®	Valent	*Bacillus amyloliquefaciens*	Paralyses nematodes
Nemasect®	Beck's	Heat-killed *Burkholderia rinojensis* plus fermentation media	Enzymes and toxins
BioST®	Albaugh	Heat-killed *Burkholderia rinojensis* plus fermentation media	Enzymes and toxins
Trunemco™	Nufarm	*Bacillus amyloliquefaciens* and cis-Jasmone	Induces systemic resistance and protective colonization
Saltro®	Syngenta	Pydiflumetofen	Inhibits nematode cellular respiration

evaluate and note the performance of various seed treatments in fields infested with *H. glycines* to develop a sense of how well the products work and under which conditions.

The overall success of management efforts should be judged not only based on yields, but also on trends in *H. glycines* population densities. Thorough soil samples should be collected from fields preceding every third soybean crop to determine if management efforts are keeping *H. glycines* population densities in check or if nematode numbers are increasing, resulting in increased chances of yield loss in future years.

Outlook: anticipating future developments

Managing *H. glycines* in the future likely will be based on growing resistant soybean cultivars and non-host crops and using effective nematode-protectant seed treatments, just as is currently being done in 2021. But the situation with conventional, non-transgenic resistance in soybean cultivars must improve if soybeans are to be grown profitably in the future. The widespread increase in virulence of *H. glycines* populations throughout the mid-west on the PI 88788 breeding line is a crisis that must be addressed quickly. Unfortunately, the emphasis in developing new soybean cultivars for the mid-west is on new herbicide resistance technologies, not diversifying the genetic base of *H. glycines* resistance.

There are novel strategies on the horizon that may expand the options for managing this serious soybean pest. New nematode-protectant seed treatments with different active ingredients and modes of action likely will become available in upcoming years. Also, Syngenta released two soybean cultivars in 2021 for use in the mid-west with *H. glycines* resistance from PI 89772, a source of resistance not used before in commercially available soybean cultivars. Additionally, the US Environmental Protection Agency granted registration to BASF in 2020 for soybeans containing a transgenic Bt trait that confers resistance against *H. glycines*. This resistance is the first of its kind, and cultivars are not yet available for use by growers.

Other means being investigated to improve soybean resistance to *H. glycines* include stacking or combining native resistance genes in a single soybean genotype and increasing in a genotype the number of copies of genes for which resistance effectiveness is related to copy number (Mitchum, 2016). Finally, increasing expression of plant defence genes and/or decreasing expression of plant genes involved in nematode feeding both are being investigated as ways to make soybeans more resistant to *H. glycines*.

McCarville *et al.* (2017) found that in field experiments conducted from 2001 through 2015 in Iowa, increases in *H. glycines* population densities were greater in dry and warm growing seasons than in cool seasons with adequate or above-average rainfall. As global climate change progresses, damage caused by *H. glycines* may be intensified in areas that experience extreme heat and/or drought. Consequently, research to improve and broaden management options for this destructive soybean pathogen is needed more than ever.

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18 Root-knot and reniform nematodes: Double trouble for soybeans in the southern United States

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Introduction

Soybean is host to a variety of plant parasitic nematodes. Yield losses due to nematodes depends on the species present, population density, cultivar susceptibility/ tolerance and intensity of drought stress to which the host is subjected (Mitkowski and Abawi, 2011). Root-knot (*Meloidogyne* spp.) and reniform nematodes (*Rotylenchulus reniformis*), along with soybean cyst nematode (*Heterodera glycines*) comprise the most damaging group of plant pathogens of soybean in the southern US. Yield suppression as much as 50% may occur when high population densities are combined with stressful environmental conditions (Kirkpatrick *et al.*, 2014).

The southern root-knot nematode (*M. incognita*) is one of most damaging nematodes in soybeans, though Javanese root-knot (*M. javanica*) and peanut root-knot (*M. arenaria*) can also damage the crop (Sikora *et al.*, 2018). Reniform nematodes are found throughout the southern US, although Mississippi, Louisiana and Alabama appear to have the greatest incidence of this nematode (Hartman *et al.*, 2015).

Economic importance of the nematodes

Plant parasitic nematodes are the most damaging group of plant pathogens of soybeans in the 17 states that comprise the southern region of the US (Allen *et al.*, 2019). It was estimated that root-knot nematodes (RKN) were responsible for yield losses of nearly 325 million kg of soybean in this region in 2018. Reniform nematode was estimated to be the third most damaging nematode of soybeans after soybean cyst and root-knot in the south, causing an estimated yield loss of over 46 million kg. Based on 2018 crop values, yield reduction caused by RKN and reniform combined resulted in an economic loss of over US$120 million to the industry.

Host range

The RKN present in the southern regions are extremely polyphagous with a host range of up to 3000 plants (Mitkowski and Abawi, 2011). Reniform nematode, on the other hand, has a host range of over 300 plant species including

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economically important crops such as cotton, cowpea, soybean and various vegetables (Robinson *et al.*, 1997).

Distribution

RKN are found throughout the southern region of the US. Reniform nematode is known to be established mainly in Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina and Texas (Hartman *et al.*, 2015).

Symptoms of damage

RKN induce knot-like swellings or galls on the roots that vary in number and size depending on the intensity of the infection and the nematode species involved. When numerous, galls tend to coalesce so that entire roots may be greatly swollen (Fig. 18.1). Galled roots are limited in their ability to absorb and transport water and nutrients which can result in stunted plants that wilt even when soil moisture is available, and that exhibit symptoms of nutrient deficiencies.

Above-ground symptoms usually appear on patches of plants in an infested field (Fig. 18.2). Patches usually occur irregularly, often caused by spread of the nematode in the direction of cultivation and/or surface water flow (Kirkpatrick *et al.*, 2014).

Symptoms of reniform damage on soybean roots are the same as those observed on cotton and discussed in Chapter 14. Above-ground symptoms on soybean are most noticeable in areas where the soil type is poor and where plants are stressed by other factors. The attachment of the reniform females with their egg mass to the root, often with soil attached, and the absence of the typical swellings seen for root-knot is diagnostic for its presence (Fig. 18.3).

Biology and life cycle

The life cycle of RKN nematodes on soybean is typical for the nematode in general (Mitkowski and Abawi, 2011; Sikora *et al.*, 2018). The life cycle is completed in 17 to 23 days at a soil temperature of 29°C, the optimum for root invasion and nematode development. Infection is greatest when soil moisture levels are just below field capacity, and extremely wet or dry conditions reduce root invasion. RKN typically survive as eggs in the southern US. The number of nematodes in the soil reaches a maximum at crop maturity. The population declines slowly through the winter and then precipitously as soil temperatures increase in the spring. This rapid decrease in the number of infective nematodes can occur several weeks before planting.

The life cycle of reniform nematodes on soybean is the same as on cotton and was described in Chapter 14.

Fig. 18.1. Root-knot galls on infected soybean roots in Alabama, USA. Author's own photograph.

Fig. 18.2. Symptoms of chlorosis and stunting of infected soybean plants infected with root-knot nematode. Author's own photograph.

Fig. 18.3. *Rotylenchulus reniformis* exposed anterior portion of white females and females with egg mass that are stained red. Photograph courtesy of Marina Rondon.

Recommended integrated nematode management (INM)

Integrated nematode management strategies are employed to maintain nematode densities below economic threshold levels. Damage threshold levels have been established for RKN and reniform on soybean in some states to determine risk associated with nematode populations (Jagdale *et al.*, 2013). The most effective means of determining population densities is through annual soil sampling with proper nematode identification by a diagnostic facility. This information, coupled with a combination of cultural practices, resistant cultivars and nematicides, can provide acceptable management of nematodes.

Crop rotation

Damaging levels of nematodes usually occur following several years of continuous planting of susceptible hosts. Growing crops that are non-hosts can lower nematode populations below damaging levels for a sustained period. As with selection of the right resistant cultivar, it is vital that the cropping sequence be matched to the nematode species present. For example, if RKN is severe, then growing rice, peanuts, wheat or some cultivars of grain sorghum can effectively lower populations, but growing cotton, maize or soybeans will likely increase the severity of the problem (Kirkpatrick *et al.*, 2014).

Unfortunately, most maize cultivars are good hosts for the southern RKN and therefore do little to reduce populations. Although rice is susceptible to the nematode, the flooded fields where the crop is grown inhibits development of nematodes. Peanut would be a good rotational crop for fields infested with *M. incognita*, but a poor choice if *M. arenaria* or certain races of *M. javanica* are present. Developing rotation sequences can be challenging when multiple nematode species, including multiple species and/or races of RKN, are present in the same field (Mitkowski and Abawi, 2011).

Crops including maize, oats, rice, grain sorghum, peanuts, sugarcane and wheat are considered non-hosts to reniform and are usually effective in a crop rotation with soybean (Hartman *et al.*, 2015). Production of non-host crops for 2 or more years can usually reduce even excessively high reniform populations below damage threshold levels to allow for the successful production of soybeans for 1 year. Fields need to be maintained weed free to prevent reniform reproduction on alternative hosts.

Resistant cultivars

There are RKN and, to a lesser extent, reniform nematode resistant cultivars available for use in the southern US. Planting a resistant cultivar generally results in yield improvement of 10–25% depending on the severity of the problem (Kirkpatrick *et al.,* 2014). Growing a resistant cultivar usually leads to a decline of the nematode population. Conversely, some cultivars are moderately resistant or moderately susceptible to RKN, and these may result in a significant yield improvement if nematode pressure is not excessive. However, most of these cultivars also allow RKN to reproduce at near normal rates, so populations remain high and may pose a risk for the following crop.

Nematicides

Chemical nematicides have successfully been used to decrease at-planting populations of nematodes and increase yields, but the cost of this practice is prohibitive for soybean production in the US (Hartman *et al.*, 2018). Several nematicidal seed treatments are labelled for use against nematodes on soybean. Seed treatments usually provide for a reduction of early root penetration of the young seedling and appear to have a variable effect on increasing yield, but rarely provide season-long control to protect the next crop in the rotation.

Cover crops

Cover crops can be grown outside of the normal agricultural growing season, and some are antagonistic to nematodes (Gill and McSorley, 2017). Cover crops are mainly planted to decrease soil erosion and improve organic matter levels and soil quality with a potential added benefit of suppressing weeds, insects and plant pathogens (Mitkowski and Abawi, 2011). There are a variety of cover crops adapted for cultivation in the southern production regions: cowpea, sunn hemp, sorghum-sudangrass, hairy indigo, sesame and some grasses have most commonly been used as summer cover crops to keep RKN populations at lower levels (Gill and McSorley, 2017). Which cover crop to choose depends on the nematode present in a field. For example, some cultivars of cowpea were effective against some species of root-knot but susceptible to others, and cowpea is a host of reniform.

Many cultivars of sorghum and sorghum-sudangrass are effective in reducing populations of RKN. Sunn hemp is a versatile crop that helps reduce populations of both RKN and reniform. Rye is a commonly used winter cover crop and is a poor host of RKN. Crop mixtures have also become popular, but choosing the right mix is critical when trying to manage RKN (T. Faske, Arkansas, 2020, personal communication). Cereal rye, black hulled oats, Cahaba white vetch and red clover are poor hosts of RKN, whereas wheat, oats, barley, crimson clover, Austrian winter peas, turnips and hairy vetch are good hosts.

In summary

Many growers are unaware they have a nematode problem until it becomes obvious, either through clear symptom expression or when they notice yields decreasing over time. Soil testing for nematodes at the end of season is not always practiced unless a nematode problem is suspected. The lack of clear identification of the species of RKN present is a serious problem for an effective management programme. Crop rotation is used by most growers; however, following an effective crop rotation programme can be challenging when farmers are confronted with erratic commodity prices, multiple pests, limited alternative crops, added equipment cost and lack of nematode resistant cultivars.

Optimization of nematode management

There does not appear to be major change on the horizon in crop rotation alternatives in the southern US. More adventurous growers try to incorporate specialty crops into their operations such as industrial hemp, malting barley, sunflowers, cowpea and sesame, among others to take advantage of niche markets or potentially emerging industries. In some cases, such as with sesame, cowpea and sorghum, there can be a benefit in reducing nematode populations when using these non-host crops.

Growing resistant cultivars is both effective and economical for nematode management but few cultivars are currently available especially for reniform nematodes. Additional sources of resistance are needed to reduce the loss of current cultivars.

Extension educators are fewer in number and less specialized, therefore new methods of communication must be utilized to meet grower demands. The COVID-19 pandemic forced extension personnel to adjust to new interactive platforms such as Twitter, Facebook, Podcasts, web-based newsletters and webinars to capture the attention of growers to educate them on current and developing issues in a non-traditional style.

Extension educators must do a better job of highlighting reniform nematodes as a threat in soybean production. Reniform is feared as a pathogen of cotton in the south, but many growers plant soybeans as a rotational crop with cotton unaware that the nematode will cause yield loss to the crop, and that populations will build up on soybeans during the season, increasing the risk of economic losses if the following crop is cotton.

Future research requirements

Adding advanced genetic techniques such as high-density genetic mapping of plant parasitic nematodes to breeding programmes may introduce new sources of resistance and increase the genetic basis for adapted soybean lines. In addition, using remote sensing techniques such as field spectrometry and aerial UAV hyperspectral imaging can be a valuable tool for improving breeding efficiency by facilitating monitoring of multiple crop traits (Oerke *et al.*, 2010; Joalland *et al.*, 2018). More information can also be found in Chapter 58 of this volume.

A method to perform molecular diagnosis of nematodes directly from soil samples without nematode extraction is available, but there are problems with accuracy of results and processing costs (Shah and Mahamood, 2017). The future of nematode taxonomy and diagnostics are dependent on molecular-based tools that will discriminate nematode species and races allowing for more focused management strategies (see Chapter 56 in this volume).

Improving upon current nematicidal seed treatments is needed to extend the longevity of activity in the root zone beyond 2 to 3 weeks and to prevent delayed nematode infection processes. Combining chemical seed treatments with biological products could help inhibit nematode penetration in the rhizosphere for longer periods of time and thereby increase crop yields.

Development of environmentally safe foliar nematicides that move basipetal to the roots along with novel methods of precision application should be considered. This can incorporate tractor-mounted or drone technology for scouting fields and eventually applying foliar nematicides to at-risk zones in a field. Site-specific aerial application of foliar nematicides using drones could be used to spot treat fields where nematodes are problematic during the season. This technology is currently being used on a limited basis for fungicides on multiple crops. Robotics has also progressed rapidly this century, and the idea of using robots to scout and sample for nematodes and apply pesticides in a precise area is not far off in the future.

Drone technology has become more affordable for some growers. Drones equipped with field spectrometry and aerial UAV hyperspectral imaging are being used for field scouting to efficiently detect field disorders, allowing growers to respond to problems more quickly. This technology will undoubtedly improve nematode management in the future as discussed in the Chapter 59 in this volume. This becomes a cost-effective way to conduct close-grid field testing using plant damage in the previous crop to focus soil sampling efforts in advance of rotations.

Technology is now available to detect changes in soil texture that supplies a map of relative probability of poor yields due to soil type and textural variability (see Chapter 59 in this volume). This technology would benefit greatly if we could layer soil texture, ground-proof nematode numbers and add historical yield maps for a field (J. Mueller, Clemson University, 2020, personal communication).

Practical methods need to be developed to identify and harness more effective biological agents within nematode-suppressive soils. These biological agents would include predators and pests of nematodes such as nematode-trapping fungi. Industry would then need to find an economically sustainable way to mass produce these organisms. A key would be to produce biological products that could survive through multiple seasons and cropping cycles.

Outlook: anticipating future developments

Climate models predict wide-ranging impacts on local temperature and precipitation patterns, with broad implications for crop yields, crop-water demand, water supply availability and farmer livelihoods (Singh and Prasad, 2016). I visualize mild winters, earlier springs and warmer temperatures during the summer, which will extend into a protracted autumn. These, combined with more tropical storms and an increase in drought stress, suggest global warming will pose a multi-faceted challenge to soybean growers in the southern US.

These dynamic changes in seasonal temperatures will negate the current decrease in population levels of nematodes during winter months because of warmer soils. Nematodes will also become active earlier in the year and stay active longer after harvest, possibly adding another generation to their life cycle. Warmer temperatures will also increase weed populations and possibly make them more diverse, offering alternate hosts to RKN and reniform before and after a crop when growers are more apt to abandon weed control measures. Changes in temperature and precipitation can also alter persistence, availability, and reduce toxicity of chemical nematicides which may influence their efficacy.

Climate change will increase the likelihood of drought stress which would lead to more severe symptoms and damage from nematodes. Increased water stress diminishes plant vigour and can modify plant physiology lowering plant resistance to nematodes (Singh and Prasad, 2016). However, a more serious threat may be the increased selection pressure resulting from acceleration of nematode development and increase in number of generations per season (Singh and Prasad, 2016). Researchers are currently developing drought resistant crops and hopefully resistance to nematodes will be part of the genetic traits incorporated into these cultivars.

Climate change will alter the spatial and temporal distributions of nematodes, likely extending southern RKN and reniform distribution northward into the mid-west, joining the soybean cyst nematode as a three-headed monster, challenging growers in this region of the US.

INM methods for nematode control, including resistant cultivars, diverse crop rotations, trap crops, green manures and biological agent amendments, take on greater significance under a changing climate. An extended growing season could allow growers to plant earlier or later depending on the crop. This would allow for the inclusion of trap crops or biofumigant crops before or after the soybean crop. There is no doubt that climate change will make growing profitable soybeans more challenging especially when dealing with RKN and reniform nematodes in the southern US.

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19 Integrated management of root-knot and other nematodes in food legumes

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Introduction

Food legumes represent a broad group of crop species and types, grown primarily as a fresh vegetable for the green (immature) pods or for dry grain harvested at pod maturity. In some crops, the young leaves of legumes such as cowpea in Africa are eaten as a spinach-style vegetable. While description and discussion of each food legume and their nematode management must be limited here, from a list of important food legume crops including bambara groundnut, black-gram, broad bean, chickpea, green-gram, lentil, moth bean, mung bean, pea, pigeonpea and runner bean, some examples will be highlighted; however, the primary focus herein is integrated nematode management (INM) in cowpea (black-eyed pea or southern pea, *Vigna unguiculata*), common bean (*Phaseolus vulgaris*), and Lima bean (*P. lunatus*). Food legumes are mostly damaged by root-knot nematodes (RKN, *Meloidogyne* spp.) as the major global nematode problem. Some relatively minor or more local associations of damage occur from lesion nematode (e.g. *Pratylenchus scribneri*) on Lima and common bean, reniform nematode (*Rotylenchulus reniformis*) and spiral nematodes (*Scutellonema cavenessi* and *S. clathricaudatum*) on cowpea, stem and bulb nematode (*Ditylenchus dipsaci*) on broad bean, and pea cyst nematode (*Heterodera goettingiana*) on pea and broad bean (Sikora *et al.*, 2018). Among these more localized nematode–legume interactions, quantifying crop loss has not been achieved with clear definition.

Economic importance

Cowpea, common bean and Lima bean suffer significant yield loss from RKN. The damage by RKN is prevalent in most growing regions and crop loss estimates are available. In the author's experience, RKN on cowpea in California, USA, affects about 20% of the planted area, limited to coarse-textured soils, and is frequently compounded by fusarium wilt disease. Losses in fields infested with *M. javanica* or *M. incognita* can range from a few per cent to more than 50% of normal yield. In the Sahel of West Africa, surveys of farmers' cowpea fields in Burkina Faso, Nigeria and Senegal revealed a high prevalence of RKN (100% of 248 fields infested in Nigeria, and about 50% in the other countries) (Sawadogo *et al.*, 2009). Problems were associated mostly with wetter production zones, or where irrigation is used on high-value vegetable cash crops such as tomatoes, peppers or aubergine grown in rotation with cowpea. In Burkina Faso, the

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potential for damage from *Meloidogyne* spp. to cowpea increased from the dry Sahelian semi-desert zone in the north (annual rainfall <600 mm/year), through the north-central Soudanian zone (annual rainfall of 600–800 mm/year), to the wet Soudanian zone (annual rainfall ≥1000 mm) in the more humid south-west (Sawadogo *et al.*, 2009). A significant increase in the frequency (56% of soil and 89% of root samples) and abundance of RKN in the humid south-west established it as the most prevalent nematode in cowpea fields in this region, even though *Pratylenchus*, *Helicotylenchus*, and *Scutellonema* were present. All cowpea fields sampled in Nigeria, where the northern cowpea zone is not as dry as in Burkina Faso, were positive for *Meloidogyne*, with *M. incognita* and *M. javanica* being the primary species present (see Sawadogo *et al.*, 2009).

Losses in common bean from RKN have been documented to range up to 60% of normal yields across different production settings (see reference to Ngundo and Taylor, 1974, cited in Omwega *et al.*, 1990). Losses to Lima bean in California are similar to that described for cowpea, where infested fields can lose up to 70% of yield (Ogallo *et al.*, 1999; Roberts *et al.*, 2008).

The reniform nematode (RN) is reported to attack cowpea in the southern USA and in the Sahelian region of sub-Saharan Africa, although the magnitude of the problem is not well known. A survey of cowpea fields in Burkina Faso revealed about 10% frequency of RN in soil samples but none in root samples (Sawadogo *et al.* 2009). A screening of a cowpea germplasm core collection revealed susceptibility to RN across the whole collection, with no resistance identified (Roberts *et al.*, personal communication).

In some food legume systems, cyst nematodes are of limited regional or local importance. The most well studied of these is pea cyst nematode, *H. goettingiana*, on pea (*Pisum sativum*) and broad bean (*Vicia faba*) which is a problem in several countries in Europe and in the USA, where pea yield losses correlate with pre-plant population densities in soil (*Pi*), and losses of 20%, 50% and over 85% were recorded. A second example is the pigeonpea cyst nematode, *H. cajani*, which attacks pigeonpea and cowpea in India and other parts of South-east Asia, but for which the extent of crop loss is unknown (Sikora *et al.*, 2018).

Host range

Unfortunately for nematode management considerations, all the main RKN species and RN have very broad host ranges across multiple plant taxa, extending to thousands of plant species (unlike cyst nematodes that typically have narrow host ranges). Broad host ranges severely limit options for deploying non-host crops in rotations with food legumes, limit application of cover cropping, and generate nematode management problems by maintaining or increasing nematode soil population densities by reproduction on weed hosts and volunteer plants from previous crops in food legume fields.

Distribution

RKN infestations are typically associated with coarse-textured sandy loam and sandy soils that favour the nematode biology with their quick draining, well-oxygenated profiles, and which exacerbate damage from infection due to higher plant water and nutrient deficits in such soils. In the Central Valley of California, USA, these RKN-conducive soils represent on average about 15–20% of the planted field areas within the region. Within fields, sandy areas associated with old river or stream beds or sandy patches in fields are often quite visible, where RKN symptoms of poor plant stand and unthrifty infected plants are evident.

Symptoms of damage

Above-ground plant symptoms of RKN and other nematode damage are not usually distinct or diagnostic, involving stunting, wilting, yellowing of foliage and general lack of vigour; in other words, symptoms that also can be attributed to inadequate water or fertilizer regimens. Typical symptoms of RKN infection below ground are distinct and diagnostic, with prominent 'root galling' (Figs 19.1 and 19.2) resulting from swelling of root cortical tissue around feeding sites housing the nematode-induced complex of giant cells. In maturing infected root systems, secondary fungal root rots are often present in galled tissue, stimulated by leakage of root exudates from galled tissue into the rhizosphere, which in turn act as attractants for fungal infection and also as

Fig. 19.1. Field-grown cowpea root systems infected with root-knot nematode, *Meloidogyne javanica*, showing severe galling symptoms in a susceptible cultivar (right) compared to minimal galling in a resistant cultivar (left). Author's own photograph.

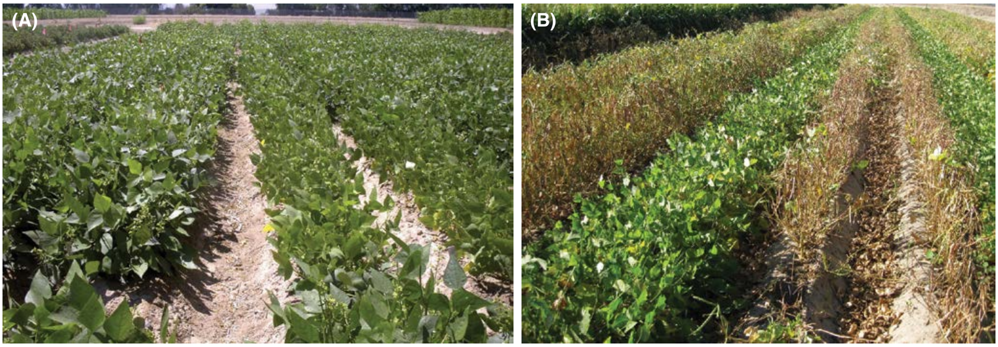

Fig. 19.2. (A) Growth differences at mid-season in Lima bean caused by infection from root-knot nematode, *Meloidogyne incognita*, in field plots in Orange County, California, USA. Note healthy growth of resistant cultivar (two rows on left) versus poor growth of susceptible cultivar (two rows on right). At harvest, the resistant cultivar had 80% higher yield than the susceptible cultivar. **(B)** Growth differences at harvest in near-isogenic lines of cowpea caused by infection from root-knot nematode, *Meloidogyne javanica*, in field plots in Orange County, California, USA. Note healthy growth of resistant line (two rows on left) with heavy pod load versus susceptible line (two rows on right) with reduced pod load. The two-row plots are separated by a single border row of Lima bean – bright green foliage). Grain yield was reduced by >50% in the susceptible line. Author's own photographs.

stimulants for sporulation and hyphal growth. Association with soil-borne fungal pathogens is also problematic, including with fusarium vascular wilts and ashy stem blight or charcoal rot caused by *Macrophomina phaseolina*.

Biology and life cycle

Driving the damaging effects of RKN on food legume crops are the multiple generations per season, especially in warm temperate, subtropical and tropical production regions. Warm soil temperatures drive multiple generations per cropping season, with a generation completed in as little as 3 weeks and often resulting in three to four overlapping generations per growing season. In year-round cultivation of successive crops, even more generations per year can be produced for species such as *M. javanica* and *M. incognita*, where soil temperatures between about 18°C and 35°C are ideal to drive the nematode life cycle. A second important feature contributing to RKN success in infecting legume crops is survival capacity between crops such as in fallow breaks. Under these conditions, even though up to 90% population density decline has been documented to occur between autumn harvest and spring planting of the next crop, the residual population levels in spring (the new *Pi*) can be devastating due to the final soil population densities (*Pf* values) in autumn being extremely high.

RKN species found in more temperate climates, such as *M. hapla*, are adapted to lower temperature regimens and produce fewer generations but can still be highly damaging (Chen and Roberts, 2003).

Interactions with other nematodes and pathogens

The co-infection of cowpea by RKN and fusarium wilt (caused by *Fusarium oxysporum* f. sp. *tracheiphilum*) forms a classic disease complex on cowpea resulting in devastating yield loss of up to about 80% in infested fields (Roberts *et al*., 1995). The nematode predisposes young plants to fungal infection, which kills seedlings and stunts or kills older plants. Numerous studies on this nematode–disease complex in field and greenhouse experiments document the problem (Roberts *et al*., 1995). Fortunately, good host resistance traits to both the nematode and fungus are available for breeders to develop resistant cultivars for managing either the pathogen alone or when together as a complex (see below).

Another interacting pathogen with RKN on cowpea is the soil fungus *M. phaseolina*, which causes ashy stem blight or charcoal rot. This disease can occur in the absence of RKN and is prevalent in water-stressed plants in rain-fed production systems such as in the drier zones of sub-Saharan Africa. It is less of a problem in irrigated legume crops. However, charcoal rot disease can be exacerbated by nematodes including RKN, and in the documented case of soybean, by soybean cyst nematode (*Heterodera glycines*). The extent to which this complex affects the broad range of food legumes is unclear, but the author anticipates a likelihood of increasing problems as hotter and drier conditions develop in our current production zones due to global warming progression.

Recommended integrated nematode management

Most food legume crops grown for dry grain are not high value and thus the margins between input costs, including for INM tactics, and crop profit are narrow. Thus, high-cost nematicides typically have not been used even in high-input developed farming systems (e.g. in California and much of the developed world agriculture). Fresh green bean vegetables for either fresh or frozen markets are exceptions to this and can support higher INM costs. Therefore, INM for the majority of food legume production relies on the integration of crop rotation, resistant cultivars (where available) and soil amendments, especially in ‘organic farming’ systems which is a growing sector for food legume production (Sikora and Roberts, 2018). Resistance developed in the food legume crop or in rotation crops can provide good protection from RKN, which is vital for successful rotations because non-host crops are limited. Resistance is available in common bean, Lima bean and cowpea. For example, common bean cultivars such as Nemasnap have good resistance to common RKN species including *M. arenaria, M. incognita, M. javanica* (Omwega *et al*., 1990) and *M. hapla* (Chen and Roberts, 2003), Lima bean cultivars UC92, resistant to *M. incognita,* and Cariblanco N, resistant to *M. incognita* and *M. javanica,* are available and grown in California (Roberts *et al*., 2008), while in cowpea several resistance genes control *M. incognita* and *M. javanica*, including virulent forms of those species. Hence, cowpea blackeye cultivars such as California Blackeye 46 (CB46), CB50, CB27 and CB5, and some southern USA types such as Pinkeye Purple Hull, Iron Clay and Nemagreen have resistance to *M. incognita* and in some cases to *M. javanica* (Huynh *et al*., 2015; Santos *et al*., 2018), and new R gene sources in African cowpea germplasm have been identified (Ndeve *et al*., 2019). The resistance in these crops is highly effective in protecting root systems and yield.

As another component of INM, earlier work demonstrated how effective resistance in rotation crops, such as cotton, can be in protecting a succeeding crop of legume such as susceptible Lima bean, by reducing the soil population density of *M. incognita* after just one season.

Likewise, resistant cowpeas grown as a cover or green manure crop or as the previous year's main crop were shown to be highly effective in protecting a following susceptible vegetable crop such as tomato, again by suppressing the soil *Pf* of *M. incognita* or *M. javanica* in the first year (Roberts *et al*., 2005). Combining these types of rotations with resistant cultivars of the target or rotation crop provides the central tactic for INM. When combined with careful selection of fields with lower infestation levels of RKN by employing pre-plant sampling surveys and boosting the organic matter content of field soils to improve soil microbial populations and overall soil health, then one has a three-component INM programme. The strong efficacy shown against RKN in these crops by the new generation of fluorine containing nematicides, which also have low mammalian toxicity, may offer an additional tactic for the INM toolbox.

Typical INM recommendations in the Pacific North-west USA for pea cyst nematode, for which resistant pea cultivars are unavailable, include avoiding fields with high nematode *Pi* (assessed by pre-plant sampling), employing long 4 to 5+ year non-host rotations between pea crops, delaying planting in spring until soil temperatures are at least above 18°C to suppress nematode infection and reproduction, and cleaning by pressure-hose all field equipment to prevent spread to non-infested fields. Good data from field and controlled experiments have established damage thresholds and functions relating relative yield to nematode *Pi* values, for use in guiding field selection and other INM options and decisions.

Optimization of nematode management and future research requirements

Optimization of these INM approaches can come through greater knowledge and demonstration of efficacy through applied field experiments. A major boost to plant breeding programmes to develop better yielding cultivars with RKN resistance, including stacking multiple R genes for broader and more durable resistance, is a key to future success. Possibly the new gene-editing tools based on CRISPR/Cas9 and other technologies ultimately may provide novel and quicker development of resistant food legumes. Certainly, the explosion of molecular marker-assisted breeding approaches is expediting resistant cultivar development. The accelerating trend toward organic farming systems, as seen in the USA and other developed country agriculture, will no doubt help to boost general soil health and the microbial balance needed to suppress RKN and other plant parasitic nematode (PPN) populations. Organic growers in California are reporting fewer problems with RKN than are typical in conventional high-input farming, even on highly sensitive crops such as carrot. This trend is considered to be due to careful field selection following preplant sampling and to improved soil health, which is achieved through organic amendments such as manures, crop debris (e.g., almond and walnut hulls) and incorporated green manures, and cover cropping. A major research focus on soil and rhizosphere microbiomes will no doubt promote improvements to soil and root health and to PPN suppression in food legumes.

Outlook: anticipating future developments

The overarching challenges to future food legume production will be management of abiotic stresses from water deficits and high growing season temperatures due to global warming trends. These stresses on plants will exacerbate damage inflicted by nematodes because they typically weaken plant stress response, and root damage from nematode infection diminishes water uptake efficiency. I also predict there may be an expansion of infestation distributions of warm-climate RKNs into higher latitudes as regional temperature patterns shift. Demands by consumers for safe food supplies are pushing crop production systems toward low-input organic farming systems where inorganic fertilizers and conventional nematicide, pesticide and herbicide applications must be excluded. These trends will magnify the need for the types of INM outlined above where reliance on synthetic chemistry solutions for nematode management will become less accepted due to consumer demands. Coupled with this, genetic modification of crop plants by transformation and gene-editing technologies to create novel resistance and tolerance phenotypes of crops for use in INM will no doubt come under considerable scrutiny. Likely rejection from a society of consumers who have little confidence in industrial scale science solutions for food production presents a significant challenge, and efforts to push harder on delivering naturally bred resistant crops will increase in importance.

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20 Sustainable management of major nematode parasites of chickpea and broad bean in the Mediterranean region

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Introduction

Food legumes are in great demand for enriching the protein content of human and livestock diets. Today, several of them are ingredients of Mediterranean specialties of high nutraceutical value and are listed on the menu of five star restaurants. Because of the association of their roots with nitrogen-fixing *Rhizobium* spp., their cultivation does not require nitrogen fertilizer and enriches the soil with nitrogen for the benefit of the following crops. Usually, legumes are used as dried grains and some, such as broad bean and pea, as green grains.

In 2018, a total of 2,695,068 ha was cultivated to legumes (except soybean) in the Mediterranean basin, mainly in rather marginal lands and under rain-fed conditions, especially in dry areas. Legume crops require low-capital input. They produce low-yield (1.12–1.44 tonnes/ha for chickpea, lentil and broad bean) resulting in low proceeds per hectare (<US$2,000/ha) for the farmers.

Chickpea

The area cultivated with chickpea in the Mediterranean countries totals 806,527 ha. Turkey, Morocco, Spain, Syria, Algeria, Italy and Greece are the main producers of the Kabuli type (creamy seeds). Chickpea has a rather deep root apparatus and therefore is cultivated in rather dry areas (about 350 mm rainfall per year). It is sown from mid-autumn to early winter (winter chickpea) or late winter (spring chickpea), the latter producing only 75% of the yield per ha obtained by winter chickpea. Harvest is in June at low altitude and much later on the Anatolian Plateau of Turkey and in the Beka'a Valley of Lebanon. Most damaging nematodes of the crop are the chickpea cyst nematode, *Heterodera ciceri* and the British root-knot nematode, *Meloidogyne artiellia*.

Broad bean

This is cultivated on 430,759 ha, especially in Morocco, Tunisia, Algeria, Egypt, France and Italy, producing an average of 2.4 tonnes grain/ha. It requires more rainfall (500–600 mm) than chickpea or supplemental irrigation. Generally, it is sown in November and harvested in June. The main nematodes damaging broad beans are *Ditylenchus* spp.

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Economic importance of the nematodes

Beside causing direct damage to host plants, nematodes may interact with other soil-borne pathogens increasing their severity and suppressing *Rhizobium* root nodulation. In the dry areas, as nematodes reduce root development and efficiency, they also increase drought stress.

In general, farmers are unaware of the nematode damage and a true estimate of damage caused by nematodes to legume crops in the Mediterranean basin is lacking. Some information about each of the main nematode species is provided hereafter.

Heterodera ciceri

The nematode reduces grain nutritional value, suppressing 20% of protein content. Yield loss of chickpea starts to occur at nematode densities above 1 egg/g soil and 20%, 50% and 80% yield losses are expected when sowing chickpea in soil infested with 4.7, 14.2 and 33 eggs of the nematode/g soil, respectively. The graphs in Fig. 20.1 relate the ratio *Pi*/*T* (*Pi* = nematode soil population density before sowing; *T* = tolerance limit of chickpea to the nematode) with % yield and are useful to estimate expected yield loss for making management decisions; they are derived by fitting the data obtained by properly designed microplot experiments with Seinhorst's equation (Greco *et al.*, 1988) and derivation of tables of nematode pathogenicity by Sasanelli (1994).

Meloidogyne artiellia

Yield loss of chickpea occurs at nematode densities of as low as 0.14 and 0.02 eggs and juveniles/cm^3 soil, for the winter and spring sown crop (Di Vito and Greco, 1988), with complete crop failure expected at 8 and 1 eggs and juveniles/cm^3 soil, respectively (Fig. 20.1).

Ditylenchus gigas, *D. dipsaci*, *D. oncogenus*

Ditylenchus gigas, earlier reported as the giant race of *D. dipsac*i, is listed as a quarantine organism by most countries. It can be destructive to broad bean especially during mild (15–20°C) and humid conditions with abundant rain, fog and dew. The tolerance limit of broad bean is about two nematode specimens/100 g soil. Plant infection originating from seeds is more severe than that from the soil. The stem and bulb nematode *D. dipsaci* damages broad bean to a lesser extent and invades mostly the basal plant parts. The economic importance of *D. oncogenus* is unknown.

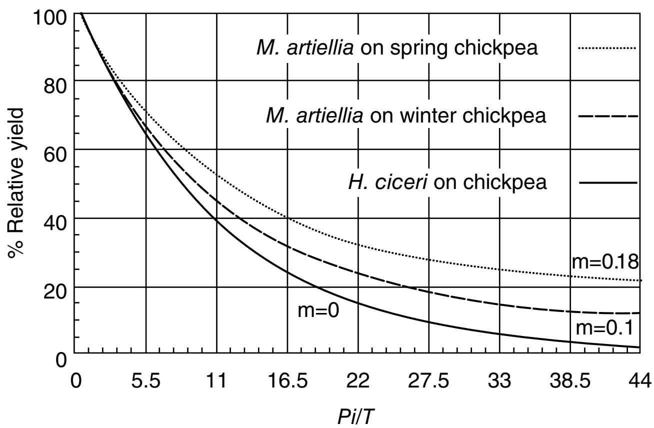

Fig. 20.1. Relationships between the ratio *Pi*/*T* for *Heterodera ciceri* or *Meloidogyne artiellia* and % relative yield of chickpea. Tolerance limits (*T*) are 1 egg/g soil for *H. ciceri* and 0.14 and 0.015 eggs and second-stage juveniles for winter and spring chickpea, respectively, for *M. artiellia*. Figure courtesy of Nicola Sasanelli, Italy.

Host range

The narrowest host range is that of *H. ciceri* and includes chickpea, lentil, pea, grass pea and, in some populations, also lucerne and *Medicago rigidula*.

The crops of economic importance parasitized by *M. artiellia* belong to the botanical families Leguminosae, Graminaceae and Brassicaceae (Di Vito *et al.*, 1985). Lupin, lentil, sainfoin and oats are poor hosts, and potato and strawberry non-hosts.

Ditylenchus species have rather large host ranges that may vary according to local populations and include about 500 cultivated and wild plants. In general, winter cereals, except oats, appear to be poor hosts, but several wild plants, such as *Avena fatua* and *Lolium perenne* may contain as many as 2930 and 16,130 nematodes per 10 g plant tissues, respectively, in February.

Distribution

Heterodera ciceri occurs in Turkey and more commonly in Syria (Hama, Idleb and Aleppo), Lebanon (Beka'a valley) and Jordan (Irbid Governorate in the North). It was not found in Algeria, Tunisia and Morocco.

Meloidgyne artiellia has been reported from several Mediterranean countries, including southern Italy (Puglia, Basilicata and Sicily) and north Syria where the most severe infestations have been observed (see Fig. 20.2).

Ditylenchus gigas is known to be widespread all over the Mediterranean area and elsewhere. *Ditylenchus dipsaci* is also widespread, while *D. oncogenus*, described from sow thistle in Italy (Brindisi), was also found in Algeria (Guelma) in broad bean with unknown economic importance.

Symptoms of damages

Chickpea plants severely infested with *H. ciceri* and *M. artiellia* show stunting, yellowing, early senescence and low yielding (Fig. 20.2). Roots of chickpea infected with *H. ciceri* bear white or yellow females and brown cysts from flowering onwards, while those attacked by *M. artiellia* show large egg masses but small or no galls.

Ditylenchus gigas and *D. dipsaci* do not parasitize roots but invade all aerial plant parts, causing brown spots, distortions and swellings (Fig. 20.3). Pods are also distorted while mature seeds show necrotic and discolored spots. *Ditylenchus gigas* infects even the top of the plant while *D. dipsaci* more the stem base.

Fig. 20.2. Yellowing of chickpea in Syria caused by *Meloidogyne artiellia* and/or *Heterodera ciceri*. Inserts: left, females of *H. ciceri* in the roots; right, very small galls and egg masses of *Meloidogyne artiellia* in the roots. Author's own photographs.

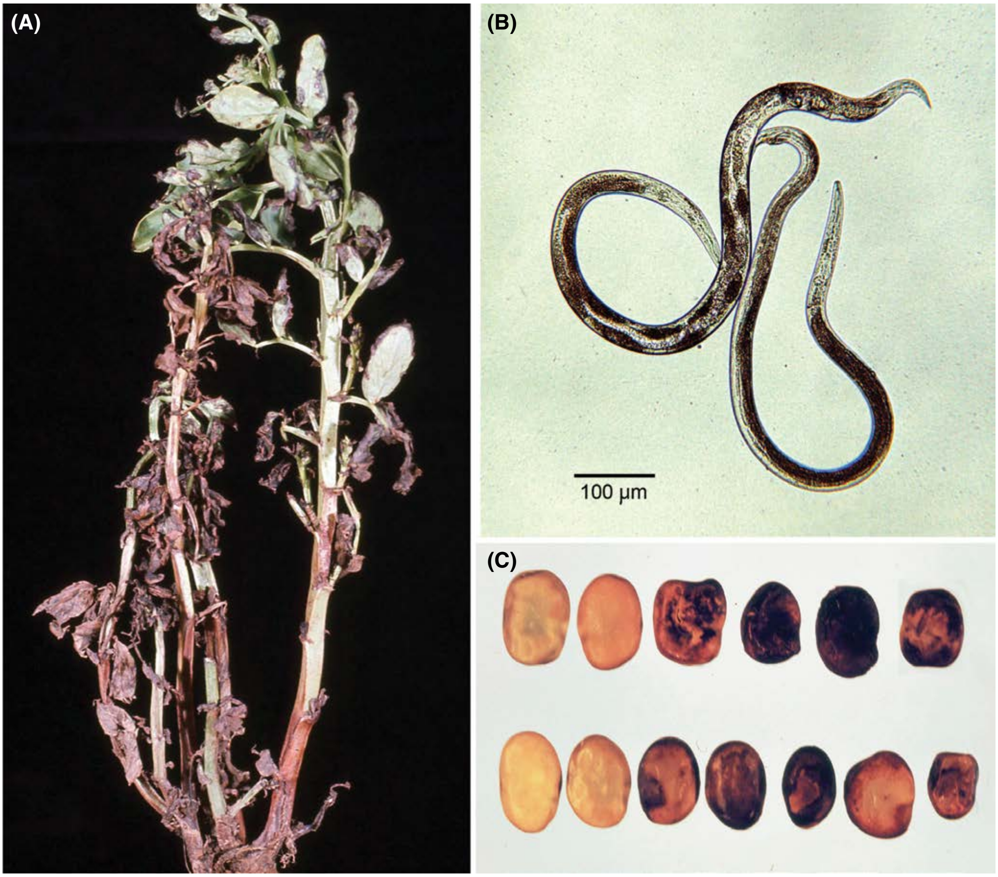

Fig. 20.3. *Ditylenchus gigas*. **(A)**: A broad bean plant showing brown spots, distortions and swellings caused by the nematode; **(B)**: vermiform specimens of the nematode; **(C)**: healthy (left) and infected (right) seeds of broad bean showing discoloration due to nematode infection. Images courtesy of F. Lamberti and A. Troccoli, Italy.

Biology and life cycle

Heterodera ciceri

Survival is by eggs in cysts from which second-stage juveniles (J2) hatch at suitable soil temperature (15–25°C) and soil moisture content and in the presence of the host plant. At plant emergence, roots are already invaded by J2, while white/yellow females (averaging 773 and 451 µm, L × W) appear on the roots after an accumulation of 212–227 day degrees (basal temperature 10°C) by mid-March (winter chickpea) to mid-April (spring chickpea). Only one generation per year is completed. Reproduction rates at low population densities vary from 297 to 4.5 folds on winter and spring sown chickpea, respectively. Although no information is available on nematode decline in the absence of a host, it should average 80–95% yearly like other cyst nematodes in the area, such as *Globodera rostochiensis*, *G. pallida* and *Heterodera schachtii*.

Meloidogyne artiellia

The short J2 have body and tail of 350–360 and 18–26 µm long, respectively. In the Mediterranean coastal areas, they invade chickpea roots in winter, at 10°C, developing into females with large egg masses by early April. Development is slow at 10°C and 30°C and reproduction is greater on winter chickpea (up to 55.5 fold) than on spring chickpea (3.4 fold). Only one generation per crop cycle is completed after an accumulation of 230–240 day degrees above 10°C. After harvest in June, the nematode population declines to 55–59, 13 and 3% at the end of July, mid-November and after 15 months, respectively. Nematode survival is by eggs in hardened egg masses, if no rain occurs after their formation, and/or coiled anhydrobiotic J2. Anhydrobiotic juveniles return to activity after rains and invade roots when the temperature becomes suitable (optimal 20–25°C).

Ditylenchus gigas

This is a vermiform (Fig. 20.3) migratory endoparasite with a body longer than that of *D. dipsaci* (1500–1900 versus 1200–1300 µm, respectively) and can be destructive especially during mild (15–20°C) and humid conditions with an elevated amount of rain, fog and dew. High temperature (>25°C) and drought suppress nematode development and damage. The nematode attains several generations per crop cycle, each taking about 19–23 days at 15°C. Very high reproduction rate (1000×) can be expected especially if plants remain wet for several hours because of abundant rain, fog and dew. At the end of the crop cycle, soil nematode densities decline rapidly, reaching undetectable levels in sandy soil. It survives during cool, hot and dry periods in soil, plant residues and seeds as quiescent fourth-stage juveniles, which become activated by the rainfall and low temperatures in the autumn.

Interactions with other nematodes and pathogens

In general, there is little chance for plant parasitic nematodes to develop and reproduce in the same root already parasitized by a very aggressive nematode. However, this author has commonly found high infestations of *H. ciceri* or *M. artiellia* associated with *Pratylenchus* spp. in chickpea roots, and concomitant large numbers of *D. gigas*, *Heterodera goettingiana* and *Pratylenchus* sp. in broad bean stands. Damage by *M. artiellia* can be compounded by concomitant infection of *Fusarium oxysporum* f.sp. *ciceri* race 5.

Recommended integrated nematode management (INM)

No specific nematode management recommendations for chickpea and broad bean are made by extension agencies. The only agronomic practice recommended is crop rotation with cereals, other cool season crops and fallow, regardless of its effect in mitigating the nematode problems. Nematode management on these legumes, usually, is not implemented even in Europe where there is a need for the inclusion of provisions regarding nematode parasites of legumes in integrated pest management guidelines.

Optimization of nematode management

It is necessary that actions are implemented to make agriculturalists and farmers aware of the importance of nematodes, their concomitant occurrence with other soil-borne problems, and of the most effective methods of management (see also Chapter 36 in this volume). Because of the extensive area of their cultivation and low profit, the integrated management of nematodes must rely on agronomic rather than on chemical approaches. However, precise identification of the nematode species is a pre-requisite for an appropriate control strategy. In the absence of the crop, the only way to determine nematode species and population density in the soil is by collecting soil samples, which should be processed by an accredited laboratory to obtain the nematode number per g or cm^3 soil. Based on these results and the graphs in Fig. 20.1, a good estimation of the expected damage can be obtained along with the appropriate management strategy that can be adopted.

More expensive management tools can only be used if the soil nematode population density at sowing is larger than the economic threshold for the crop. This threshold corresponds to the level of nematode population at sowing at which the value of the yield increase following the treatment equals the cost of the treatment; it varies greatly according to market price of the grain yield and cost of the different control options. The best approach would be applying a control option at the nematode soil density at sowing at which the difference between the value of the yield increase and the cost of the treatment is the largest (level of maximum economic return) (Ferris and Greco, 1992).

In the presence of a crop, observation of the roots, from flowering onwards, may give good information on the presence/absence of nematode infection and it is highly recommended. If the roots show galls, the causal nematode is quite certainly a species of *Meloidogyne*; if lemon-shaped sedentary white or yellow specimens are observed, then they are of a species of *Heterodera*. For broad bean, if stems, leaves and pods show discolorations and distortions the causal agents could be a species of *Ditylenchus*. In any case, a close examination of the nematode specimens by an experienced nematologist is necessary for identification to species level. It is highly recommended that these observations are made on the previous crop to forecast the impact the nematodes may have on the following host crop.

The most important nematodes, along with appropriate methods of control, are discussed hereafter. More information can be found in Sikora *et al.* (2018).

Heterodera ciceri

This can be efficiently controlled by long crop rotations in which chickpea should follow non-host crops after 3 to 4 years. Deep ploughing the soil two to three times in summer after harvest would increase nematode decline because of the killing effect of high summer temperatures. Control by granular nematicides and soil solarization and fallow are effective but not feasible because of the low value of the crop. No chickpea cultivar resistant to *H. ciceri* is available.

Meloidogyne artiellia

The control of *M. artiellia* is more difficult due to its rather large host range. However, deep ploughing in summer and growing chickpea once every 3 to 4 years after summer crops, such as tomato, cotton, maize, sunflower, melons or other cucurbits, and non-host cool season crops such as sugar beet, would strongly suppress nematode soil populations.

Ditylenchus gigas and *D. dipsaci*

Plant infection originating from seeds is more damaging than that from the soil. The use of certified seeds free from nematodes, wide spacing between plants, weeding, sowing in windy areas and avoidance of sprinkler irrigation are prerequisites for a successful broad bean crop. Dipping seeds in cool water for 2 to 3 hours and then in warm water at 58–60°C for about 15 minutes, before drying them in the shade, will kill most of the quiescent nematodes. Crop rotation with winter and summer non-host crops followed by fallow from fall to mid-spring is highly recommended. Other control means may not be convenient for grain production but can be profitable for the production of green pods or certified seeds.

No cultivar of broad bean with good agronomic traits that is resistant to nematodes is available. However, some local cultivars from North Africa and Syria have shown some degree of tolerance/resistance to *D. gigas*.

Future research requirements

The yield potential of chickpea has been estimated at 6 tonnes/ha (Varshney *et al*., 2017, in Zwart *et al*., 2019), but because of several adverse factors the world average is less than 1 tonne/ha. No breeding programmes are in progress in the Mediterranean area to introgress nematode resistance genes in cultigens of legumes. However, there is awareness that genetic control by tolerant and resistant varieties is the best long-term approach (Thompson *et al*., 1999). Resistance sources have been found in lines, plant introductions and wild relatives of many legumes (Zwart *et al.*, 2019). Unfortunately, most of these sources are in species not crossable with cultivated species using classical breeding techniques. Therefore, more sources of resistance should be explored and new techniques of genetic engineering should be fostered to transfer multiple resistance in to legume cultigens.

Outlook: anticipating future developments

Climate change will certainly affect nematode spread and damage. If the increase in average ambient temperature continues in the future, the warm season root-knot nematodes *M. arenaria*, *M. incognita* and *M. javanica*, present in the region, are expected to extend their distribution, increase the number of generations per year and nematode populations in the soil and, therefore, their damage to crops even in areas where today they do not constitute a problem for cool season legumes. On the other hand, *M. artiellia*, *H. ciceri* and species of *Ditylenchus* would become less aggressive because of their intolerance to elevated temperatures.

The Mediterranean diet, of which chickpea and broad bean are important protein components, has been selected as a United Nations Intangible Cultural Heritage asset. The promotion of the Mediterranean diet should encourage the consumption of more legumes and their demand in the market, resulting in an expansion of their production areas and possibly in an increase in their price and revenue for farmers. The legume industry will benefit greatly if legumes are grown in fertile lands using precision agriculture techniques.

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21 Managing *Meloidogyne arenaria* in peanut with old and new tools in the south-eastern USA

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Introduction

The peanut root-knot nematode *Meloidogyne arenaria* is the most important nematode pathogen of peanut in the south-eastern US. Georgia is the number one producer of peanuts in this region, with 334,929 ha harvested in 2017. The neighbouring states of Alabama and Florida harvested 78,144 ha and 75,596 ha in 2017, respectively. Among the numerous diseases affecting peanut in Georgia, nematodes (primarily *M. arenaria*) were ranked second in importance based on percentage reduction in crop value for 2017, behind southern stem rot (*Sclerotium rolfsii*) and ahead of tomato spotted wilt virus (Little, 2017).

Economic importance of the nematode

Economic losses in peanut from *M. arenaria* damage were estimated at 4% of total crop value for Georgia in 2017. This translates into US$33 million in crop losses and an estimated additional US$8 million for the cost of managing this nematode (Little, 2017). In Florida, loss is estimated at 6% or US$10 million and an additional US$7.25 million in management. In addition to direct losses from the nematode, infection of peanut by *M. arenaria* can increase the risk of aflatoxin contamination which may lead to downgrading of farmer seed stocks and a lower price per tonne for farmers. When *M. arenaria* is left unmanaged, it can result in up to 50% yield loss for an entire field with some areas of the field experiencing 100% loss.

Host range

Meloidogyne arenaria has a broad host range which includes other crops common to the region, including some cultivars of soybean, tobacco, cowpea and most vegetable crops. Some winter cover crops are good hosts for this nematode; however, they are mostly legumes, which are not commonly planted prior to peanut. Small grains such as rye and wheat are more commonly planted; these are relatively poor hosts and populations of *M. arenaria* have not been found to increase on them in the field. Among the weeds known to be a problem in peanut, common lambsquarter (*Chenopodium album*), smallflower morning glory (*Jacquemontia tamnifolia)*, annual ragweed (*Ambrosia artemisiifolia*), Palmer amaranth (*Amaranthus palmeri*), sida

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(*Sida spinosa*), spotted spurge (*Euphorbia maculata*), common purslane (*Portulaca oleracea*), phasey bean (*Macroptilium lathyroides*) and cutleaf groundcherry (*Physalis angulata*) are all hosts of *M. arenaria* (Rich *et al*., 2009).

Distribution

Meloidogyne arenaria is the dominant root-knot nematode in peanut throughout the lower southeastern states; in the more northly range of peanut production, *M. hapla* is dominant. In Georgia and Florida approximately 25% of the peanut production area is infested with *M. arenaria* and in Alabama approximately 40% is infested.

Symptoms of damage

Root-knot nematodes often show a patchy distribution in abundance, with sandier areas of the field having higher nematode densities than less sandy areas, and this is reflected in both above- and below-ground symptoms (Fig. 21.1). Early in the season, above-ground symptoms of *M. arenaria* infection are subtle; plants can be stunted, foliage may appear less green and vines may be slow to cover the soil between rows compared to other areas of the field (Fig. 21.2). As the peanuts near maturity, heavily infected plants may be chlorotic, show premature wilting, and even death before harvest when conditions are hot and dry (Fig. 21.1).

Below-ground symptoms are more diagnostic of *M. arenaria* infection and include the formation of galls on the roots, pegs and pods (Fig. 21.3). In the first few months of infection, root galls will be discrete; however, as the season advances, galls begin to coalesce along the root in heavy infestations. Galls can easily be confused with nodules formed by the nitrogen-fixing bacteria (*Bradyrhizobium* spp.). Nitrogen-fixing nodules are uniformly round swellings attached to the root surface and easily detached, whereas nematode galls are irregular swellings of the actual root and cannot be removed without destroying the root. Galls on pegs and pods are more easily identified than galling on roots but may not always occur even though the roots are galled (Fig. 21.4). Galling on pegs results in pod loss during harvest and poor pod development.

Biology and life cycle

The second-stage juvenile (J2) and eggs are the only stages present in soil, except for an occasional male, and all other stages are within the root. The J2 is the infective stage of root-knot nematodes. After infecting a susceptible plant

Fig. 21.1. A peanut field with a severe *Meloidogyne arenaria* infestation late in the season with symptoms patchy in distribution. Photograph courtesy of Zane Grabau.

Fig. 21.2. Early symptoms of *Meloidogyne arenaria* infestation at mid-season in a severely infested field. Note yellowing stunted plants in foreground. Photograph courtesy of Zane Grabau.

Fig. 21.3. Galling on roots and pods from *Meloidogyne arenaria*. Photograph courtesy of Patricia Timper.

Fig. 21.4. Galling from *Meloidogyne arenaria* on pods and pegs of peanut. Photograph courtesy of Patricia Timper.

root, *M. arenaria* establishes a permanent feeding site and develops through two more juvenile stages before becoming an adult female. Males are only produced under some conditions and do not mate with females. Females produce their eggs in a gelatinous matrix which binds them together in a single mass containing between 200 to 350 eggs. Adult females begin to produce eggs 25 to 30 days after J2 infect roots and approximately four to five generations can occur during the growing season in the subtropical south-east region. Over the winter months, populations of J2 in soil decline substantially. However, the nematode can overwinter as eggs or J2 in soil or within the roots of cover crop or weed hosts. In the subtropics, it is estimated that two to three generations of *M. arenaria* can develop on an alternative host plant during the non-crop cycle from autumn to spring.

Interactions with other nematodes and pathogens

Meloidogyne arenaria interacts with *Cylindrocladium parasiticum* to increase the symptoms of cylindrocladium black rot (CBR) as well as plant mortality. In years with wet, cool springs, the CBR–nematode interaction can lead to considerable yield loss. However, this disease has not been a problem in recent years and may become less frequent with climate change. Infection of both roots and pods of peanut by *M. arenaria* has been shown to increase the risk of pre-harvest aflatoxin contamination, a potent and highly regulated carcinogen (Timper *et al*., 2004). This is a concern in non-irrigated peanut, as heat and drought stress are the primary risk factors for aflatoxin production by *Aspergillus* spp.

Recommended integrated nematode management (INM)

Predictive sampling is useful for determining whether *M. arenaria* is above the damage threshold and thus requires a management intervention. Soil samples for the nematode are typically collected in the autumn when populations of J2 are high. Because of winter attrition and poor extraction efficiencies, spring samples can result in an underestimate of nematode populations. Soil samples can be sent to a diagnostic laboratory specializing in nematode analysis. The damage threshold for *M. arenaria* in Georgia is 10 or more J2/100 cm^3 of soil based on samples collected in the autumn.

Crop rotation

For several decades, crop rotation has been widely used in much of the south-east for managing nematodes and other soil-borne disease in peanut. Cotton and maize are common and effective rotation crops for reducing populations of *M. arenaria*. Cotton is a non-host and maize is a poor host for the nematode. Pasture bahiagrass, a crop some growers have integrated into row crop rotations, is also a non-host. When these crops are planted between peanut crops, nematode populations decline to very low levels because of starvation in cases of a non-host or inability to produce enough eggs to sustain the population in cases of a poor host. While 1 year of a non-host crop can improve peanut yields compared to continuous peanut, a rotation that includes two years of poor or non-host for *M. arenaria* before planting peanut is recommended for both reducing nematode populations as well as soil-borne diseases. A weedy field or planting a susceptible winter cover crop may offset the benefits of crop rotation. The extent of grower adoption of rotations that effectively manage *M. arenaria* varies across the region. For example, in parts of north-central Florida, growers continuously plant peanut due to a lack of cotton processing facilities and deep sand, which is not conducive for maize production. Economics are typically the main barrier to lack of grower adoption of crop rotations; market price and other considerations are often more important than nematode management.

Nematicides

Nematicides are also widely used to manage *M. arenaria*. Older products such as the non-fumigants (granular or liquid formulations) aldicarb and, less commonly, oxamyl are still in use. The fumigant (applied as a gas) 1,3-dichloropropene (1-3-D) is also commonly used. Fluopyram was the first of a new class of non-fumigant nematicide chemistries (benzamides) to be brought to market for peanut, with other products in this class expected to become available in the near future. For organic labelled products, commercial formulations of live *Purpureocillium lilacinum* (synomym *Paecilomyces lilacinus*) have been available for several years and are known to provide some nematode control. Recently, commercial formulations of heat-killed *Burkholderia* bacteria have been labelled for nematode management in peanut but testing has been limited.

Nematicides are intended to temporarily reduce nematode populations and infection, protecting the crop from some damage and increasing yield. Nematicides often do not help with year-to-year nematode management because nematode populations often rebound by harvest after nematicides dissipate. In an ideal INM plan, nematicide application would be the last management choice when predictive sampling shows that nematode populations are above the damage threshold despite crop rotation, and planting a resistant cultivar is not an option. In practice, nematicide application is commonly used because it is flexible. Additionally, rotation and resistant cultivars for *M. arenaria* may not be effective against other common nematode pathogens of peanut such as lesion (*Pratylenchus brachyurus*) and sting (*Belonolaimus longicaudatus*) nematodes. Finally, non-fumigant nematicides can be deployed for management of multiple organisms–insects in the case of oxamyl and aldicarb and fungal diseases with fluopyram.

Fumigation with 1,3-D is typically the most effective nematicide available, although application requires specialized equipment and is more expensive than non-fumigants (up to US$370/ha in product cost alone). Aldicarb and fluopyram can also be effective and are typically less expensive than fumigation (US$100/ha for at-planting application or US$200–250/ha for both at-plant and post-plant applications). Application of non-fumigant nematicides is generally integrated with planting operations, reducing operations costs. Precision or variable-rate nematicide application to only regions of a field with high nematode pressure is one option for reducing application costs.

Resistance

In the last 10 years, peanut cultivars with a high level of resistance to *M. arenaria* have been commercially available. These cultivars often have high or moderate resistance to multiple diseases including tomato spotted wilt virus, increasing their usefulness to growers. However, resistance is not as widely used as it should be by growers, many of whom believe that planting non-resistant cultivars with higher yield potential and applying nematicides is more profitable than planting the resistant cultivars without the need for nematicides. Based on the 3-year (2017–2019) yield average from Georgia variety trials, the most widely planted susceptible cultivar (Georgia-06G) yielded 485 kg/ha more than the newest nematode resistant cultivar (TifNV-High O/L) and 724 kg/ha more than older resistant cultivars. Growers who practice good crop rotation and manage their weeds can reduce field populations of *M. arenaria* to low levels, allowing them to plant a susceptible cultivar with application of a nematicide; however, growers that have high populations of *M. arenaria* are recommended to plant a resistant cultivar or rotate with a non-host crop. In Georgia, even growers using resistant cultivars continue to rotate because of other soil-borne diseases. Resistant cultivars are not effective against lesion or sting nematodes, so in fields with damaging levels of those nematodes, integrated approaches are needed. Root-knot nematode resistant cultivars are estimated to be used on less than 5% of peanut acreage in the south-east based on certified seed production.

Optimization of nematode management

A wider adoption of resistant cultivars by growers is needed. As newer resistant cultivars with higher yield potential are developed, many more growers should be able to reduce the use of costly nematicides by planting nematode resistant cultivars. Moreover, research and extension are needed to demonstrate the economic and agronomic cost-benefit of planting a resistant cultivar at various *M. arenaria* population levels.

Increasing knowledge of nematode pressure, through traditional scouting or precision tools like remote sensing (drones) and soil mapping, as well as using that information to efficiently deploy nematode management, is perhaps the most important step in optimizing nematode management. Currently, many growers do not base nematode management decisions on evidence of nematode infestation (soil counts or infected plants), so nematode management practices are inefficient in terms of crop loss due to unmanaged nematode damage or, conversely, to unnecessary and costly management. More widespread and precise scouting of fields would allow growers to deploy nematode management practices that are appropriate for the infestation level in their field. Creating management zones within fields, corresponding to areas of high and low nematode pressure, could improve predictive sampling. Methods to create management zones include mapping soil properties (electrical conductivity can be mapped as a proxy for soil type using ground equipment), using remote sensing to map areas of differential crop health, and yield monitors to precisely map variable yield within a field. Creation of these management zones using precision tools could then be supplemented by predictive nematode sampling, divided by zone, to confirm current nematode pressure. The final step in this process is the grower deploying different management strategies (e.g. presence or absence of nematicide application) in different management zones within a field depending on nematode pressure.

Future research requirements

New nematode resistant cultivars that are higher yielding and have multiple disease resistance are needed for wider adoption by growers. Because there is a risk of populations of *M. arenaria* that can overcome the existing source of resistance developing in some field sites, new cultivars that incorporate additional sources of resistance such as the resistance found in the wild peanut species *A. stenosperma* are needed. Nematode resistance is currently only available in runner peanuts, which are the predominant variety grown in the south-east and are generally processed for oil or peanut butter. Future research should integrate nematode resistance into specialty peanuts such as Virginia and Valencia peanuts, which are grown on fewer acres but are high value.

Continued development and evaluation of new nematicides is needed and expected. Older nematicides present more toxicity risk than newer nematicides to handlers and the environment. This creates uncertainty about continued availability of older nematicides, thus the need for new nematicides to diversify or maintain options for growers.

Profitable rotation crops that are resistant to *M. arenaria* are needed. Cotton and maize have long been excellent options for rotating with peanut in the south-eastern USA; however, additional rotation crops would provide growers with more options. Bahiagrass, grain hybrids of pearl millet and sorghum, and perhaps bioenergy crops such as sweet sorghum with resistance to *M. arenaria* may be good options for some growers in this region. As other new crops emerge, such as the biofuel crop *Brassica carinata*, their impact on *M. arenaria* will need to be quantified.

Pasteuria penetrans is a bacterium parasitic on root-knot nematodes. The bacterium reduces infection of roots by the J2 and prevents egg production; infected females produce millions of bacterial spores instead of eggs. Although naturally present in the region, *P. penetrans* only reproduces when the nematode infects a host plant. Rotations with non- or poor hosts for the nematode prevent spore production and lead to low levels of the bacterium. High levels of *P. penetrans*, either naturally occurring or applied as a product, may permit rotations that include another host of *M. arenaria*. A yearly rotation of peanut, aubergine and maize resulted in intermediate levels of *P. penetrans* compared to continuous peanut with the highest level and a peanut, maize, cotton rotation with the lowest level (Timper, 2009).

More research is needed to identify INM options for organic peanut producers. Like conventional producers, organic producers currently rely on crop rotation and resistant cultivars to manage *M. arenaria*. However, these options will not suppress populations of lesion and sting nematodes. Organic producers need additional options such as biological control and rotation crops that can control multiple nematode pests.

Outlook: anticipating future developments

Peanut production areas may expand northward and *M. arenaria* may displace *M. hapla* in the northern production areas. The lack of water, both in terms of rainfall and irrigation, will be an issue for agriculture worldwide, and nematode infection increases water stress issues. Incorporating drought-tolerant rotation crops such as pearl millet and sweet and grain sorghum with resistance to *M. arenaria* may reduce water usage compared to cotton and maize rotations in a peanut cropping system. For growers in the south-eastern USA to remain competitive in the world market, continued efforts are needed to identify effective, low-cost and susceptible options for managing *M. arenaria*.

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22 The war against the pod nematode, *Ditylenchus africanus*, on groundnut in South Africa

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Introduction

Groundnut cultivation occurs under both rain fed and irrigated conditions in the summer rainfall areas of Southern Africa. These areas include the western and north-western Free State, North West, Northern Cape, Limpopo and Mpumalanga provinces. Both commercial producers and smallholder farmers cultivate this protein- (65%) and oil-rich (91%) legume, but for different purposes. Commercial producers plant groundnut to be used as a cash crop and small-scale farmers as a vegetable crop (DAFF, 2010). However, a steep decline of groundnut production has consistently been experienced in South Africa since the 1990s with the exception of 2001 where a total of 200,000 ha were planted that produced 676,000 MT. During the 2018/2019 growing season a total of 20,050 ha produced 20,030 MT (Grain SA, 2020).

Although groundnut production and kernel quality can be limited by many biotic and abiotic factors, a worldwide assessment of nematodes indicated that a number of plant parasitic nematodes are associated with groundnut, which can lead to global annual losses of up to 12% (Timper *et al.*, 2018). The nematodes associated with groundnut in South Africa are listed in the SAPPNS database and include the testa nematode *Aphelenchoides arachidis*, various *Meloidogyne* and *Pratylenchus* spp. and the economically most important, seed-borne nematode, *Ditylenchus africanus*. Other plant parasitic nematodes associated with South African groundnut include *Criconemoides*, *Helicotylenchus*, *Longidorus*, *Rotylenchus*, *Nanidorus*, *Neodolichorhynchus*, *Rotylenchus*, *Rotylenchulus*, *Scutellonema*, *Tylenchorhynchus* and *Xiphinema* spp.

Economic importance

Ditylenchus africanus is considered to be one of the most economically important factors that hamper South African groundnut production because of its qualitative effect on the kernels and severe losses in yield and income (McDonald *et al.*, 2005). *Ditylenchus africanus* penetrates the pod at the connection point of the pod to the peg. This causes the cells in that region to weaken so that the pod breaks off and remains behind in the soil at lifting of the crop. In severely infested fields, *D. africanus* may cause losses of 40–60% due to this pod loss.

However, the main effect of *D. africanus* on groundnut is qualitative (McDonald *et al.*, 2005).

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South African law specifies the grading of groundnut consignments into choice edible, standard edible and diverse or crushing grade (Anonymous, 1997), which is based on its content of percentage unsound, blemished and soiled kernels (UBS%). Each grading class in descending order earns a corresponding descending income, specified by supply and demand of that particular season. The lower the UBS%, the higher the grading and income. The economic importance of *D. africanus* is determined by the loss of income of a consignment compared to that of a consignment graded as choice grade. Symptoms caused by *D. africanus* increases the UBS%, which leads to downgrading of groundnut consignments, often to crushing grade (McDonald *et al.*, 2005) and is highly correlated with the number of *D. africanus* present in the testa of the seed (Venter *et al.*, 1991).

Host range

Ditylenchus africanus can survive in low numbers on a variety of crops such as cotton, cowpea, dry bean, grain sorghum, lucerne, lupin, maize, pea, soybean, sunflower, tobacco and wheat, as well as weeds that include cocklebur, feathertop chloris, goose grass, jimson weed, khaki weed, purple nutsedge and white goosefoot (De Waele *et al.*, 1990). However, it causes damage only to groundnut (De Waele *et al.*, 1989).

Distribution

Ditylenchus africanus is present over the whole groundnut producing area of South Africa (De Waele *et al.*, 1989). This nematode seems to be endemic to South Africa and has not yet been reported in other areas of the world (Timper *et al.*, 2018).

Symptoms of damage

Symptoms caused by *D. africanus* resemble that of black pod rot caused by *Chalara elegans* and are similar to those caused by *Aphelenchoides arachidis* (De Waele *et al.*, 1989). No above-ground symptoms are visible. The initial symptoms appear at the primary infection site located on the peg near the connection point of the peg to the base of the pod. Tissues on the surface of the connection point appear dark brown and corky and upon removal of the peg, brown and necrotic in the infection site. From there on the nematode penetrates the hull endocarp through openings at the base of the exocarp or at the pod apex and feed on the parenchyma cells surrounding the vascular bundles just below the surface of the pod. Feeding on the vascular bundles causes pods to develop darkened veins and appear dead (De Waele *et al.*, 1989). Breakdown of the hull further increases water seepage into the pod and causes severely infested pods to split open.

Infected seed appear shrunken with dark brown or black micropyles (De Waele *et al.*, 1989). Infected seed testae are flaccid, have a distinct yellow discoloration on their inner layer and can easily be removed by gentle rubbing. Destruction of the seed testa caused by feeding leads to leaching of chemical compounds necessary to inhibit seed germination and results in the initiation of hypocotyl growth, premature germination before harvest (Fig. 22.1) and occurrence of second-generation seedlings in the field. Feeding in or near the vascular bundles causes darker vascular strands which increases the unattractive appearance of the seed (Fig. 22.2). *Ditylenchus africanus* do not penetrate the cotyledons but do feed on the embryos, which turn olive green to brown (De Waele *et al.*, 1989).

Biology and life cycle

This nematode thrives in temperatures of 25°C and higher, at which it completes its life cycle from egg to adult within 6 to 7 days. Most eggs produced at 28°C start to hatch after 3 days, and within 6 days 90% of the eggs have hatched, thus producing numerous generations within a single growing season. At harvest, 90% of the *D. africanus* population is found in the pods and the rest of the population in the roots and surrounding rhizosphere (Basson *et al.*, 1991).

Anhydrobiotic juveniles, adults and eggs surviving in hulls left behind in the field after harvesting are the primary means of carry-over from one groundnut crop to the next. One-third of these anhydrobiotic nematodes become active

Fig. 22.1. *Ditylenchus africanus* infested groundnut exhibit premature germination of seed before the producer can harvest. Author's own photograph.

Fig. 22.2. Infected seed appear unattractive with dark, vascular strands. Author's own photograph.

after rehydration and will re-infest and damage a subsequent groundnut crop, even at small initial population densities (Basson *et al.*, 1992). Eggs are the preferred survival strategy in seed, during which storage time and winter months of at least 28 to 32 weeks has no negative effect on surviving nematodes (Basson *et al.*, 1993).

Interactions with other nematodes and pathogens

No information is available in the literature on interactions of *D. africanus* with other pathogens. Personal observation indicated that this nematode might have a close relationship with *Aspergillus* spp. that causes aflatoxin in groundnut.

Recommended integrated nematode management (INM)

It is difficult to control *D. africanus* because of its short life cycle, high reproduction potential, high viability and its ability to survive in small numbers on crops other than groundnut and a variety of weeds (De Waele *et al.*, 1990). Nematicides registered for the control of nematodes on groundnut in South Africa are not totally effective, especially because of the multiple generations of *D. africanus* that occur in a single growing season. Furthermore, *D. africanus* only starts to proliferate at pegging, which falls into the withholding period of most of the registered nematicides (Basson *et al.*, 1992). Production of *D. africanus*-free, certified seed in South Africa is further hampered by the omnipresence of this nematode in groundnut producing areas (De Waele *et al.*, 1989), the unpredictable efficacy of nematicides (Basson *et al.*, 1992) and the unavailability of resistant groundnut cultivars (Timper *et al.*, 2018). Heat and chemical methods of control are not suitable because of the negative germination effect on the soft, moisture sensitive and easily damageable seed. Although a well-planned crop rotation system can aid in the production of a high-quality groundnut yield, the effective management of *D. africanus* is hampered because of its ability to survive in small numbers on crops other than groundnut and re-infest and damage a subsequent groundnut crop, even at small initial population densities (Basson *et al.*, 1992).

Optimization of nematode management

Rotation with resistant groundnut cultivars may be the only alternative for the control of *D. africanus* on groundnut (De Waele *et al.*, 1990). Steenkamp *et al.* (2010) identified sources of resistance, but this still has to be incorporated into a groundnut breeding programme. Should resistant cultivars not be implemented, it seems likely that *D. africanus* will contribute to a further decline in groundnut production and possibly spread to other countries in the region.

Future research requirements

This nematode is almost impossible to control and cannot be kept below damage threshold levels with current management strategies. Since host-plant resistance may be the only viable management method, it is necessary to incorporate sources of resistance into groundnut breeding programmes in order to develop groundnut cultivars resistant to *D. africanus*. The identification of markers closely associated with the *D. africanus* resistance trait goes hand in hand with the development of resistant groundnut cultivars. The quest for appropriate markers is necessary for the successful introgression of sources with *D. africanus* resistance and to ascertain that the level of resistance is sustained once commercial cultivars emerge from the resistance-breeding groundnut programme.

South African groundnut producers experience significant aflatoxin challenges on some groundnut consignments. This challenge opens up yet another pathway to follow in terms of research, which is to study the interaction of *D. africanus* with *Aspergillus* spp. Knowledge of the relationship between plant parasitic nematodes, the *Aspergillus* spp. group and aflatoxin contamination should enable researchers and the groundnut industry to devise suitable management strategies for minimizing or eliminating aflatoxin concentrations in groundnut consignments.

The major source of resistance to *D. africanus* was found in a Malawian groundnut cultivar. This raises the question: Why would resistance develop in groundnut cultivars outside South Africa that have presumably not been under selection pressure from *D. africanus*? Therefore, the third research effort is to conduct surveys outside South Africa, especially in adjacent countries, to determine the presence of this nematode. It should also be determined where *D. africanus* has the potential to spread to and thrive in other parts of the world in order to develop appropriate protocols for the avoidance thereof.

Outlook: anticipating future developments

Southern African temperatures are increasing faster than the world average, with some stations recording a temperature increase of 3°C to 4°C since records began during the mid-20th century (Johnston, 2019). Undeniably, these higher temperatures will be accompanied by drought, changing rainfall patterns and changes in pests and diseases. Available information indicates that plant parasitic nematodes have a neutral or positive response to CO_2-enrichment effects and show a potential rapid build-up, which will interfere with the plant's response to global warming. To absorb the impact of these higher temperatures and lower rainfall, new crop cultivars adapted to these changes should be developed. Novel crops and markets not previously considered in a production area now have to be evaluated, which means that groundnut may be shifted to cooler areas not previous planted to this crop. Since *D. africanus* thrives at higher temperatures, is seed borne and has a high reproduction and damage potential, they will spread, with the seed, to these new areas and continue to flourish.

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SECTION IV:

Fruit and Nut Crops

23 Improving the management of plant parasitic nematodes in banana: Integration of technologies and responding to the demand of the consumers and markets

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Introduction

Bananas are the most important fruit in the marketplace and are a staple food in many areas of the world. There are over 1200 cultivated varieties with a total worldwide production of 139,470,376 tonnes, of which 79,617,907 tonnes belong to the Cavendish group, representing 57% of world banana production (Lescot, 2020). In addition, banana represents an important source of income as well as an important source of employment in more than 120 countries in the tropics and subtropics (Jones, 2000). Presently, there are two major constrains in Cavendish commercial banana plantations: black Sigatoka caused by *Mycosphaerella fijiensis* and plant parasitic nematodes. The spread of banana wilt *Fusarium oxysporum* race 4 is also becoming a very important limiting factor. There are several nematode species that damage banana crops. The most frequently detected and economically important are the burrowing nematodes (*Radopholus similis*), the spiral nematodes (*Helicotylenchus multicinctus*), the root-knot nematodes (*Meloidogyne* spp.) and the lesion nematode (*Pratylenchus* spp.).

Economic importance

Worldwide, almost 100% of banana production destined for the export market are monoculture of Cavendish cultivars (Grande Naine, Williams, and Valery). All of these cultivars are highly susceptible to the burrowing nematode, *R. similis*. Where this nematode is present at high levels of infestations, 100 to 200 plants per ha per year topple over due to the poor root systems. Losses of over 15% in production is common due to the fact that the damaged bunches of toppled plants cannot be exported.

Host range and distribution

Radopholus similis has been associated with most of the edible diploid (AA, AB) and triploid (AAA, AAB and ABB) banana cultivars (Gowen *et al.*, 2005). However, the most affected genotypes are the cultivars of the subgroup Cavendish (Pocasangre *et al.*, 2015). One of the main reasons why *R. similis* is not reported as the main parasitic nematode in West, East and Southern Africa is because most of the cultivars planted in Africa are cooking bananas or plantains, which

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are more resistant to *R. similis*. Therefore, the burrowing nematodes is a Cavendish issue. Its worldwide dissemination was a result of infested suckers being distributed by transnational banana companies during 1950 and 1970 when Gros Michel was replaced by Cavendish cultivars. In addition, *R. similis* has been also associated with over 250 plant species, mainly weeds belonging to Poaceae, Euphorbiaceae and Solanaceae (Quénéhervé *et al.*, 2000). In Latin America, the nematode has also been reported in cover crops like *Arachis pintoi*. The eradication of *R. similis* in commercial plantations with rotation is difficult, and perhaps impossible, due to the economics of production and thereby the speed of the plantation renovation process. Replanting new plantations does not allow for fallow periods. Therefore, there are always infested roots and corms left in the soil in the land where the renovation is implemented. This means the new plantation is re-infested several weeks after the planting.

The banana industry, in the beginning, mostly grew the cultivar Gros Michel that is tolerant to *R. similis*. However, Gros Michel is highly susceptible to tropical race 1 of *F. oxysporum* f.sp. *cubense* and the fungus destroyed most of the commercial plantations. Therefore, Gros Michel was replaced by the Cavendish cultivars that are resistant to the fungal wilt disease but highly susceptible to *R. similis*. At that time the most popular Cavendish cultivars were Valery and Giant Cavendish, both very tall plants that still encounter losses due to toppling caused by both nematodes and high winds. Therefore, a new generation of short Cavendish cultivars, including Dwarf Cavendish, Grande Naine, and Williams and others of less importance (Parecido al Rey, Zelica), were developed. However, It is important to stress that the expansion of commercial plantation around the world as well as renovation of fungal wilt damaged plantations was done with suckers infested with *R. similis*. Currently, there is a new generation of several lines of improved Cavendish cultivars (e.g. Gal, Galil, Duroi, and Oscar Arias lines) that have been selected and provided by commercial laboratories. However, all of them are highly susceptible to *R. similis*, and most of the suitable land for banana production is infested with *R. similis*, so that the host–pathogen relation will continue forever in commercial plantations.

Symptoms of damage

The disease caused by *R. similis* has several names, but the most common is black head and toppling over of the plant. Most of the damage caused by the burrowing nematode is concentrated in the root system, with less in the rhizome of the mother plant and suckers. The damage in the roots is dependent on the nematode present, which in most cases is *R. similis* that affects the internal tissue of the roots producing necrosis and reddish lesions (Fig. 23.1). The damage to the root due to *H. multicinctus* and *Pratylenchus* spp. is more superficial with small necrotic lesions, and that of *Meloidogye* spp., the root-knot nematode, includes deformation and galling of the root system.

The damage caused by these nematodes affects the anchorage of the plants and results in the toppling over of the plant (Fig. 23.2). Nematode root damage also affects the uptake of nutrients and water (Gowen *et al.*, 2005). There are several other reasons for the plant toppling over, such as soil compaction and flooding of the soil that causes necrosis of the roots due to secondary fungal and bacterial infection. Nematode damage and resulting necrosis and root rotting coupled with periodic high winds during the bunch bearing period can also be responsible for the plant toppling over.

Biology and life cycle

Radopholus similis is a migratory endoparasitic nematode that penetrates mainly through the

Fig. 23.1. *Radopholus similis* internal tissue damage with necrosis and reddish lesions. Photograph courtesy of R.A. Sikora.

Fig. 23.2. Toppling over of banana plants due to extensive *Radopholus similis* root damage. Author's own photograph.

root tips. It completes its entire life cycle inside the root cortex (Sarah, 2000; Gowen *et al.*, 2005; Sikora *et al.*, 2018). There are several biotypes of *R. similis* attacking bananas and there is a relationship between pathogenicity and their reproduction rate (Tarté *et al.*, 1981). Females and all juvenile stages are infective, and males are present but do not cause damage. The life cycle is completed in 20 to 25 days at an optimal temperature range of 24–32°C. *R. similis* is not affected by soil conditions due to a strictly endoparasitic biology.

Interaction with other nematodes and pathogens

In general, banana root samples in commercial plantations contain several nematodes species affecting the root system. *R. similis* is the predominant nematode parasite, followed by *H. multicinctus*, then *Meloidogyne* spp. and *Pratylenchus* spp. (Pocasangre *et al.*, 2015). However, it is important to note that in new banana plantations *Meloidogyne* spp. and *H. multicinctus* are the most frequent nematodes. After prolonged cropping, *R. similis* predominates due to its virulence and rapid reproduction, so that the other two nematodes cannot compete in the root system.

There is evidence that *R. similis* infested plantations become more susceptible to the soilborne diseases: Panama disease caused by *F. oxysporum* f.sp. *cubense* and moko (*Ralstonia solanacearum*). However, more research is needed to better understand the interaction of *R. similis* with these root pathogens. Conversely, there are unique interactions between *R. similis* and a broad spectrum of non-pathogenic mutualistic fungal endophytes in the root system that suppress *R. similis* densities (Pocasangre *et al.*, 2000).

Recommended integrated nematode management (INM)

Integrated management of nematodes in commercial plantations basically does not exist on a large scale mostly due to the perennial nature of the crop. Management of the most destructive nematode, *R. similis*, has been traditionally carried out with only a minimum focus on integrated control (Pocasangre *et al.*, 2001, 2015). Historically, the burrowing nematode had been managed by using soil fumigant nematicides (i.e. Nemagon) and a number of red label granular nematicides.

Dependency on nematicides

Nematode management depends on the use of granular nematicides. The most popular granular nematicides are organophosphorus (Counter, Rugby and Mocap) and carbamates (oxamyl [Vydate™]). There are new and less toxic nematicides on the market, but they are more expensive than the older established products. Therefore, farmers and commercial companies use the lower priced nematicides, often without considering the social and environmental impact issues. Currently, high pressure from the supermarket chains stimulated by consumer fear for pesticide residues exists and this may lead to the withdrawal of the red label nematicides because of their high toxicity to both humans and the environment.

The granular nematicides are applied two or three times per year at a cost ranging from US$350 to US$500 (Pocasangre *et al.*, 2015). It is important to note that the efficacy of these nematicides in *R. similis* management is very limited due to the short-term activity of the pesticides (only a few weeks after the application) and the fact that some only inactivate *R. similis* over this time period. Some of the newer nematicides entering the market, however, do cause direct mortality and have systemic properties, but again in small areas around the matt and only with short durations of activity. Repeated nematicide applications in commercial plantations has also led to problems of biodegradation of some active ingredient by the soil biota (Cabrera *et al.*, 2010). Additional environmental problems are caused by high rainfall in banana production areas that result in nematicide run-off into massive drainage systems and additional loss by leaching.

Currently, most of the commercial plantations are managing nematodes using two or three applications of nematicides, often in rotation, of mainly red label products such as Counter, oxamil, Rugby, and Mocap. However, because of international and national pressure, restrictions on their use in agriculture has been enacted in some countries. Use of new-generation nematicides with green or blue labels and low toxicological profiles seldom happens due to their high price.

Optimization of nematode management

There are many technologies that potentially could be used to expand nematode management from the present total dependency on granular nematicides. Some of these are used in the production of organic banana (Holderness *et al.*, 2000).

Improvement of sampling and damage thresholds

It is important to note that control of plant parasitic nematodes is very complex and that there is a great deal of inconsistency in the data collected on control activity using nematicides. Therefore, in some farms, management with nematicides is considered effective whereas in others they are reported to be less effective. This has led to constant discussions on the importance of measuring pre-treatment population densities in the root and their relationship to final yields. The most critical point seems to be sampling protocols and sample size that do not represent the root system distribution in the soil near the matt (Fig. 23.3). Because soil and root samples are often small (approximately 5000 cm^3

Fig. 23.3. The 360° view of the distribution of the root system of a mature banana plant in Costa Rica. Author's own photograph.

of soil), the true density of the nematode is often not properly measured and leads to inconsistent interpretation of efficacy. The sampling of the follower sucker is an alternative sampling approach (Fig. 23.4). In addition, damage threshold levels are still used that were established in very old research studies that do not relate to current banana cultivars and production systems. It is, therefore, important and necessary to develop improved sampling methodology and develop new thresholds for a better understanding of the nematological problem in the banana industry and the impact of management inputs, especially the need and use of nematicides.

Remote sensing

There is a need to use remote sensing for the mapping of nematode distribution in established plantations with known infestations. Soil and root sampling could be reduced and thereby reduce costs to the plantation.

Biological control

There is a need to test the wide range of biological-based products, including antagonistic fungi (e.g. *Trichoderma, Pochonia* and *Paecilomyces*) and strains of antagonistic bacteria (Sikora *et al.*, 2018).

Biological enhancement of tissue culture plantlets

There is also the option of treating nematode-free tissue culture derived planting material with mutualistic fungal endophytes that improve plant growth as well as reduce nematode reproduction (Sikora *et al.*, 2008).

Rotation between plantation rehabilitation

Because well-established and older plantations always have plants heavily infested with *R. similis*, nematode management is focused on these poor-yielding plantations. In addition, every year at least 10–15% of these old plantations are replanted, which is the ideal moment to optimize integrated nematode management. For example, short periods of fallow can be used to reduce nematode population densities along with the use of organic amendments to improve the soil.

Fig. 23.4. Sampling the roots of the follower sucker for more exact damage estimation. Author's own photograph.

Organic amendments

The addition of large amounts of organic matter (6 tonnes/ha/year) to improve beneficial microorganisms and overall soil health while enhancing the physical properties of the soil should be standard before plantation rehabilitation. Organic matter reduces soil compaction and improves water infiltration by increasing the fine pores of the natural drainage system and is known to have negative effects on plant parasitic nematodes.

High-density, short-cycle production

Banana production is examining the use of high-density planting with 2000+ plants/ha, grown in short cycles of 3 or 5 years in attempts to manage nematodes as well as tropical race 4 of fusarium wilt, which is an emerging constrain to the whole industry. In future it will be mandatory to begin renovation of plantations every 5 years. This new 5-year regeneration programme will be an important opening for the incorporation of new methodology for plant parasitic nematode management and thereby support the trinity of sustainability (environmental, social and economic).

Resistance and tolerance

There are a number of banana clones which are more resistant to nematodes and to fusarium wilt than the Cavendish. The most important are: Pisang Mas (Musa AA), Lady Finger (Musa AAB), Red Bananas (Musa AAA) and the somaclonal Formosana (Musa AAA). These could be planted in the 4- to 5-year planting cycles, and even rotated with Cavendish cultivars.

Bio-banana production

Organic banana production is located in both Latin America and the Caribbean and is solely in the hands of smallholder farmers who sell their banana crops to exporters. All of the organic banana is produced in dry climatic zones with long periods of drought to reduce or avoid black Sigatoka. The integrated management of plant parasitic nematodes in organic banana production is mainly carried out using organic mulches, including different kinds of composts and Bokashis produced with crop residues of mainly banana, sugarcane, rice, cocoa and coffee. In addition, the farmers often apply farm-based composts enriched with a cocktail of microorganisms. Currently, there are several commercial products based on strains of *Trichoderma*, *Pupureocillium*, *Pochonia* and effective microorganisms. However, most of these products are registered as bio stimulants and not as commercial biocontrol products. It should be noted that there is little information related to the efficacy of these commercial products. Another strategy that some farmers are applying is intercropping banana with cocoa and coffee. This system is normally used for banana destined for local markets. In most cases the banana crops are not certified as organic and therefore cannot be exported as bio-banana.

Future research requirements

It is very clear that the producers, field workers, and consumers are moving to reduce or eliminate highly toxic pesticides from the banana production environment. The new trend is toward fewer toxic molecules and strengthening the use of biological control products with other cultural control technologies for tackling the plant parasitic nematode problems.

There is definitely a need to improve current sampling methodology, because the small soil samples are often only to a depth of 30 cm and a distance of 13 cm from the pseudostem, which are not representative of the root system of the plant. In some cases, this type of sampling does not yield sufficient root material for nematode analysis. New approaches using a 360° examination of the root system around the plant (Fig. 23.3) is needed to better understand the vertical and horizontal distribution of nematodes affecting the root system for improved and targeted application of control products. This is important because conventional sampling only takes the follower suckers into consideration (Fig. 23.4).

Remote sensing for the mapping of nematode distribution according to densities and damage caused could lead to a reduction in nematicide use in the plantations. This is technology is outlined in Chapter 58 in this volume. This could also help in high-density planting for monitoring shifts in damage potential.

Research on soil solarization using plastic mulches in the fields could be an option for nematode and other soil-borne pathogen management. Obviously, organic banana production, where soil solarization can be used, could be supported by the application of additional organic mulch to stimulate soil beneficial microorganisms. There is also a need to re-analyse the threshold of *R similis* in bananas and upgrade thresholds under modern banana production systems. The use of site sampling methodology using GPS for a better understanding of the pattern of damage caused and distribution of plant parasitic nematodes in the plantation needs to be researched for more targeted control applications.

Outlook: anticipating future developments

With the advancement of new technology in remote sensing, big data analysis and mechatronics, I strongly believe that more efficient methods of managing nematodes will appear. The focus must be targeted toward less toxic pesticide use and the implementation of less toxic compounds with the simultaneous increase of biocontrol technologies such as enhancement of tissue culture planting material. This technology shift should be possible in the new 4- to 5-year intensive production systems of the future. In addition, it is vital to break the single cultivar-based monoculture and promote new potential banana cultivars (a few are mentioned above) that are more resistant to nematodes and fusarium wilt than the Cavendish cultivar.

The current changes in the banana industry are being driven by changes in consumer and market interests that should result in more modern banana production systems that enhance social, environmental and economic aspects of the trinity of sustainability.

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24 The shifting sands of banana nematode communities under mixed cropping conditions

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Introduction

Best known for their distinctive shape and colour, bananas are synonymous with supermarket shelves across the globe. Such bananas are grown in large-scale mechanized systems in commercial, single cultivar plantations (see Chapter 23). However, a vast range of cooking and sweet banana types are also grown for household and local consumption across the tropics. These bananas are cultivated under a great diversity of conditions, interspersed with various crops in mixed cropping systems. Produced largely by smallholders, they may be grown as isolated plants (mats) beside the house, interspersed among forest trees or in fields of 1–2 ha. Some fields may be larger, and in some areas, fields of cooking bananas may merge one into the other forming an expansive contiguous blanket across the landscape. Dessert bananas are eaten raw and serve multiple purposes, including as an infant weaning food. The cooking types though, serve as staple starch food sources, with millions of people depending on them for food. Cooking bananas include plantains, which are generally roasted, while other types are boiled or steamed. Some bananas are also used to produce a sweet juice, which is also fermented to make wine or beer. The importance of cooking bananas as a staple food source is often overlooked. Enset, a member of the banana family, does not produce a bunch but instead the stem and corm are used as a key staple food crop in southern Ethiopia.

Bananas are propagated vegetatively and take ~12–18 months to produce a bunch after planting. Ratoon stems (suckers) are produced successively from the sides of existing plants, the rate depending on a range of criteria such as genotype, climate, soil and pest and disease challenge. Bunches vary in weight but may be up to 30–40 kg, which are produced on a stem that may be as short as ~2 m or up to several metres in height. A strong root system is necessary for anchorage and to maintain stem turgidity to prevent stem snapping. When this network of roots is compromised, the success of the crop is jeopardized. Toppling or snapping of the stem prior to maturity of the bunch can render the whole harvest unusable. Infected roots are also less efficient at accessing nutrients and water.

Economic importance

As with many crops in mixed cropping systems on smallholder plots, determining realistic economic damage estimates for pests and diseases is

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a challenge. Bananas are faced with a number of serious pests and diseases, plant parasitic nematodes amongst them, which more often occur simultaneously. Indeed, rarely, if ever, are bananas affected by a single nematode pest, but moreover are challenged at any one time by a combination of species and in tandem with other problems. Some nematode species are viewed as more aggressive and damaging than others, with the burrowing nematode (*Radopholus similis*) generally recognized as the most important nematode pest of bananas. Populations of individual species can also vary in aggressiveness; *R. similis*, for example, can differ significantly (Plowright *et al.*, 2013). The wide array of species, variation in pathogenicity between populations and the multiple infections that prevail dictates that we consider this community effect, as opposed to single species infection, for banana production under mixed cropping conditions.

The devastation that nematodes impose on banana productivity cannot be underestimated. A key factor is that toppling often occurs late in the development of the bunch, following a lengthy period of production. Lost bunches due to plant toppling is dramatic and obvious, while significant losses from nematodes are also realized through reduced bunch size, a lengthened period to harvest, or through a reduction in the productive life of fields. Overall losses depend on a range of factors, such as the level of infection, as well as on host susceptibility and environmental influences. The combined diverse effects of nematode infection, however, can result in substantial losses to production.

Most evidence of crop loss is derived from field experimentation, which is then extrapolated to farmer conditions. Nearly threefold increases in yield following the management of nematodes have been reported, although losses in the range of 30–60% are more usually recorded (see Sikora *et al.*, 2018). Yield losses averaging 29% were recorded for 17 banana, plantain and hybrid cultivars over two cropping cycles when planted into fields infested with nematodes dominated by the burrowing nematode in Nigeria (Dochez *et al.*, 2009). The plantains as a group, however, appear to be particularly prone to nematode infection, substantially reducing yields and especially plantation longevity. In West Africa, nematodes are a critical factor in reducing the number of crop cycles to a single crop even. In East and Central Africa, there is strong evidence that the introduction of *R. similis* in the 1960s had a major influence on East African highland banana production (Price, 2006).

Host range

Radopholus similis infects a wide range of hosts such as vegetables, ornamentals, tree crops and tuber crops, and can result in the slow decline of many plant species. Distinctive symptoms and damage are particularly noted on anthurium, aroids (e.g. swamp taro), black pepper, citrus, coffee, ginger and palms, while various weed species will act as hosts.

Helicotylenchus multicinctus is variously recorded from a wide host range, including weed species, although there is little information on its status as a pest.

Pratylenchus coffeae is highly polyphagous, with hosts that include numerous economically important crops, such as citrus, coffee, grapevine, vegetables, ornamental foliar plants, yam (*Dioscorea* spp.), in addition to occurring on many broadleaf weed species.

Pratylenchus goodeyi appears to have a relatively broad host range, although there is little information on its status as a pest on other crops. Beans and maize have been considered good hosts of *P. goodeyi*, but this seems to be cultivar dependent.

Rotylenchulus reniformis, another polyphagous tropical species, has a broad range of known host crops on which it commonly occurs, such as cotton, legumes, various vegetables, ornamentals and fruit tree crops. Particularly good host crops include pineapple, sweet potato, papaya and edible aroids.

The species of *Meloidogyne* most reported occurring on banana are *M. arenaria, M. incognita* and *M. javanica*. These include the most polyphagous nematode species known, especially *M. incognita* and *M. javanica*, which can infect a vast botanical range of cultivated and non-cultivated plants. Their infection is a major source of damage for many crops and together they represent a most formidable threat to tropical agriculture (Coyne *et al.*, 2018).

Distribution

Species compositions on banana will vary depending on location, climate, soil, banana type and genotype but, as with most tropical crops, occur as a simultaneous combination of species.

Bananas are associated with a range of species, of which a handful pose the greatest threat. These key species very much have a global distribution, with one major exception, *Pratylenchus goodeyi*. This species is mainly restricted to Africa and cooler (higher altitude) conditions and is the major nematode species occurring on enset in Ethiopia. *Radopholus similis*, however, tends to be viewed as the most important species, although this very much depends on the location and situation. For example, in the East and Central African highlands, *R. similis* tends to be the principal species, but is undoubtedly found in combination with *Helicotylenchus multicinctus* and others. And then at the higher cooler altitudes (over ~1400 m), *P. goodeyi* replaces the more thermophilic *R. similis*. More recently, however, *P. goodeyi* has been increasingly found on banana under hotter, more tropical conditions in lowland East Africa. In the West African lowlands, *P. coffeae* has superseded *R. similis* and is now viewed as the more damaging species. *Pratylenchus coffeae* is a cosmopolitan species and found across banana growing areas of the world, as is *H. multicinctus.* The semi-endoparasitic nematode, *Rotylenchulus reniformis* is also commonly found on banana, sometimes as the principal species, such as in some areas of India. *Meloidogyne* spp., although not generally considered as a key nematode pest of banana, does occur regularly, and in combination with other species can be associated with significant damage. *Helicotylenchus multicinctus,* together with *Meloidogyne* spp., is commonly problematic on plantain in West Africa. *Meloidogyne incognita* and *M. javanica* appear to be the principal species involved, although it is likely that other species occur. Other species may locally attain pest status but in general are not considered serious pests. However, the complex of *Pratylenchus* species that infect banana may be more diverse than currently understood, as some species are morphologically inseparable. *Pratylenchus speijeri*, for example, was first recorded as *P. coffeae*. Accurate species diagnostics can have major implications to their management, especially in respect to genetic host resistance.

Symptoms of damage

The most obvious, visible symptom of nematode damage on banana is a fallen (toppled) banana stem, usually heavily laden with a maturing bunch. The stem of a plant that has toppled due to nematode root damage will be intact, with the roots and corm uprooted and exposed. Due to the reduced functionality of infected root systems water uptake is affected, which can reduce the turgidity of stems, especially during periods of low water availability, leading to stem snapping. Nematode infection additionally reduces potential bunch size, as well as delaying the time to harvest, and reducing the productive life of banana fields. Infected plants may be stunted with reduced girths.

Feeding and migration of nematodes within root cells causes tissue damage leading to necrosis, which gradually extends as the nematodes feed, multiply and migrate. Mild necrotic spots coalesce and develop into ever-larger red-brown necrotic patches, which blacken, and eventually the root, or root area, dries and dies (Fig. 24.1). These are recognized symptoms for migratory endoparasitic nematodes, which may differ somewhat depending on the species and aggressiveness of the population. *Helicotylenchus multicinctus* generally feed close to the outer edges of

Fig. 24.1. Split banana roots exposing tissue necrosis and root deterioration due to lesion nematode feeding. Author's own photograph.

the root cortex, while *Pratylenchus* spp. and *R. similis* will feed throughout root tissue except the central stele, which remains unaffected, except perhaps in very young roots. Although lesions caused by nematodes feeding close to the surface, such as by *H. multicinctus*, may appear superficial, they still lead to yield loss (Fig. 24.2). Different genotypes of banana may also differ in their reaction to individual species. *Meloidogyne* spp. and *R. reniformis* differ from the migratory endoparasites as they are sedentary endoparasites, and once juveniles establish a feeding site within the root the female remains in the same feeding location as she develops.

In banana roots, the position of female *Meloidogyne* nematodes can usually be observed in sliced roots as a blackened halo surrounding a microscopic white spot (the female). With time, the tissue deteriorates and necrotic patches form that can be clearly seen when roots are split open. Infected roots tend to be swollen around the area of infection, often with cracking of the surface, but are not usually highly deformed or knotted, as on other crops.

Externally, banana cord roots that are infected with nematodes become blackened, often with some external cracking along the root (Fig. 24.3). Severely infected roots become withered, dry out and die.

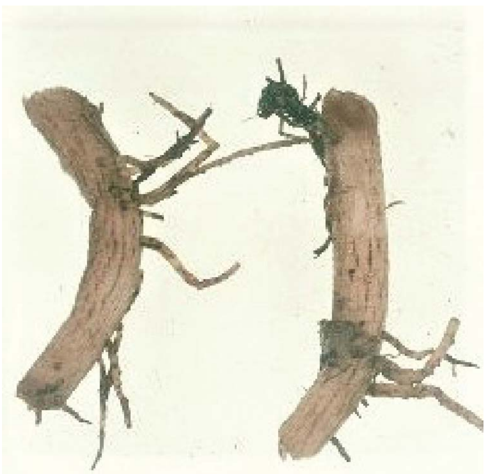

Fig. 24.2. Shallow, superficial cortical lesions caused by *Helicotylenchus multicinctus*. Photograph courtesy of R.A. Sikora.

The corms of infected plants also become necrotic and blackened. Initially, circular necrotic spots occur at the junction of the roots with the corm, extending as nematode feeding extends (Fig. 24.4). Large areas of blackened corm tissue signify high levels of nematode infection.

Biology and life cycle

The migratory (*R. similis*, *Pratylenchus* spp.) and sedentary endoparasites (*Meloidogyne* spp., *R. reniformis*) complete their life cycle within the root tissue. With severe necrosis and root death, nematodes will exit to locate new roots. After entering the roots, usually near the root tip, the migratory nematodes cause cavities in the root

Fig. 24.3. Banana roots blackened and cracked due to lesion nematode infection. Author's own photograph.

Fig. 24.4. Banana corm peeled back showing necrotic areas due to heavy infection with lesion nematodes. Author's own photograph.

as they feed. The migratory nematodes lay eggs within root tissues and the life cycle is completed within ~3–4 weeks, depending on temperature and species. Female sedentary endoparasites produce a gelatinous external egg mass. In thick fleshy cord roots, *Meloidogyne* spp. are encapsulated within the root and do not protrude externally (Fig. 24.5), while *R. reniformis* are semi-endoparasitic and so the female partly protrudes from the root. Hatched infective juveniles locate a suitable feeding site where the female then develops. Optimal temperatures for *Meloidogyne* spp., *R. similis* and *P. coffeae* are around 25–30°C, unlike *P. goodeyi,* which is closer to 20°C and is generally more tolerant of cooler temperatures.

Interactions with other nematodes and pathogens

As all the various nematode species infect similar sites, feed in a similar manner and have similar requirements for space and food, there is obvious competition for space and nutrients. The more aggressive species will outcompete less competitive species. However, there is limited understanding on how the various interactions between the different species ultimately affect and damage the crop.

In general, prior infection with nematodes appears to predispose banana to additional disease infection. In particular, the incidence of two important diseases of banana, Panama disease, caused by *Fusarium oxysporum* f.sp. *cubense*

Fig. 24.5. Split cord root with encapsulated *Meloidogyne* spp. (females). Author's own photograph.

and Xanthomonas bacterial wilt (BXW) caused by *Xanthomonas vasicola* pv. *musacearum* is higher in the presence of nematodes. Other soil-borne pathogens will also be facilitated, and the colonization of the lesion by secondary weak fungal pathogens, such as *Fusarium* spp. and *Rhizoctonia* spp., will result in the development of necrotic lesions (see Fig. 24.1). Damage to the stem by the banana weevil will exacerbate nematode damage symptoms and losses.

Recommended integrated nematode management

Without doubt the single most important intervention in managing nematodes pests is the use of healthy, uninfected planting material (Tenkouano *et al.*, 2006). This has many benefits in being pest and pathogen free. Even when planting healthy material into infected fields, benefits will be realized, although eventually this advantage will erode. When planting into fields previously cropped to banana it is advised to leave fields fallow or plant an alternative crop for at least 6 months. Given the diversity of nematodes involved it is difficult to select a suitable crop that is not host to at least one of the species. Establishing the most prevalent species present will therefore help in determining suitable fallow crops. Smallholder farmers are well known for exchanging planting material, which is a primary source of nematode dissemination. Creating farmer access to healthy planting material is to be encouraged, therefore, in reducing the overall nematode (and other pathogen) problem. Healthy planting material is available to farmers as tissue culture plantlets, macro-propagated material or by disinfestation of suckers (Tenkouano *et al.*, 2006). The removal of infected roots, paring the corm and immersion in hot or boiling water is a practical and suitable method of sucker disinfestation for smallholder farmers (Sikora *et al.*, 2018). Enhancing plantlets with beneficial microorganisms has received growing attention for its potential in nematode management but has yet to receive significant uptake for use with smallholder farmers. Similarly, biological control products are emerging for use in bananas. Limited availability, as well as a lack of understanding by farmers, limits the adoption of new products, including improved, resistant cultivars. Chemical nematicides can be effective but tend to be costly, so farmers with limited resources or access to credit tend not to use them. Instead, pesticides that are unreliable or adulterated, which are more affordable, tend to be used. This further reduces farmer confidence of pesticides, however, through their limited impact on pests.

Combining healthy planting material with host resistance has obvious positive benefits. Resistance against some of the key nematode species has been identified and some good progress made, but knowledge of this by farmers is generally lacking. The genetic improvement of bananas, through conventional breeding, is also not straightforward and is hindered by numerous obstacles, such as sterility. Selecting for broad resistance against all the various nematode species is challenging, including combining this with desired agronomic and quality traits. To reach this desired outcome the Breeding Better Bananas programme in East Africa has focused specifically on improving hybrids with resistance against key pests and diseases (http://breedingbetterbananas.org/, accessed 10 March 2021). Some good progress has been made in a few breeding programmes, in particular against the burrowing nematode. Generating cultivars with useful host plant resistance against nematodes through genetic modification has also been attempted with some good results, but government confidence to accept these remains a concern with only a few countries currently accepting genetically modified crops.

Optimization of nematode management

The single most important intervention in managing nematode pests is the use of healthy, uninfected planting material. Enhancing this with microbial antagonists and combined with host resistance is ultimately preferred (Coyne *et al.*, 2018). Selection of new fields or delayed planting into previous banana fields is recommended. These practices alone should have substantial impact on suppressing nematode-related losses in smallholder banana farms.

Future research requirements

The development of high-yielding hybrids with host resistance, which is active against a broad range of nematode species, as well as other key diseases, is necessary to reduce banana losses in these mixed cropping systems. Improved cultivars also need to have preferred consumer and agronomic traits in order for them to be acceptable by farmers and consumers. Identification of effective microbial antagonists and access to new products should be a research emphasis. The development of efficient, healthy (and improved) seedling delivery systems, in tandem with the introduction of resistant cultivars, is essential.

Outlook: anticipating future developments

The rapidly evolving and shifting population dynamics of nematode species on banana in these mixed cropping systems is intriguing as well as a concern for their future management through host resistance. The diversity of species against which to develop durable resistance presents an initial challenge, but the apparent rapid shift in species dynamics is alarming, such as *P. goodeyi* becoming heat tolerant in East Africa or the rise of *R. reniformis* in India. A changing climate may stimulate or create shifts in nematode challenge that are difficult to keep abreast of, especially given the inherent challenges of breeding bananas. The spectre of other aggressive diseases, such as fusarium wilt tropical race 4 or bacterial wilt, devastating whole swathes of banana presents a real threat that demands that we address multiple pests and diseases simultaneously. The genetic modification of existing preferred cultivars offers a practical option but necessitates government acceptance. The development of new genetic and molecular techniques will, however, continue to provide the means to exploit useful genes, possibly using more acceptable mechanisms, such as gene editing.

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25 Pre-planting solutions for the slow decline of citrus caused by *Tylenchulus semipenetrans*

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Introduction

The citrus nematode, *Tylenchulus semipenetrans*, is the causal agent of the slow decline of citrus. The nematode is well adapted to reproduce on citrus and very high densities are required to damage the trees and reduce yield. Symptoms of nematode damage in infested sites develop slowly in non-bearing citrus trees and they become apparent on fruit-bearing trees 5–8 years after planting. Diseased trees show retarded growth, vigour and root mass compared to healthy trees. Symptoms are more evident in replanted citrus orchards and when healthy seedlings are interplanted between nematode infected trees in established orchards.

Citrus is cultivated in 298,000 ha in coastal areas and river valleys in Spain, with an annual production of 7,520,000 tonnes. Oranges (47%), mandarins (37%) and lemons (15%) are the main products that are marketed primarily for fresh consumption.

Economic importance

Tylenchulus semipenetrans is the most economically important plant parasitic nematode in the citrus-growing regions worldwide. In Spain, 80% of the orchards are infested with *T. semipenetrans.* Mature citrus trees can perform relatively well in soils with low citrus nematode infestation levels, but young trees planted on heavily infested orchards suffer severe damage resulting in unthrifty growth and low yields (Verdejo-Lucas and McKenry, 2004; Duncan, 2009). The citrus nematode is one of the components of the citrus root disease complex in which root-rotting fungi, poor water management, salinity and other predisposing factors (tristeza, replant problems) are involved. Therefore, it is important to determine which component is the limiting factor and this should be corrected before considering nematode management. Nematode densities are generally negatively related to tree condition and fruit yield. Damage thresholds (minimum nematode densities that suppress tree growth and yield) are influenced by tree age, scion–rootstock combination, the alternate bearing habit of citrus, soil type, and other diseases or management practices. Nematode densities exceeded the damage threshold in 40% of the mandarin orchards in north-east Spain (Sorribas *et al.*, 2008). Yield increases in response to nematicides usually range from 10–30% depending on infection level and orchard management. Management actions are recommended

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when nematode densities in spring exceed 400 females/g root and 10,000 juveniles/250 cm^3 soil, although chemical control is only used occasionally.

Host range

Tylenchulus semipenetrans has a narrow host range that includes all *Citrus* species and most hybrids of citrus with *Poncirus trifoliata*. Grape, olive, lilac (*Syringa vulgaris*) and persimmon (*Diaspyros* spp.) are also infected by the nematode. Citrus rootstocks and their hybrids support different reproduction rates of the nematode, and such differences in host status could be exploited to regulate increases in nematode densities. However, large areas are planted on the same rootstock in a given region. Parasitic variation among populations occurs; thus, populations collected from Troyer and Carrizo citrange rootstocks showed higher reproduction rates than those from sour orange.

Distribution

The citrus nematode is the dominant parasitic nematode in citrus in Spain. Nematode dissemination may occur with infected nursery stock, heavy rain, run-off water from adjacent infested orchards and contaminated equipment. Nematodes are distributed in aggregates within an orchard with great variation in soil densities within and also between orchards. In replanted citrus orchards, *T. semipenetrans* is not detected on newly planted trees during the first 2 years after planting susceptible rootstocks, and it has an irregular distribution during the following 2 years (Le Roux *et al*., 1998; Sorribas *et al*., 2003). Thereafter, nematode populations increase once the trees have produced a canopy, and continue increasing with time, reaching maximum levels at tree maturity (8–12 years after planting).

Symptoms of damage

Above-ground symptoms include slow growth, lack of vigour, leaf chlorosis and curling, and twig dieback in severe cases (Fig. 25.1). Therefore, accurate diagnosis of the slow decline disease requires nematode sampling and analysis owing to the lack of specific symptoms.

As for below-ground symptoms, nematode infected trees have fewer and shorter feeder roots with numerous rootlets. Heavily infected roots are thicker and darker and have a dirty appearance because soil particles stick to the gelatinous matrix on the root surface (Fig. 25.2). Because symptoms may not be apparent on lightly infected roots, infected nursery stocks may easily go undetected.

Biology and life cycle

This highly specialized sedentary semi-endoparasite requires 6–8 weeks to complete its life cycle at soil temperatures of 24–26°C. Juveniles and males are vermiform and free in the soil. The immature female penetrates into the root cortex to initiate a permanent feeding site consisting of several nurse cells. Mature females have an enlarged

Fig. 25.1. Trees of mandarin ‘Clemenules’ on ‘Carrizo’ citrange infected by *Tylenchulus semipenetrans* in a 6-year-old commercial orchard. **(A)** Good tree condition with no symptoms of nematode damage. **(B)** Tree with moderate vigour and some symptoms of decline. **(C)** Poor tree condition with advanced symptoms of decline. Author’s own photographs.

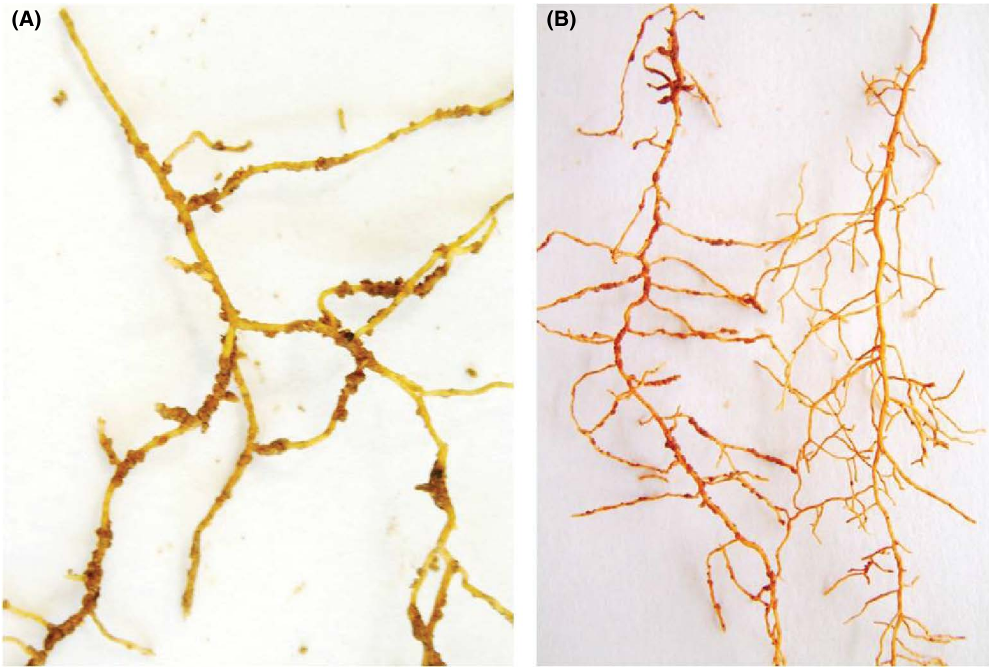

Fig. 25.2. Citrus roots infected by *Tylenchulus semipenetrans*. **(A)** Dirty appearance due to the adhesion of soil particles to the gelatinous egg masses deposited by females on the root surface. **(B)** Non-infected (right) and citrus nematode infected roots (left). Author's own photographs.

posterior end that stays exposed on the root surface where eggs are laid into a gelatinous matrix.

Nematode densities are regulated by tree phenology and changes in soil temperature and moisture. They are subjected to seasonal fluctuations with one or two distinct and predictable annual periods of maximum population growth that coincides with active periods of root growth, usually one in spring and a second in autumn. Higher densities of *T. semipenetrans* in roots are found in the spring rather than autumn (Sorribas *et al.*, 2008). Soil moisture is inversely related to population growth. Although *T. semipenetrans* infects citrus over a range of soil edaphic conditions, they prefer silt and loamy sand textures. Nematode densities are higher in alkaline than in acidic soils.

Interactions with other pathogens

The citrus nematode and soil-borne pathogenic fungi are ubiquitous in the citrus rhizosphere, where they parasitize the root cortex reducing citrus root mass and this interaction contributes to citrus decline (Duncan, 2009). For instance, levels of *Phytophthora nicotianae* were inversely related to those of *T. semipenetrans.* Pre-infection of citrus roots by the nematode also can reduce the rate of infection by *P. nicotianae*. The pathogenicity of *Fusarium solani* to citrus may be increased by the nematode when soil temperatures are favourable for the fungus.

Recommended integrated nematode management

Management should start by preventing the spread of the nematode to uninfested areas using field sanitation, nematode-free rootstocks and nematode-free nursery soil. The presence of *T. semipenetrans* in newly planted orchards is probably the result of introductions via contaminated nursery stock. Citrus nurseries in Spain are established in virgin soils far away from established orchards. Currently, nurseries are using pasteurized substrates and containerized production systems in greenhouses to improve tree establishment after transplanting, which prevents nematode spread. Sampling will determine nematode densities and the need for management measures.

Pre-plant measures

Pre-plant measures are the most effective measures for nematode control in perennial citrus because they reduce initial densities before establishing an orchard, and they will promote young tree establishment, yield increases, and reduce the need for repeated post-plant treatments.

Site preparation by soil tilling can accelerate the mortality rate of nematodes due to desiccation and/or direct exposure to sunlight. Sub-soiling orchard soils at periodic intervals before re-establishment reduced *T. semipenetrans* densities by 90% (Sorribas *et al.*, 2003). Fallowing for 1 year, which is also recommended before replanting orchards in Spain, reduces nematode densities. However, nematodes may survive in deeper layers of sub-soil for long periods of time where soil moisture and temperature fluctuations are minimal. In addition, the nematode can survive for many years in remnant roots from the infected trees removed from the field. These roots should be removed as much as possible because they are reservoirs for the nematode. Constraints of fallowing and extensive tillage however include soil erosion, soil structure impairment, labour and equipment costs, and a reduction of beneficial organisms.

Pre-plant fumigation maintained *T. semipenetrans* under detectable levels for several years (Le Roux *et al.*, 1998; Sorribas *et al.*, 2003). It must be considered when replanting trees in old citrus orchards to prevent damage to newly planted trees from pathogenic fungi and nematodes. Historically, the broad-spectrum soil fumigants, methyl bromide, 1,3-dichloropropene and metam sodium, were used but they are no longer authorized for citrus in Europe. Fumigant efficacy is affected mainly by moisture content of the soil, but also by soil porosity, temperature and dose. Phytotoxicity, poor distribution in soil, lack of persistence, human toxicity, reductions in non-target beneficial organisms, and the cost of application need to be considered (Verdejo-Lucas and McKenry, 2004).

Resistant rootstocks to *T. semipenetrans* significantly reduce nematode reproduction in comparison with susceptible rootstocks. They can be useful for replant situations in nematode infested soils because population increase occurs more slowly than on susceptible rootstocks (Sorribas *et al.*, 2003). *Poncirus trifoliata* is the only source of nematode resistance incorporated into commercial citrus rootstocks. *Poncirus trifoliata* and the hybrid Swingle citrumelo provide effective resistance against *T. semipenetrans*, tristeza virus (CTV) and *P. nicotianae* in many regions. Both rootstocks, however, are intolerant to calcareous or alkaline soils which prevent their use in most regions of Spain. The CTV-tolerant Troyer and Carrizo citranges were used to replace the CTV-susceptible sour orange but they are more susceptible than sour orange to Spanish populations of *T. semipenetrans*. Various hybrids of mandarin × *P. trifoliata*, tolerant to calcareous soils, have shown resistance to *T. semipenetrans*. They reduce female and egg densities in roots by 85% or more (Galeano *et al.*, 2003). The resistance involves a hypersensitive response to nematode feeding and subsequent formation of wound periderm, and higher accumulation of lignin or suberin-like deposits around the nematode. Furthermore, fewer individuals reach the mature female stage on resistant rootstocks. An increased shift in sex ratio also occurs, with a higher percentage of males over juvenile stages on some resistant rootstocks. Biotypes of *T. semipenetrans* pose a limitation to rootstock choice. Three biotypes are recognized: 'Citrus', 'Mediterranean' and 'Poncirus' (Inserra *et al.* 1980). All three reproduce on citrus but they differ in their ability to reproduce on *P. trifoliata* and olive. The 'Mediterranean' biotype is the most widespread in Spain, although the 'Poncirus' biotype was detected in small areas planted to this rootstock.

Post-plant measures

Post-plant measures aim at regulating population increases and to diminish citrus damage. They should be applied coinciding with active periods of root growth because the life cycle of *T. semipenetrans* is regulated by tree phenology. Measuring the densities of females in roots in spring are more consistent than juveniles in soil to evaluate the effectiveness of nematicides and rootstocks, seasonal activity of *T. semipenetrans* and its economic importance.

Non-fumigant nematicides decrease nematode densities on citrus although positive yield responses have occurred with no reduction in *T. semipenetrans* densities (Fig. 25.3A), and reduction of populations may happen without a measured yield response (Duncan, 2009). Repeated applications over seasons are usually needed to maintain nematode densities at low levels and consistent yield increases. Split applications of the maximum recommended dose of the liquid formulations are suggested to increase their efficacy. The solubility in water of the nematicide is an important issue because it affects the movement of the active ingredient and its distribution in the soil profile (Verdejo-Lucas and McKenry, 2004). Irrigation is often recommended before nematicide application in drip-irrigated orchards to get better distribution of the product in the soil.

Little effect of management of *T. semipenetrans* on citrus yield may be observed the first year after nematicide treatments because citrus trees can allocate carbohydrate to vegetative growth before fruit growth so that yields may or may not increase in the first year after nematicide treatment (Duncan, 2009). Oxamyl increased the numbers of mandarin fruits after

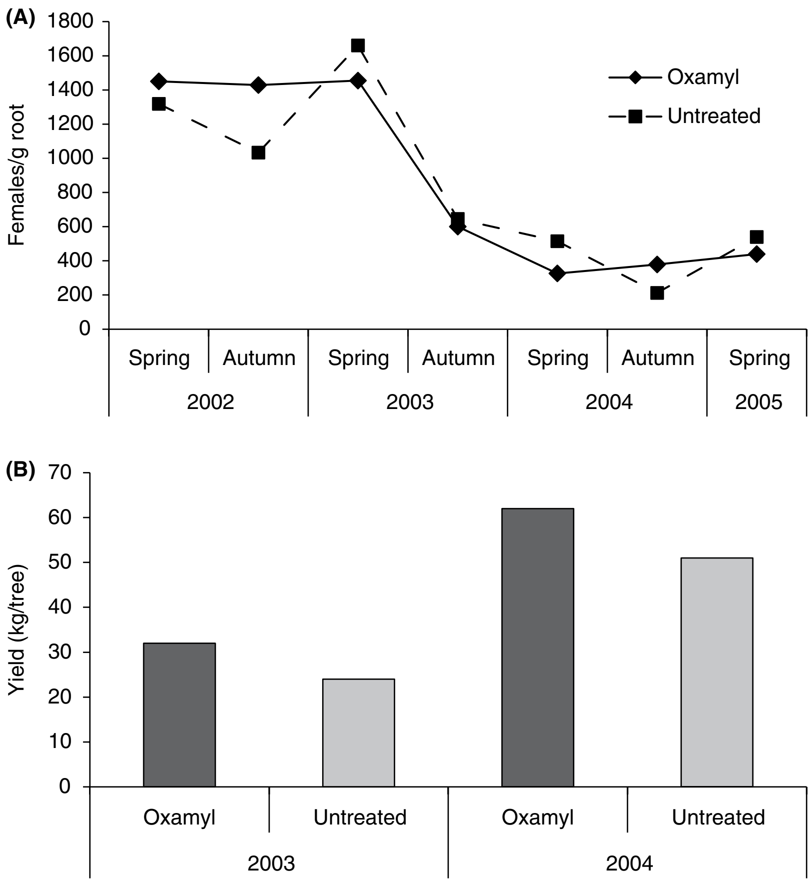

Fig. 25.3. (A) Densities of females per gram of root of *Tylenchulus semipenetrans* and **(B)** yield of mandarin 'Clemenules' on 'Carrizo' citrange in a 6-year-old replanted citrus orchard treated three times annually with oxamyl 10L at 60 l/ ha (seven split applications at 3-week intervals). Stratified block design, two treatments (oxamyl treated or untreated plots), five replications/treatment, six trees/replicate. Nematode densities assessed annually before nematicide treatment (spring) and 1 month after finishing the nematicide treatment (autumn). Author's own figures.

two and three treatment programmes with an overall yield increase of 28% and 22%, respectively (Fig. 25.3B). None of the nematicides currently available in Spain (azadirachtin, fenamiphos, fluopyram, fosthiazate and oxamyl) are presently registered for use in citrus. This will affect yield in the future if new nematicides do not enter the marketplace.

Biological control

A diversity of microbial antagonists occurs naturally in citrus orchards worldwide. *Pasteuria* (50%) and *Paecilomyces lilacinus* (35%) were frequently isolated from *T. semipenetrans* in north-eastern Spain (Gené *et al*., 2005). Fungal egg parasitism was related directly to the number of females and inversely to the number of eggs. The maximum level of fungal parasitism of nematode eggs in field soil infestations was estimated to be 45%. Additional measures need to be designed to increase this level of naturally occurring biological control. A bio-nematicide containing *Paecilomyces lilacinus* strain 251 (now *Purpureocillium lilacinus*) as the active ingredient is registered for use in citrus in Spain. Juveniles, females and eggs of *T. semipenetrans* are parasitized by *P. lilacinus* and culture filtrates of this fungus immobilize juveniles although the efficacy of the culture filtrate in the field is unknown. The presence of *Pasteuria* was shown to be positively related to the number of juveniles in soil.

Agronomic and cultural practices

Orchards generate high yields under optimum growing conditions even in the presence of nematodes. Environmental conditions that stress the trees can result in suboptimal production, and eventually yield losses. Controlling weeds has little direct impact on *T. semipenetrans* because of its high host specificity but weed control will improve tree growth by reducing competition for water and nutrients. Mulching the tree row in new plantings can help tree establishment by reducing weeds, water evaporation, herbicide use and moderating extreme daily soil temperatures. In established orchards, mulching trees with anti-weed nets prevented weed growth with no effect on nematode densities.

Optimization of nematode management

Pre-plant fumigation is not common in citrus because uprooting trees and 1-year fallowing greatly reduce *T. semipenetrans* densities (Sorribas *et al*., 2003), so the citrus nematode is not perceived as a problem by most growers. Successful pre-plant management often involves combination of two or more tactics before replanting an orchard. Combining site preparation, soil fumigation and resistant rootstocks was more effective in delaying root infection of newly planted trees for 5 years than combining two tactics (Fig 25.4). Site preparation plus fumigation prevented root infection for 3.5 years, whereas site preparation plus a resistant rootstock reduced infection for 5 years. Female density attained the threshold level on the susceptible rootstock after 3 years but did not on the resistant rootstock for the first 9 years after replanting. However, continuous exposure to citrus nematode inoculum over time may reduce the resistance level of some rootstocks originally described as resistant to *T. semipenetrans* (Verdejo-Lucas and McKenry, 2004).

In established orchards, new modern nematicides with low toxic profiles should be investigated under conditions prevailing in Spanish citrus orchards (i.e. alkaline soils). Degradation of most oxime-carbamates and organophosphates is enhanced at pH >7.0. Abamectin and azadirachtin, however, have proven effective against *T. semipenetrans* in sandy soil (El-Tanany *et al*., 2018). Biological nematicides will be useful for organic orchards but they need further testing because temperature and soil moisture affect their performance. There should be an attempt to apply chemical and biological nematicides sequentially in the same season. The follow-up biological would not be subjected to a security interval between treatment and harvest.

Future research requirements

Changes in production systems leading to higher planting densities per hectare will require

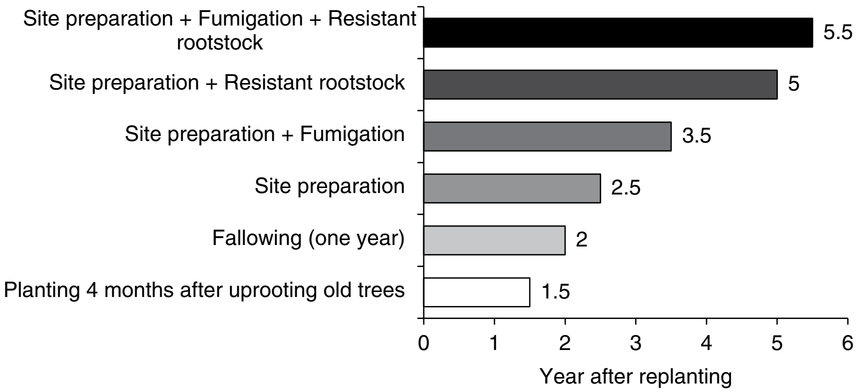

Fig. 25.4. Combination of pre-plant management tactics and their efficacy in delaying (years) root infection by *Tylenchulus semipenetrans* of mandarin 'Orogrande' on citrange 'Carrizo' in a replanted orchard infested with the nematode. Site preparation: Soil sub-soiling and three passes of cultivator. 2 × 2 factorial design, six replications/treatment. One factor: fumigation with 1,3-dichloropropene at 600 l/ha. Second factor: resistant rootstock (Forner Alcaide no. 5) and susceptible rootstock (Carrizo citrange). Author's own figure.

combining dwarfing characteristics with rootstock resistance or tolerance to key pathogens and abiotic stresses in the region. Extreme changes in weather patterns due to a changing climate leading to severe drought conditions will limit citrus cultivation in some areas because citrus trees require supplemental irrigation in Mediterranean climates and its availability and cost would compromise the profitability of citrus. With the restrictions imposed on the use of chemical nematicides, the need for rootstocks adapted to the new demands of climate stresses need more research input and funding. Large plant populations must be examined to detect segregating types expressing resistance. Molecular markers linked to resistant traits will accelerate the labour-intense and time-consuming screening processes. The aggregate distribution of the nematode could be detected with drone and remote sensing technologies and used for site-specific application of nematicides and biologicals.

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26 Sting nematode management in Florida strawberry

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Introduction

Strawberry (*Fragaria* × *ananassa*) is an economically important fruit crop for the United States, with a total production of 1.3 million tonnes across 20,000 ha in 2018. Florida produces 15% of the total strawberry produced in the US. It is currently grown on 4450 ha within a 34 km^2 area with an economic impact exceeding US$700 million annually to the Florida economy. The estimated cost of strawberry production in Florida is close to US$75,000 per hectare.

Economic importance

The most economically important nematode pest of Florida strawberry is the sting nematode (*Belonolaimus longicaudatus*), and occasionally the root-knot nematode (*Meloidogyne hapla*) or strawberry crimp nematode (*Aphelenchoides besseyi*) when introduced within bare-root strawberry transplants from US or Canadian nurseries. Sting nematode, a migratory ectoparasite, is estimated to occur on as much as 40% of Florida strawberry acreage.

Strawberries are typically produced on raised plastic-mulched covered beds in most areas of Florida (Fig. 26.1). These beds are routinely fumigated with a multi-purpose fumigant at the time they are plastic mulch covered for broad-spectrum soil pest control. Following the loss of methyl bromide in 2013 and reliance upon other broad-spectrum fumigants, sting nematode within the Florida production acreage was observed to increase in area and severity. During this time, as much as 9% of total Florida acreage was estimated to harbour the nematode, causing an estimated US$13.4 million loss in production. Now after the widespread adoption of vertical management zone approaches (deep shanking) for sting nematode control, the problem and its associated losses has been largely resolved (Noling *et al.*, 2016). The amount of damage in sting nematode infested fields not treated with nematicides are considerably higher, often resulting in a 40–100% crop failure, depending on specific circumstance.

Host range

Belonolaimus longicaudatus has a very wide host range, including a variety of wild and commercially cultivated plants. Many different small grain and forage crops, fruits, ornamentals and turfgrasses have all proved to be suitable hosts for sting nematode. Most vegetable crops grown

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Fig. 26.1. Strawberry raised beds under plastic with gradients of *Belonolaimus longicaudatus,* sting nematode damage. Author's own photograph.

in Florida are damaged by the sting nematode. Cucumber and okra are symptomless hosts but support sting nematode reproduction. Tobacco and watermelon are poor hosts, showing little or no evidence of damage in the field to sting nematode. Many weeds also serve as good hosts. Bermuda and crabgrass are native weeds supporting nematode carry over during the summer and have even allowed population increase. Due to market windows and the specializations required, very little crop rotation is practiced in Florida strawberry as a nematode management strategy.

Distribution

The sting nematode appears to be a native pest of the sandy soils of the lower coastal plains of the south-eastern US. It has such a preference for sandy soils that it fails to exist in significant numbers in soils containing even small amounts of silt, clay or organic matter content.

The higher numbers and greater distribution of this ectoparasite in Florida is probably not only related to the predominance of fine sandy soil, but also due to the warm subtropical environment. In addition, sting nematode appears to be very sensitive to sudden changes in soil conditions such as rapid drying.

Biology and life cycle

Sting nematode reproduction is greatest in sandy soil, at temperatures of 25–30°C (75–85°F) with constant, but moderate, moisture levels. Under suitable conditions, a life cycle is completed in about 28 days.

Symptoms of damage

Strawberry production problems caused by sting nematode tend to occur in more or less defined areas where transplants fail to grow-off normally (Fig. 26.1). Infested areas consist of spots that vary in size and shape, but the boundary between diseased and healthy plants usually is fairly well defined. Affected plants become semi-dormant, with little or no new growth. Leaf edges turn brown, progressing or expanding from the edges to midrib to include the entire leaf. Leaves seldom become chlorotic, although cases have been reported and observed in which leaf yellowing occurs when essential nutrients are present in limited supply at the end of the season.

Any loss of sting nematode control typically results in a higher incidence of plant stunting in the field due to root damage. Sting nematode kills the root meristem and halts root growth. Lateral roots will develop, but *B. longicaudatus* will migrate to these lateral roots and damage them as well. This causes an abbreviated and stubby-looking root system (Fig. 26.2). Roots that are damaged by sting nematodes are undeveloped and prevented from extracting water and nutrients from the soil for proper plant growth. The plants have short, stubby root systems and may exhibit discolored leaves that are yellow or reddish, indicating nutrient deficiency. With time and continued feeding, necrotic lesions form laterally along the sides of roots, progressing to overall decay in root mass and loss of oldest, mature leaf tissue.

Fig. 26.2. Stubby root symptoms caused by *Belonolaimus longicaudatus* feeding. Photograph courtesy of J. Hamill, University of Florida.

Damage assessment and field monitoring

A gradient of plant stunting is typically observed to radiate outward from field areas where soil nematode densities are highest. Plant stunting and yield losses are very well correlated and defined by soil density and time in which upward movement from deeper soil horizons, below the traffic pan, occurs into the plant bed. The patchiness and spatial variability in plant stunting (and thus yield) does not appear suddenly before harvest but reflects a slowing of growth during the time from planting in October to final harvest in March/April. To account for differences in plant size, new technologies based on georeferenced field coordinates (GPS) is being used to spatially characterize sting nematode damage based on individual plant canopy size within the field (Noling and Cody, 2014). Given this ability to monitor nematode damage and field distribution, yield loss maps have been developed based on indirect measures of plant yield using plant canopy size or more recently, using hyperspectral reflectance or digital colour imaging technologies to characterize canopy cover or greenness on a field basis (Noling *et al.*, 2015) (Fig. 26.3).

Overall, field scale changes in strawberry crop productivity due to sting nematode and chemical treatment have been effectively determined, on a farm-by-farm and even industry-wide basis, from post-harvest assessments of counts of different plant sizes and canopy greenness measurement (Fig. 26.4). The methodology is currently being used for crop loss assessment, providing growers guidance and quantitative performance data on alternative nematode management strategies.

Recommended integrated nematode management

Today, in subtropical Florida, fumigants are extensively used in high value cropping systems such as strawberry. Fumigant-use decisions are not exclusively based on nematodes but on the overall spectrum of soil-borne pests present. In most cases, fumigants are used in combination with other soil pest and crop management strategies but are relied upon for their superior

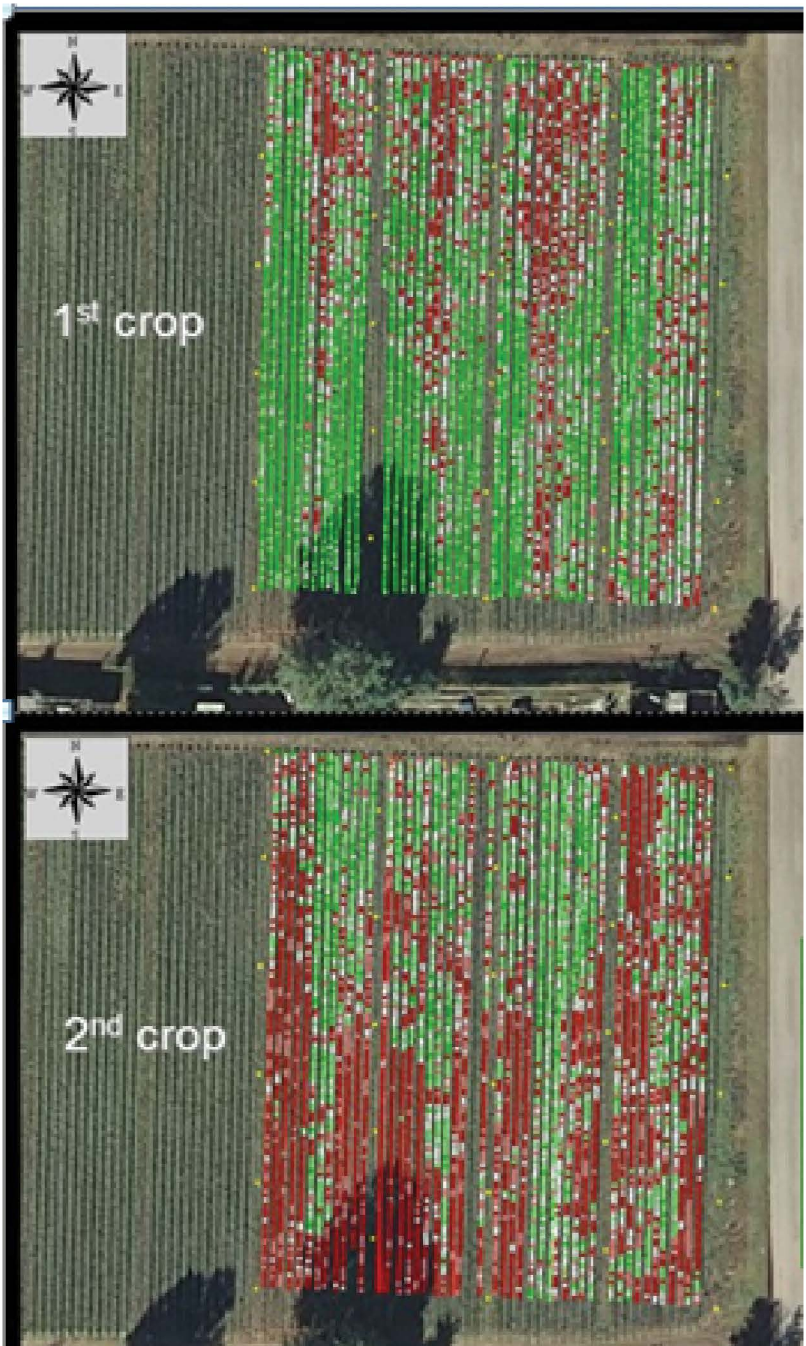

Fig. 26.3. Two field examples of hyperspectral reflectance and other imaging technologies of *Belonolaimus longicaudatus* damage distribution on strawberries used for improvement of integrated management. Author's own images.

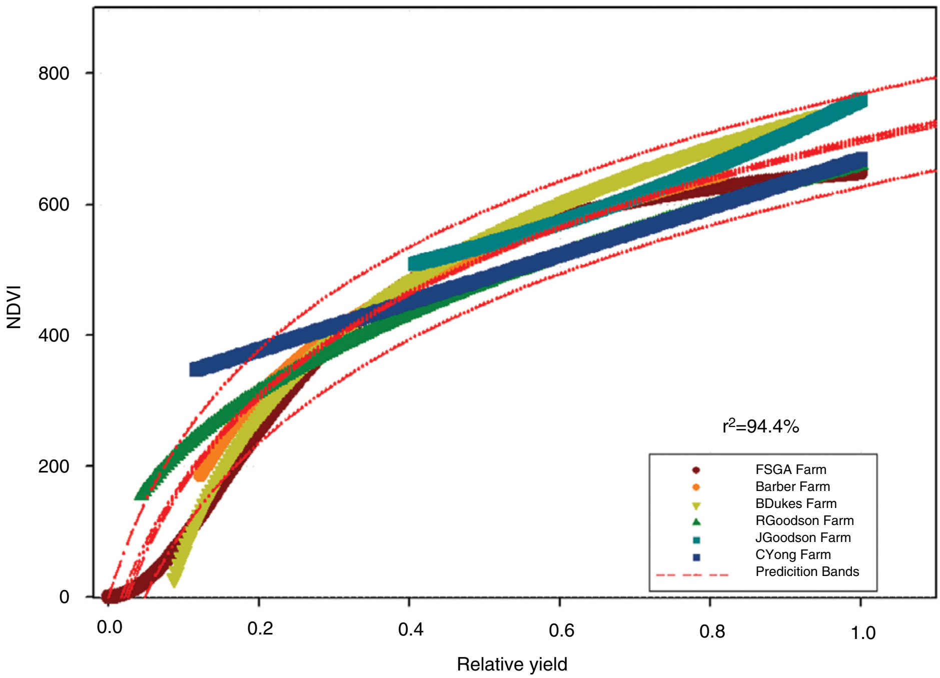

Fig. 26.4. Relationship between relative strawberry yield based on counts and yield contributions from plants of four different plant sizes per field row and normalized difference vegetation index (NDVI) within six commercial strawberry fields infested with sting nematode in Florida, USA. Author's own figure.

broad-spectrum pest control efficacy and consistent enhancement of crop growth, development, yield and quality.

For Florida strawberry growers, sting nematode management is considered and implemented now as a year-round programmatic activity requiring integration of a variety of cultural, chemical and agronomic practices in fields where the nematode occurs. Because strawberry must be vegetatively propagated and transplanted into the field, Florida growers pay special attention to the nursery source of strawberry transplants to ensure they will not arrive infested with nematodes. With the loss of methyl bromide and other important fumigant products on a global scale, increasing imports of nematode- and disease-infested strawberry plant material have been observed in Florida on an annual basis. Nematode introductions have been observed from all major regions of transplant production in the US and Canada. After introduction, post-plant remediation treatments with commercially available products have generally not been able to resolve further issues of plant decline and yield loss during the season. In many cases the infested crop is destroyed prematurely to avoid spread into non-infested areas.

Resistance

Sting nematode resistant and/or tolerant strawberry cultivars that provide for early yield and possess both acceptable flavour and marketability attributes are currently not available for use in Florida. Germplasm resistance to ectoparasitic nematodes like sting have yet to be naturally identified, whereas some genetically modified strawberry plants were reputed to have expressed resistance to sting nematode via additions of a statin gene.

Weed management

Weed densities and changes in weed spectrum have increased in Florida strawberry compared to the weed control previously provided by methyl bromide. For example, black medic (*Medicago lupinus*) is a new, winter leguminous weed that is becoming increasingly important to Florida strawberry production. As a hard-seeded legume, none of the currently available soil fumigants provide acceptable black medic control. As an excellent host to sting nematode, black medic has been observed to significantly increase field populations of sting nematodes, particularly in the row middles and plant holes. In this regard, unmanaged, post-plant weed growth can have a very destabilizing effect on overall nematode population growth and crop loss. Herbicides are included as a critical component of integrated sting nematode management.

Deep shank summer broadcast: vertical management zones

In most strawberry field surveys, a compacted zone (traffic pan) is observed to occur just below the base of the raised bed. The presence of subsurface traffic pans was shown to unavoidably cause changes in the downward percolation of water, permeability and diffusion of fumigant gases, and root penetration into deeper soil profiles. Since the traffic pan almost completely restricts downward diffusion of fumigant gases when applied above the restrictive layer, application below the layer reduces damaging populations of sting nematodes which would have otherwise survived the bed fumigation treatment. Within infested fields, increases to crop production of 25–30% are typically achieved when fumigants are placed under the traffic pan (Noling *et al*., 2016).

In situations where sting nematode is a reoccurring problem within a strawberry field, Florida strawberry growers now include a summer broadcast (August–September) fumigant treatment with 1,3-dichloropropene (1,3-D) using deep shank application technologies (Fig. 26.5) to a soil depth of 40–50 cm (Noling *et al*., 2016).

After application, the moist soil is shallowly disked and roll compacted to provide an additional measure of fumigant containment within the soil. This treatment of the zone below the traffic pan is applied in addition to autumn in-the-bed strip fumigant treatments (above the traffic pan) and crop termination treatments of the previous strawberry crop in the spring (April–June). Adoption of the vertical management zone deep shanking treatment in combination with in-the-bed autumn fumigant treatment has largely resolved sting nematode problems in Florida strawberry (Noling *et al*., 2016). Without the deep shank treatment, all the soil fumigants currently available have pest control and crop yield inconsistencies associated with long-term use.

In sting nematode infested fields, Florida strawberry growers are accustomed to using a 63:35 ratio of 1,3-D and chloropicrin (Telone C35™), at a rate of 439 kg/ha. As incidence and severity of soil-borne diseases have increased in recent years, so has the chloropicrin content of the 1,3-D and chloropicrin formulation used. Currently a formulation of an 80:20 ratio of chloropicrin to 1,3-D (250–275 kg/ha) is used when reoccurring soil-borne disease problems such as *Macrophomina phaseolina*, causal agent of charcoal rot, has been observed within the field. None of the fumigant formulations above have proved to be consistently effective against sting nematode when a vertical management zone approach is not implemented.

Plastic mulch considerations

After a fumigant is soil applied, the bed is covered with a plastic mulch to provide, among other things, an additional measure of fumigant containment to soil. When soil-borne pests and diseases are problematic, Florida growers now extensively utilize totally impermeable plastic mulch films to enhance fumigant dosage (CxT) within the plant bed. Because of their excellent barrier properties, fumigant application rates are frequently reduced, which reduces production costs, decreases soil aeration times and potential phytotoxicity problems to transplants.

Early crop destruction/crop termination

The opportunity to enhance nematode control with soil fumigation and minimize losses in crop yield due to nematodes is dependent upon the adoption of early crop destruction after final harvest (Fig. 26.6).

Fig. 26.5. Bedded drip or shank applications in the autumn (August–September). Author's own photograph.

It is one of many integrated strategies, both chemical and non-chemical, which Florida growers implement to incrementally manage nematode populations within their fields. After final strawberry harvest in the spring (March), the crop is destroyed quickly to eliminate nematode food sources and population increase, and greater difficulty in achieving nematode control in the subsequent strawberry crop. In most fields where sting nematode is a significant and reoccurring problem, strawberry growers terminate the strawberry crop at the end of the production season in March with a crop termination chemigation treatment using drip irrigation delivered fumigants. Even with a single irrigation tape per bed, benefits from long injections of crop termination chemicals for sting nematode management have been expressed in improved health, vigour, size and yield of the following season's crop. Short injections fail to treat a significant portion of the plant bed.

The treatment is now a foundation component of an integrated pest management programme for sting nematode management, targeting nematode populations that have increased to high levels and the destruction of the plants' root system which serves as sustenance for continued nematode growth and reproduction. It is an incremental approach to reduce population density at a time when most nematodes are largely confined within the raised beds (i.e. 62% of the field), rather than all over (100%) after the plastic is removed and the field is disked. For crop termination, a bottom-up

Fig. 26.6. Crop destruction with herbicides. Author's own photograph.

approach with a drip applied fumigant is preferred to a top-down approach with a herbicide or foliar defoliant spray. Significant increases in strawberry yield are generally observed in the following crop in response to early crop destruction/crop termination treatments.

Double cropping Cucurbitaceae and Solanaceae

After strawberry crop termination in the spring, a second double crop of melons, squash, cucumber, maize, tomato, onion or pepper is often planted by many growers to capitalize on the cost of plasticulture inputs and to generate additional crop production revenue. For many small acreage growers, the additional revenues generated sustain the family farm. Sting nematode has a very wide host range and can severely damage most of the spring double crops currently being planted in Florida.

Cover cropping

As an alternative to summer fallowing, which is frequently used after a second curcurbit crop, crop rotation with a poor or non-host cover crop is currently used as an effective means of reducing soil populations of sting nematode. Cover crop rotations with Sunn hemp (*Crotalaria juncea*) is widely practiced in Florida strawberry fields and has been shown to reduce sting nematode populations. Sunn hemp is densely seeded (34 kg/ha) and quickly established with overhead irrigation and kept as free as possible of grasses and other undesirable host weeds which serve as excellent carry-over hosts. Sorghum-sudangrass is still used to some extent but is a poor choice for a summer rotation on land infested with sting nematode. Iron clay pea was once widely used as a summer cover crop in the major strawberry producing areas of Florida until it was shown to increase some sting nematode populations.

Double cropping strawberry

Florida growers also double crop strawberry after strawberry, reusing the same mulch and single tape drip system. For double-cropped strawberry with an existing bed and plastic mulch cover, the choice for method of fumigant application becomes very simplified because now only a chemigated drip, rather than chisel applied fumigant, can be used for bed treatment. A full summer of solar heating of a black plastic mulch covered bed provides an appreciable level of thermal control of the nematode. Soil temperatures in Florida can cycle between 29°C and 48°C on a daily basis for the duration of the summer for all bed shoulder locations and, to lesser degree, with bed middle locations. In the absence of food, nematodes that do not die from heat stress are more likely to die from starvation in the stale, double-cropped beds, particularly in those fields where a crop termination treatment has been deployed.

Outlook: anticipating future research requirements

The current reality is that soil fumigation is now extensively relied upon, in a vertical management zone approach, to resolve sting nematode problems in Florida strawberry (Noling *et al.*, 2016). With such a heavy reliance upon soil fumigation as the foundation for nematode, weed and soil-borne disease management, any future changes in fumigant registration and availability will pose potentially dramatic economic consequences. In this regard, recent reviews by different state and federal regulatory agencies suggest that additional restrictions, including expanded buffer zones and localized reductions of volatile organic compounds being released into the atmosphere, will continue to restrict fumigant use. Additional personal protective equipment requirements for field workers will also encumber the use of the remaining fumigants in the US. With the loss of methyl bromide and the imperfections of the replacement tactics, new nematode and soil-borne diseases have emerged, and historically important soil-borne diseases have re-emerged in Florida strawberry. New broad-spectrum fumigants are being evaluated globally for use in commercial agriculture. Registration of any of these new fumigants will, however, be difficult to achieve given growing environmental activism and consumer demands for greener chemistry. Strawberry losses due to nematodes and other soil-borne diseases are likely to increase in Florida, because nematode, weed and disease control has yet to be demonstrated with an integrated, non-fumigant chemical programme.

Florida growers will continue to focus on the integration of a variety of sting nematode management tactics to sustain strawberry production. However, strawberry resistance to sting nematode is unlikely to be developed in the near future due to misinformed health concerns about genetically modified organisms, the absence of identified plant genes capable of conferring resistance to sting nematode, and because the breeding programme for new strawberry cultivars in Florida continues to prioritize early yield and post-harvest quality, flavour improvement and cultivar resistance to prevalent foliar and soil-borne diseases.

In the absence of fumigants, the challenge is to develop novel soil disinfestation techniques that will address multiple pests and pathogens in order to sustain farm profitability well into the future. Hyperspectral reflectance and other imaging technologies will continue to enhance large-scale evaluation of these novel alternative management systems for nematode control (Fig. 26.3). The loss of weed control associated with soil fumigant alternatives poses a major threat to nematode management by weed hosts of nematode pests. International competition and market forces are demanding cheaper pricing and higher fruit quality from Florida growers. However, worldwide strawberry production acreage (particularly Mexico) is dramatically increasing annually, often benefiting from government subsidies, and lower land, labour and operational costs. Florida growers are recognizing that this is evolving into a system where foreign fruit floods the market earlier each year, annually threatening farm profitability and sustainability.

With increased restrictions on use of soil fumigants and herbicides, soilless strawberry production in Florida is likely to increase, but not to the degree it has done in Western Europe, where production now exceeds 1600 ha. The ability to grow crops in a soilless system with reduced fertilizers and pesticides is advantageous in a regulatory environment mandating best management practices to achieve minimum resource use. However, increasing production cost is an impediment to any deviation from the current system. Moreover, soilless systems, or other indoor, permanent structure farming techniques, are especially vulnerable to hurricane-force storms. The cost of these systems and increased urbanization of what were once rural farming communities will constrain the grower's ability to remain competitive abroad and within US markets.

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27 Ectoparasitic nematodes: Emerging challenges to wine grape production in the Pacific Northwest of North America

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Introduction

Plant parasitic nematodes are a constraint to the production of wine grapes worldwide. In the Pacific Northwest (PNW) of North America, including British Columbia (BC) in Canada and Oregon (OR) and Washington (WA) in the United States, the impact of plant parasitic nematodes, specifically ectoparasitic nematodes, on wine grape production has not been extensively studied or documented. Wine grape production in this region is relatively young (30–40 years old), and most vineyards were originally established on native ground or in areas where something else had been produced (tree fruit or annual crops).

There are some important distinctions between the OR, WA and BC wine grape growing subregions that will affect how nematodes are managed. Most of the production in OR occurs in the relatively moist Willamette Valley west of the Cascade mountains, with a high proportion of sites on clay loam soils. Production in WA and BC occurs east of the Cascades in a semi-arid environment, with many sites on coarse textured soils. Consequently, production in WA and BC is entirely dependent on irrigation while production in western OR is rain fed. Production in WA and BC differ in that rootstocks have been used in BC since the industry began expanding in the 1980s, while vineyards in WA have until very recently been planted primarily with self-rooted *V. vinifera*.

This region now supports a combined US$521 million wine grape industry, and large parts of it are slated for replanting because of vine age and the presence of phylloxera. The potential impact of the ectoparasitic nematodes *Mesocriconema xenoplax* and *Xiphinema americanum* s.l. (in the broad sense; here within referred to as *X. americanum*) will be management challenges in vineyard replant situations.

Economic importance

The impact of nematodes is often overlooked because they are cryptic in nature and at times subtle. However, for a perennial crop that can cost US$23,000 or more per ha to plant and does not begin to produce marketable fruit until the third year, a subtle decrease in yield potential can have a significant long-term impact on profitability. Using an online production cost estimator established by the Washington Winegrowers Association (http://www.nwgrapecalculators.org/intro.php, accessed 30 October 2020) with default settings and maximum annual yield of

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11 and 22 tonne/ha reached in the fourth year of production, we estimated that it would take 13 years and 7 years to achieve a positive cumulative net system balance, respectively. For a 10% annual loss of yield due to nematodes, these values shift to 19 and 8 years, respectively (Fig. 27.1).

Distribution

Both *M. xenoplax* and *X. americanum* are reported to be widely distributed in the region; however, distribution varies across the region (Table 27.1). *Xiphinema americanum* occurs at high percentages (>50%) across the region. Population densities of these nematodes in vineyards are not high, which is typical for this group of nematodes. It is likely that there are several species of *X. americanum* species in the region. In a nematode survey in BC (Graham *et al*., 1988), *Xiphinema* species were found in 80% of survey samples, with *X. bricolensis* being widespread and *X. pacificum* identified in only two samples. Preliminary morphological and molecular work with *Xiphinema* populations collected from WA wine grape vineyards indicate four to five species are present, potentially including *X. pachtaicum*, *X. utahense*, *X. rivesi* and *X. tarjense*. The implications of this diversity on virus vectoring ability (see below) is unknown.

Mesocriconema xenoplax distribution in the PNW is more variable than for *X. americanum*. This nematode was present in approximately 80% of vineyards in both OR and BC. In contrast, *M. xenoplax* was present in only 14% of vineyards in WA. As noted above, wine grape production in BC is more similar to eastern WA than western OR. Given the similarity in BC and WA climates and production practices, reasons for the low occurrence of *M. xenoplax* in WA are unclear.

Symptoms of damage

Ectoparasitic nematodes generally do not present specific signs or symptoms on grapevines. *Xiphinema index*, which is not established in BC, OR or WA, has been observed to cause stunting and swelling of root tips, resulting in an overall stubby root system. Such symptoms on grape roots have not been documented, however, for *X. americanum* in the region. Economic damage attributed to *Xiphinema* species is largely the result of their role as vectors of nematode-transmitted polyhedral viruses (see below).

At the scale of vineyard blocks, it is difficult to relate current nematode population densities with productivity or symptoms of decline in existing vineyards. Because most of the vineyards in WA and BC were first planted in the past 30 years, most of the populations of *M. xenoplax* and *X. americanum* that are now present likely developed after these existing vineyards were first established. Consequently, the nematode populations would have developed along with other factors such as winter injury, viruses and trunk diseases, obscuring influences of the nematodes.

For woody perennial crops generally, nematodes have greater impacts on young, recently replanted trees or vines than on already established plants that have well developed systems of coarse and structural roots. Field microplot studies, which effectively mimic such impacts on newly planted or replanted vines, have demonstrated that *M. xenoplax* can have severe effects on early growth and yields of self-rooted vines over multiple years without presenting specific signs or symptoms (Pinkerton *et al*., 2004; Fig. 27.2A). These impacts were observed regardless of at-plant population densities, which ranged from 30 to 3000 *M. xenoplax*/kg soil (Fig. 27.3). The insensitivity to at-plant population densities is attributed to the potential for rapid population development of *M. xenoplax*. Therefore, investing in pre-plant fumigation or any other such 'one-shot' pre-plant treatment for *M. xenoplax* may not have the extended benefit seen with other nematodes on other perennial fruit crops.

Biology and life cycle

Research on the biology and life cycle of ectoparasitic nematodes in PNW vineyards is lacking. However, the vertical and horizontal distribution of nematodes in deficit-irrigated vineyards of WA has been evaluated. From a spatial perspective, *M. xenoplax* and *X. americanum* have very different distributions within

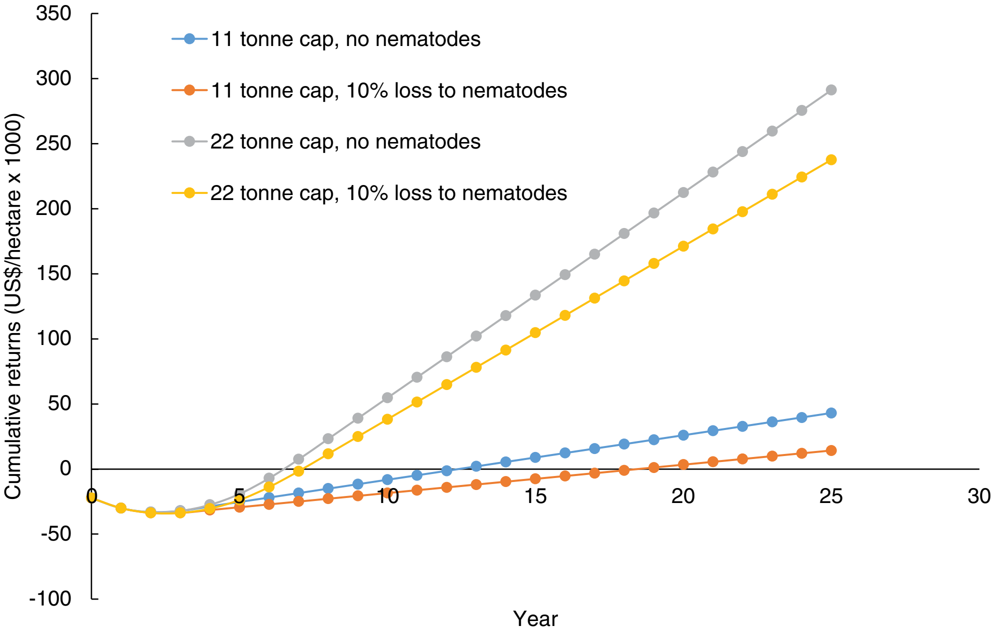

Fig. 27.1. Estimated cumulative returns (US$ per hectare) through time from simulated vineyards replanted without plant parasitic nematodes and with 10% yield reduction due to plant parasitic nematodes, and with fruit load managed for fruit quality at 11 tonne/ha and 22 tonne/ha. Author's own figure.

Table 27.1. Occurrence, mean and range of the ectoparasitic nematodes *Mesocriconema xenoplax* (*Mx*) and *Xiphinema americanum* (*Xa*) in wine grape vineyards in the Pacific North-west of North America, including British Columbia (BC) in Canada, and Oregon (OR) and Washington (WA) in the United States. Recreated from Pinkerton *et al*. (1999), Zasada *et al*. (2012) and Forge *et al*. (2021).

Nematode	Mean (max) nematodes/250 cc soil			% Occurrence relative to total number of samples collected		
	BC	OR	WA	BC	OR	WA
Mx	258 (2038)	NA	28 (160)	82	81	14
Xa	85 (1085)	NA	25 (284)	77	94	59

NA, not available.

these vineyards (Howland *et al*., 2014). *Mesocriconema xenoplax* was aggregated under emitters with a shallow distribution coinciding with the presence of grape fine roots and soil water content. This was in contrast to *X. americanum* that was randomly distributed within the vineyards and to depths of 1–1.2 m. It is likely that the distribution of these ectoparasitic nematodes is similar in BC where vines are similarly drip irrigated. This is in contrast to vineyards in OR that rely on winter rain instead of irrigation to support vine growth, and therefore nematodes and grape roots would likely not be aggregated under drip emitters. *Mesocriconema xenoplax* population densities were also found to increase with irrigation frequency and with N fertilization rate (Forge *et al*., 2019).

Interactions with other nematodes and pathogens

Grapevine fanleaf virus has been introduced into vineyards in the region, but the absence of

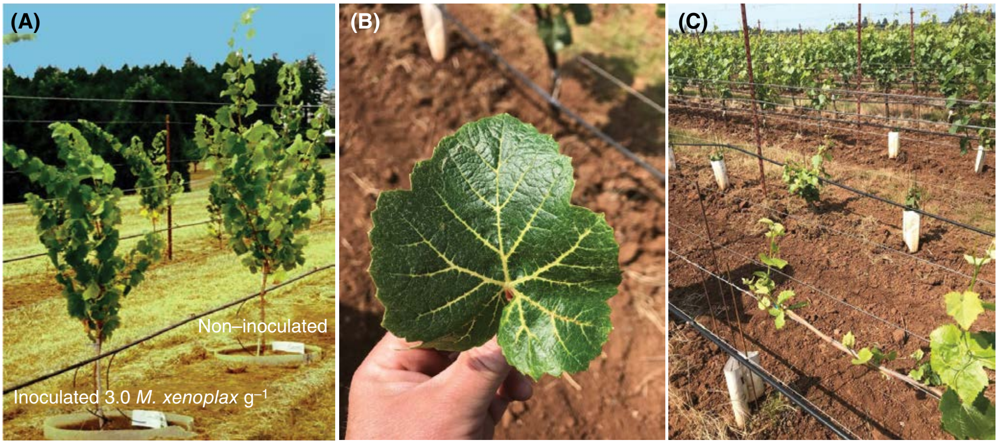

Fig. 27.2. (A) Impacts of *Mesocriconema xenoplax* (3 nematodes g^{-1} soil inoculum density) on overall vine growth. **(B)** Leaf symptoms of tomato ringspot virus, vectored by *Xiphinema americanum.* **(C)** Impact of tomato ringspot virus on young vine growth. Author's own photographs.

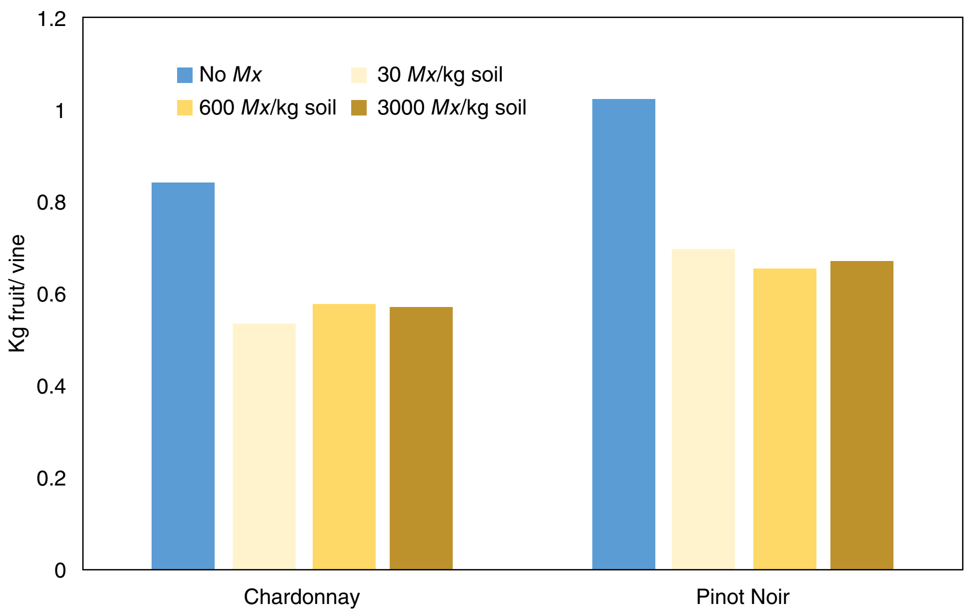

Fig. 27.3. Yield of grapes in fourth year after planting Chardonnay and Pinot Noir grapevines in microplots infested with variable population densities of *Mesocriconema xenoplax* (*Mx*). Figure is re-drawn from data in Pinkerton *et al.* (2004).

its vector, *X. index*, has prevented it from becoming a significant issue. In contrast, tomato ringspot (ToRSV) and tobacco ringspot (TRSV) viruses are vectored by a number of species in the *X. americanum* species complex. While these viruses are not widespread in PNW vineyards, when introduced the loss to production is significant. The viruses result in virus-induced grapevine decline (chlorotic mottling, weak shoot growth, reduced fruit clusters and yield) which causes extensive damage to own-rooted *V. vinifera* cultivars and interspecific hybrids (see Fig. 27.2B,C). Because of the sporadic incidence of TRSV and ToRSV in the PNW, it is suspected that the viruses have been inadvertently introduced into the region on contaminated nursery material. However, once the virus is introduced into a vineyard with *X. americanum* present, the ability to remediate this situation is very difficult and can take years at a significant cost to production.

Recommended integrated nematode management (INM)

Chemical control options

There are no proven effective, registered nematicides currently available. Fumigation is used, but it is not widespread. Cost is a major deterrent as well as an inability for use in sustainable programmes.

Cultural practices

The use of nematode-suppressive cover crops, particularly the crucifer green manure cover crops with biofumigant properties, has become a popular subject of nematode management in vineyards. Planting biofumigant green manure crops preceding replanting is generally not feasible due to high land prices and economic costs to operations of having land out of production through a growing season. Some growers and consultants in the region, and researchers in other regions (Kruger *et al*., 2015) have been experimenting with growing crucifer species as alley cover crops and, in some cases, as companion crops in the vine-row of established vineyards, usually without incorporating the crops as green manures. However, research to date indicates that the use of nematode-suppressive cover crops in the alleys does not translate to control of populations already established in the root zone. Additionally, such crops can be hosts for target nematode species and unless the crop is incorporated as a green manure to optimize the biofumigant effect, their use can have the unintended consequence of increasing population densities. The majority of research on nematode-suppressive cover crops has targeted species other than *M. xenoplax* and *Xiphinema* species of concern in PNW vineyards. Research on the basic host status of potential cover crops for these nematode species needs to be performed before apparently nematode-suppressive cover crops can be recommended in the region.

Rootstocks

In WA, replanting will increasingly involve switching to the use of rootstocks in response to new recognition of the prevalence of nematodes as well as the recent discovery of phylloxera in the region. In BC, where rootstocks have been used widely since the 1980s, but for reasons other than for nematode resistance (Reynolds and Wardle, 2001), nematode resistance will become an increasingly important criterion in the choice of rootstocks for replanting vineyards. In OR, rootstocks are used widely ever since phylloxera was found in the state and resulted in extensive replanting in the 1990s. The focus of most previous work on development of nematode resistant rootstocks has been the root-knot nematode species *Meloidogyne incognita* and *M. javanica*, and *X. index* (Ferris *et al*., 2012), none of which are present in the PNW (Ferris *et al*., 2012). Few of the rootstocks reported to possess resistance to *M. incognita, M. arenaria* or *X. index* have been assessed for resistance for *X. americanum* (Table 27.2). While resistance to *M. xenoplax* has been evaluated for most rootstocks, few appear to express resistance to *M. xenoplax* (Table 27.2), and even for those rootstocks there are contradictory results among studies and among geographically distinct populations within a study (Table 27.2; Pinkerton *et al*., 2005; Ferris *et al*., 2012). Furthermore, the one relatively recently released rootstock with solid resistance to *M. xenoplax*, UCD-GRN1, is apparently susceptible to temperatures below −5°C and would therefore not be viable in WA and BC growing conditions. While resistant rootstocks have great potential for long-term nematode management, their use in the PNW will require additional research. As the endoparasitic root-knot nematode species *Meloidogyne hapla* is also relatively widespread in the region (Zasada *et al*., 2012), it will be important to continue to assess the resistance of rootstocks to *M. hapla* (Zasada *et al*., 2019) along with the ectoparasites that are the focus of this chapter.

Table 27.2. Susceptibility of grape rootstocks to ectoparasitic (*Mesocriconema xenoplax*, *Xiphinema americanum*) and endoparasitic (*Meloidogyne hapla*) nematode species of concern in the Pacific Northwest of North America.

Rootstock	*M. xenoplax*[a]	*X. americanum*[b]	*M. hapla*[c]
3309C	S, HS, s	S	R
SO-4	ND, S	ND	ND
Riparia Gloire	S, S	ND	R
101-14 Mgt	S, HR,[d] s	ND, S†	R
Teleki 5C	MS, S	S	ND
Kober 5BB	S, S	ND	ND
420A Mgt	S, HR[d]	ND	R
44-53M	MR, S	ND	ND
1103P	S, HS, s	ND, S†	ND
110R	S, R, s	ND	R
Schwarzmann	MS, S	MS	ND
Dog Ridge	S	MR, S‡	ND
Ramsey (sc)	S	S	R
Harmony	S	S	R
Freedom	MS	MS, S‡	R
St. George	S, HS	ND	R
UCD-GRN1	R	ND	ND
UCD-GRN2	MS	ND	ND
UCD-GRN3	MR	ND	ND
UCD-GRN4	MR	ND	ND
UCD-GRN5	R	ND	ND

R, resistant; HR, highly resistant; HS, highly susceptible; MR, moderately resistant; MS, moderately susceptible; S, susceptible; ND, no data.
[a]Within column for *Mesocriconema xenoplax*, the first ratings are from Ferris *et al*. (2012) and ratings after comma are from Pinkerton *et al*. (2005), and then from Schreiner *et al*. (2012) (lower case s).
[b]Within column for *Xiphinema americanum*, first ratings are from Ferris *et al*. (2012) and ratings after comma are from East *et al*. (2021)† or McKenry *et al*. (2001)‡.
[c]Within column for *Meloidogyne hapla*, ratings are from Zasada *et al*. (2019).
[d]Pinkerton *et al*. (2005) indicated that California populations were more aggressive than Oregon and Washington populations.

Outlook and future research requirements

A number of factors are stimulating replanting of vineyards in the PNW of North America. In some areas, vineyards have reached their productive lifespan in a relatively young wine producing region. Shifts in consumer preferences and the widespread discovery of phylloxera in WA vineyards are also driving this replanting. Nematodes were not considered to be significant issues when many of the vineyards in the PNW were initially planted. As a result of limited availability of microclimatically appropriate sites and high land prices for such sites, future renewal of vineyards in the PNW will increasingly involve replanting into previous vineyard sites, many of which will now harbour potentially damaging populations of *M. xenoplax* and *X. americanum*. Consequently, development of an INM programme for vineyards in the region is urgently needed.

As any INM programme must be based on accurate pest identification, future progress will depend on research to clarify the taxonomic relationships of species of *Xiphinema* present in the region, and how they differ in their potential to vector viruses of concern. We propose that replanting with resistant or tolerant rootstocks should be the cornerstone of a regional vineyard INM programme, as rootstocks can potentially provide the most sustainable approach for mitigating the impacts of nematodes in the long term. Previous research has identified a range of rootstocks with resistance to populations of *X. americanum* and limited resistance to *M. xenoplax* in other regions. It will be crucial to examine the host–parasite interactions of these potential rootstocks with regional populations of *X. americanum* and *M. xenoplax* before such rootstocks can be recommended with confidence in the PNW. This challenge is amplified by the need for non-fumigant chemical, biological and cultural practices that are cost effective and do not require valuable land to be out of production for a growing season. We anticipate that as advances are made in the development of new nematicides and non-fumigant pre-plant soil treatments, such practices can be layered onto the foundation of rootstock selection.

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28 *Mesocriconema xenoplax* predisposes *Prunus* spp. to bacterial canker

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Introduction

In 1952, Dewey Raski described a unique ectoparasitic nematode with a 'massive spear' found in a Fresno, California, vineyard. Over the next three decades the genus name of this unique stranger, *Criconemoides xenoplax*, was changed six times while the species name remained unchanged. Since its first discovery it has been recognized as a problematic parasite of *Prunus* spp. In 1970, Norman Ross of UC Cooperative Extension and Harold Lembright of Dow Chemical were demonstrating tools and methodologies to protect against bacterial canker (BC), particularly in the coarse-textured soils of Merced County. There was a single incidence involving the smaller *Criconema mutabile* found damaging apricots in a medium-textured soil of Kern County (McKenry, 1990). *Mesocriconema xenoplax* is typically less important in medium-textured soils but can cause plant damage in highly porous clay loam soils if the rootstock is highly susceptible to *M. xenoplax* (e.g. Marianna 2624 or Myrobalan 29C).

Distribution

The widespread availability of susceptible *M. xenoplax* hosts make it a common parasite because prunus currently occupies about half the 1.376 million ha (3.4 million acres) of tree and vine crops in California. While most nematode pathogens only reduce plant vigour and yield, it is root parasitism at high population levels by *M. xenoplax* that are present at 95% of BC locations. Additionally, 85% of the smallest root tips may be missing or dead 2 years after planting (McKenry, 1996). *Pseudomonas syringae* pv. *syringae* (PSS) coupled with wind and rain delivery into tree wounds is essential for pathogen success. Trees frequently respond with root suckering below the graft union when exposed to *M. xenoplax* in this disease complex (Fig. 28.1). One question needing an answer relates to the possible inactivation of normal hormonal functions associated with the smallest root tips (McKenry, 1996).

To avoid confusion: Pratylenchus vulnus and *Phytophthora* spp. together can be responsible for springtime or summertime death, but root-rotting causes no tree suckers below the

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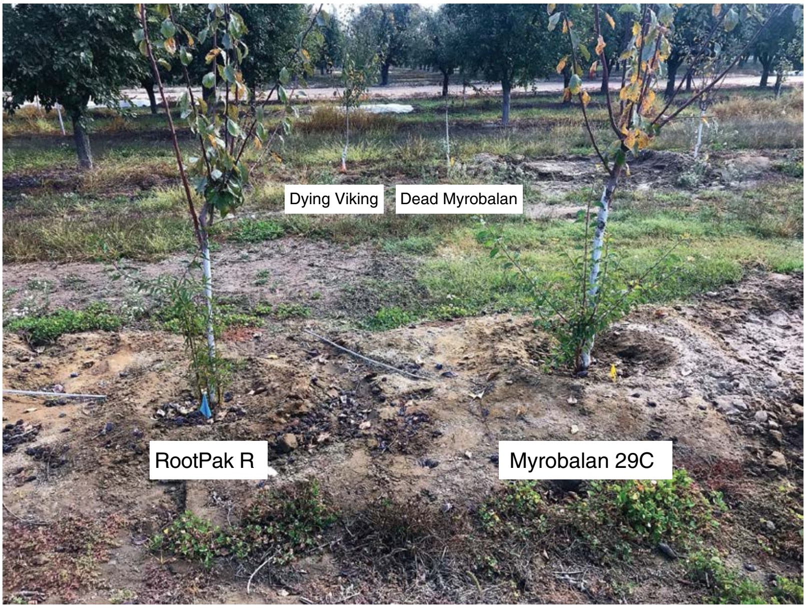

Fig. 28.1. Prune trees in the foreground depict an untreated RootPak R (left) and Myrobalan 29C (right) both showing sucker development associated with the presence of *M. xenoplax* in a plantation with a history of bacterial canker complex. The trees in the background middle depict dying Viking (left) and a dead Myrobalan 29C (right) with no suckers apparent. Author's own photograph.

graft union. It is the population abundance of *M. xenoplax* that results in springtime bud death due to BC. *Pratylenchus vulnus* appears to play no role in the incidence of BC.

Economic importance

When diagnosing tree health problems, population levels below 500 nematodes per 250 cm^3 from moist soil extracted by centrifuge are seldom a useful predictor of *M. xenoplax* damage. But if double that soil population is present in coarse-textured soils and this nematode feeds on woody, non-prunus crops, such as *Vitis*, the absence of leaf and fruit buds among normally fruitful canes can be observed. In addition, yields, plant growth decline and leaf purpling may occasionally occur along the perimeter of autumn leaves. These symptoms are not easily observed because foliar-applied fertilizers can hide incidents of poor root uptake. If 1000 *M. xenoplax*/250 cm^3 occurs in soil around *Juglans*, *Vitis* or *Prunus* spp. it provides the reason to focus on the presence of *M. xenoplax*, regardless of the host. High nematode population levels, as indicated above, do result in reduced crop yields among woody perennials. Uniquely, a good post-plant remedy in California was 'Enzone' (sodium tetrathiocarbonate) which could result in notable plant growth benefits within 30 to 60 days after a spring treatment if applied via drip irrigation where *M. xenoplax* was the major pest problem. Also unique is the ability of high *M. xenoplax* populations in coarse-textured soils to predispose prunus to the lethal disorder that this author prefers to call bacterial canker complex (BCC) because of the many different environmental and management events that may accentuate or reduce BC incidence.

Economic importance of bacterial canker hotspots

The PSS organism and trunk gumming symptoms may have already appeared during the second year after planting but by year three, sucker growth from beneath the graft union coupled with noticeable dead or missing blossoms and leaves associated with limbs, will be observable and will commonly reappear annually with varied intensity. A knife blade drawn beneath the bark of impacted limbs from their tips to the trunk can reveal shallow discolorations and oftentimes fermenting odours, especially if peach scion or rootstock is involved (Teviotdale, 1996). Within a few months the dead limbs are usually removed from the tree and the problem by early summer appears to be gone as neighbouring unaffected limbs expand to fill in the damaged site until the next spring or the spring after that, depending on the intensity of autumn chills and winds. Subsequently, the intensity of limb loss increases, indicating the overall size of the hotspot and consequently the complexity of BCC becomes apparent. Referring to it as a 'hotspot' is appropriate because limbs and entire trees within adjacent land may be completely unaffected by BC for decades, but such trees also commonly support only half the number of *M. xenoplax* or the soil type may involve subtle differences from site to site. Soil types (soil textures throughout the surface 150 cm) may become a useful predictor of nematode population levels associated with *M. xenoplax*.

Bacterial canker complex

Prunus scions yield fresh, canned or dried peach, plum, prune, nectarine, apricot, cherry, almonds or selections of hybrids or mutations of prunus. Harvests begin in spring and end in autumn. But for each of the hundreds of different scions there is only a single harvest period each year, thus it is usual to have dozens of different scion selections harvested for each crop throughout the 5-month warm period. The prunus nut crop is almond which can be stored after dehydration, much the same as prunes, apricots or peaches which can be dried or canned and stored. But importantly, there are also unique complexities among these trees relative to BCC incidence.

Complexity 1

The first important complexity relative to BCC is that not all these various scions exhibit the same sensitivity to the disorder. Also, some scions eventually recover from the annual death events while others do not. Some of these differences may relate to fungal control (e.g. *Hirsutella* spp.) impacting *M. xenoplax* numbers or *Meloidogyne* numbers if the rootstock is Lovell peach in the presence of *Dactylella oviparasitica* (Stirling *et al.*, 1979).

Complexity 2

Parentage of the *Prunus* rootstocks can play a role. Plum and almond rootstocks can be among the best hosts for *M. xenoplax*. The same can be said of almond parentage but only 10% of the world's almond varieties have been explored to date. Many of these may be poor hosts in their first few years but then become very supportive hosts by year six.

Complexity 3

Soil types are indicative of soil textural differences found at each different depth to 150 cm. For alluvial deposits, this information is vital. Sandy soils (e.g. sand dunes) are by far the most supportive for *M. xenoplax,* especially sands that are too sandy to even support *Meloidogyne* spp. Also supportive are the loamy sand to coarse sandy loam soils that support *Meloidogyne* spp. and can also compete with *M. xenoplax* once silt or clay content exceeds 12%.

Complexity 4

The fact that *M. xenoplax* resistance mechanisms have been found but are not necessarily thermophilic suggests they provide a form of tolerance (De Ley, 2012). Viking and Guardian rootstocks provide 6–7 months of resistance mechanism, while two peach rootstocks, Lovell and HBOK1, remain resistant at higher temperatures and offer 12 months of resistance mechanism each year. None of these offer high levels of resistance to *M. xenoplax* but do provide notable tolerance to *M. xenoplax* if planted after a pre-plant treatment, and particularly if

D. oviparasitica is prevalent, there may be no *Meloidogyne* spp. that survive on Lovell rootstock (Stirling, 1979).

Complexity 5

Relative to BCC incidence it was suggested that nutritional inadequacies due to nematode feeding may be more important than high population levels of *M. xenoplax*, but a 4-year study has mostly refuted this notion (Cao *et al.*, 2006).

Recommended integrated nematode management (INM)

Note that the autumn–winter population counts are typically higher than those in the warmer seasons. Soil population levels held to less than 500/250 cm^3 per sample are usually not impacted by BCC. The complexities listed above elucidate some ways that populations may be naturally reduced. The best three integrated management tools include: (i) pre-plant soil fumigants applied to deep-dried soil; (ii) post-plant treatments that reduce nematode population levels without plant damage; and (iii) selection of suitable prunus rootstocks for the specific prunus scion that will be grown while also providing a mechanism of resistance that reduces population levels by at least half when compared to Nemaguard rootstock (a California Standard). BCC results in limb death and eventually tree death. Death of trees older than 5 years in coarse-textured soil results in having to contend with an even larger problem: the replant problem (RP) for which there are two components. The grower now needs to make decisions about the eventual size of the hotspot. Will it occur across the entire field, randomly in a few tree rows or individual trees that are widely distributed? No predictor of the eventual size and longevity of a new BCC incident can be completely trusted because of prunus complexities.

The following four case studies represent management options where not all the INM tools are necessary.

Field site 1 Dinuba, California, USA

In 2009, 3 years after issues forced a lousy Telone II application, I was contacted about 250 dead or dying nectarine/Nemaguard trees on 4 ha (10 acre). It was BCC.

Three remedies tested: (i) 250 clones of HBOK1 (Harrow Blood × Okinawa) rootstock were available for large-scale field testing; (ii) pre-plant methyl bromide was available for 100 individual tree sites to compare against 150 non-fumigated tree sites; (iii) post-plant nematicide 'Movento' (Spirotetramat) was registered in 2010 for California use and we knew by then it could provide 50% nematode reductions for 5 to 6 months. A hopeful means for preserving the healthy 4-year-old trees which remained alive in the orchard.

Results: This orchard continues to be perfectly intact and economically viable in 2020. By 2016 it was apparent that one Movento treatment per year in spring or autumn during root flush was adequate if no post-treatment irrigations occurred within 9 days after the application. A pre-plant treatment was also needed to avoid the rejection and nematode components of RP. Essentially, HBOK1 peach without pre-plant fumigation was too closely related to Nemaguard Peach thus the pre-plant biocide doubled HBOK1 tree size in year two but within 3 years HBOK1 growth was similar across the entire field because the rejection component of RP commonly lasts only one year.

Field site 2 Easton, California, USA

I was contacted in 2011 by the grower of 100 ha (250 acres) of prunes who struggled for decades with a single hectare of a BCC hotspot (McKenry, 2019).

One remedy tested: Half the trees (improved French prunes/Myrobalan 29C) in this hotspot could not be kept alive more than 3 or 4 years. I had suggested annual treatments of Movento foliar spray and no irrigations for 9 days after treatment.

Result: In 2014 I revisited the site because the grower was aware of my work with a non-fuming pre-plant biocide and the Movento treatments I had recommended had not increased tree longevity.

Three remedies then tested: In an area of 84 tree sites, half the trees received pre-plant aqueous cyanamide solutions delivered to 150 cm depth. The remaining 42 trees were untreated except for the use of five different prunus rootstocks including Krymsk1, Lovell, Viking, RootPak R and Myrobalan 29C. If treated trees began to show nematode presence after 1 year we would then treat with a new post-plant nematicide in years 2 and 3.

Results: Regardless of rootstock, our preplant biocide trees doubled their height and girth compared to adjacent untreated trees (Figs 28.2 and 28.3). It was also apparent that tolerance in Lovell or Viking rootstocks compared to Myrobalan 29C could show a 50% drop in population levels of *M. xenoplax*. Just as dramatic as these two rootstocks, the use of our yet unnamed post-plant nematicide performed very well in an orchard where Movento had not performed well because it does not adequately protect young root systems of perennials.

Field site 3 Hanford, California, USA

I was contacted in January 2020 regarding nematode presence in a December 2019 almond planting following 1 year of fallow and no preplant fumigation in a 16 ha (40 acre) very fine sandy loam orchard.

Only one new remedy tested: I had already learned that Movento can only do a great job if the trees are already bearing so I tried my newer post-plant material which is especially useful for non-bearing trees. It impacts any ectoparasitic

Fig. 28.2. These young root systems received protection from nematodes and the root component of the replant problem within a zone 150 cm deep and 150 cm wide for the length of the drenched row. High densities of *M. xenoplax* remain beyond the treated zone. Improved sources of plant resistance plus new strategies for post-plant nematicides are essential. At the end of the 2020 season, we had lost only one of the 42 pre-plant treated trees and it was not due to nematodes or replant problems. Author's own photograph.

Fig. 28.3. In these untreated rows there was no uniformity of growth, thus all 42 untreated trees were removed at the end of the year 2019. Myrobalan 29C was the most vigorous of the five rootstocks but three were dead by the time of their removal. In the foreground the dead tree is a Viking and the next tree is a yellowing Myrobalan 29C about to die. Author's own photograph.

stage as deep as 4 feet. However, this is a fine sandy loam soil having 10–20% clay particles so *M. xenoplax* is unlikely to ever be in abundance, and plenty of soil samples confirmed the problem in the future will be *P. vulnus.* The grower only needs a post-plant nematicide useful during the two non-bearing years of almond; then it will be time for Movento once annually.

Results: It is now August 2020 and the author can reveal that these 16 ha of almond were not impacted at all by what I call the rejection component of RP. Since 2006 I have been expounding on *starving* the old soil ecosystem and then *switching* to a very different rootstock (S&S) as an alternative to soil fumigation (McKenry, 2015, 2017). This grower had removed a failing 4-year-old walnut orchard then waited a full year and then replanted almonds on Nemaguard Peach Rootstocks. The rejection component of RP was solved but my new task is to now protect against the nematode component of RP and for that I now have two very different post-plant nematicides to evaluate.

Various sites since 2006

At a UC Davis Conference in 2006 I first spoke about starving the rejection and nematode components of RP by applying glyphosate to old Nemaguard trees, waiting a full year and then switching to Hansen 536 (peach × almond) rootstock. Attempt this only if the soil is not coarse-textured because Hansen 536, after a few good years, can later become an excellent host for *M. xenoplax*.

Results: A few nursery people and pest control advisers who attended the conference have occasionally stated that it might work in additional settings.

Optimization of nematode management

Starve and switch is the future when combating the replant problem. Growers of orchards and vineyards in the future need new tools and workable strategies to replace the well-known values of fumigants. The S&S event for field site 3 provides a recent example that killing old *Juglans* roots, waiting a year, and then replanting *Prunus* can provide freedom from the rejection component of RP, but one still must be cognizant of the nematode component of RP. The rejection component of RP has also been mitigated within grapes (McKenry and Bettiga, 2013). Grape rootstock 10-17A (*Vitis simpsonii* x Edna) does not benefit from fumigations using methyl bromide or Telone C-35 when replanting vines. For contrast, eight other new *Vitis* rootstocks have very much benefited from soil fumigation in that they were all a bit too closely related to the *V. vinifera* history for that land. While screening walnut rootstocks my lab found a nematode tolerance mechanism against *P. vulnus* when using our new walnut Paradox rootstock, VX211 (*Juglans regia* × *Juglans hindsii*). We quickly learned that by planting VX211 when following *J. hindsii* we were able to avoid most of the rejection component of RP. However, it became very important to fumigate the land to be planted to VX211 if the previous orchard involved any seedlings or clones of Paradox. Think of S&S as one more approach within INM.

Future research requirements

Too many grants are offered with minimal attention to real-life field settings and/or scientists lacking adequate diversity in their backgrounds. To be successful with rootstock selection plant breeders need interactions with pathologists/nematologists.

Outlook: anticipating future developments

Several approaches were listed above. Meanwhile, as food consumption doubles and chromatography improves, logarithmically let's consider 'healthy' consumption as the goal.

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29 A threat to stone fruit and grape production: Tomato ringspot virus (ToRSV) transmission by *X. americanum* s.l. (sensu lato)

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Introduction

The soil and topography of south-central Pennsylvania are ideal for tree fruit. For many years, the state's fruit industry grew and prospered primarily by producing processing apples. However, as markets changed and processing fruit became less profitable, growers began to diversify into more lucrative fresh market stone fruit and a small but growing demand for wine grapes.

As stone fruit acreage increased, it became apparent that a disease known as prunus stem pitting (PSP) was widespread in the region. PSP is a lethal disease caused by the tomato ringspot virus (ToRSV) and transmitted by the dagger nematode. All peach varieties and most other stone fruit are susceptible to ToRSV. The same virus also causes a decline in certain wine grape varieties. In order to develop strategies to prevent the occurrence of these diseases it is important to understand the interaction between *Xiphinema americanum* s.l. and ToRSV.

Economic importance

Orchards and vineyards are long-term investments. They are expensive to establish and require years of maintenance before a marketable crop is produced. Nevertheless, once in full production and the initial investment recovered, a grower can anticipate a profitable return for many years. Orchards and vineyards in Pennsylvania are expected to remain in production at least 25 years but often remain profitable for much longer.

Proper site preparation is key to profitable fruit production. If the intended crop is known to be susceptible to ToRSV then it is critical to test for the presence of dagger nematodes and ToRSV. If the site tests positive, then additional steps to control these pathogens must be taken before planting. This will delay planting and add to the cost. However, if growers do not take these precautions they may find that their orchards are not as profitable as anticipated and have a shortened productive life due to tree mortality. In extreme cases, orchards have declined even before the cost of establishment was recovered.

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The financial loss to the grower by removing declining orchards extends beyond the cost of removing trees and lost income. When orchards or vineyards are destroyed, not only is the current source of income removed but there will be the added cost of renovating the site and planting a new crop. The new orchard will require another 3 to 4 years of maintenance before a marketable crop can be produced.

The initial level of infestation will determine how quickly the orchard or vineyard becomes unprofitable. Typically, a grower will not know that their crop is infected until several years after planting. Often the problem begins with the appearance of a few diseased trees, but the number increases each year until eventually the orchard is not profitable enough to justify keeping. This may take a number of years or could happen within a few seasons. Orchards with 70% tree mortality 4 years after planting have been reported (personal observation).

Calculating the total economic impact of PSP to the fruit industry is complicated by a number of variables, including the market value of different peach varieties, the size of the orchard, cropping system, yield and how long the orchard remains in production. However, using estimated average production costs, establishing a 10-acre peach block would require about US$109,260 in the first 4 years. This would cover the cost of site preparation, purchasing trees, planting, pruning, weed and pest control and harvesting. A small crop could be harvested in the third year and a near full crop harvested in the fourth year with an anticipated income of US$92,202. Therefore, a grower will not recover his investment until the fifth year. After the initial investment is recovered, the profit on a 10-acre peach block after maintenance and harvest costs are covered would be an anticipated US$25,402 per year (Harper and Kime, 2020–2021). This profit margin would begin to shrink each year as trees are lost due to PSP.

Host range and distribution

Xiphinema americanum or the dagger nematode is common throughout the mid-Atlantic region and has an extensive host range including many woody and herbaceous monocots and dicots. In one study, the results from 434 random soil samples sent to the Penn State University nematode testing service were summarized. The samples represented 1980 acres (801 ha) of land and a range of cropping systems including orchards, field crops, ground covers, pastures and fallow. Dagger nematodes were recovered from every cropping system and only 14.9% of the samples were free of the nematodes. Of the 369 samples with dagger nematodes, the counts ranged from 1 to 409 nematodes per 100 cm^3 of soil. Cropping systems with the highest numbers included fallow, orchards and lucerne, each with some populations over 200 nematodes per 100 cm^3 soil. However, most samples had very low numbers and the average was only 28.8 nematodes per 100 cm^3 of soil (Halbrendt, 1992). Other research attempting to identify a natural suppressive ground cover for *X. americanum* s.l. indicated that only *Tagetes patula* appeared to be a poor host for dagger nematode (personal observation).

ToRSV is also common throughout the mid-Atlantic region and can be recovered from many broadleaf weeds and woody plants. One of the best-known examples is dandelion (*Taraxacum officinale*) that may be important for distributing the virus since the wind-blown seeds also carry the virus (Mountain *et al.*, 1983). Although it has a broad host range, it appears that ToRSV does not infect monocotyledon plants and is not recovered from grass or grain crops.

It is interesting to note that native plants infected with ToRSV typically do not show symptoms of infection. It could be that co-evolution of ToRSV and native vegetation has resulted in plants that are tolerant of infection and thereby serve as a reservoir of ToRSV in nature. Peaches, which are not native to North America, have not co-evolved with the virus and die when they become infected (Nyzcepir and Halbrendt, 1993). Some apple cultivars are resistant to ToRSV while others are susceptible. It has been shown that when apple cultivars resistant to ToRSV are grafted to rootstocks susceptible to the virus the trees succumb to a disease known as apple union necrosis and decline. However, other rootstock/scion combinations do not develop the disease (Biggs, 2019). This disease has largely been eliminated from commercial apple production by avoiding susceptible rootstock/scion combinations.

Symptoms of damage

Dagger nematodes are ectoparasites that feed primarily on the tender root tips and root hairs. Root tip swelling and stunting has been reported when high numbers feed on small herbaceous plants such as strawberry but feeding damage does not appear to be a problem on woody plants (personal observation). In orchards and vineyards, dagger nematodes are only a problem as a vector of ToRSV.

Initially, peach trees infected with ToRSV may appear slightly stunted but otherwise healthy and produce a normal crop. However, in the autumn, leaves will lose colour and drop earlier than non-infected trees. The following season, fruit will develop but the tree will suddenly collapse in mid-summer. The tree will lose its foliage but the unripe fruit will remain hanging (Figs 29.1 and 29.2). If tree bark is removed near the soil line it will be thicker and have a corky appearance compared to healthy trees. The underlying wood will not be smooth, rather it will have pits and grooves which gives the disease its name. The degree of pitting and grooving in the wood will vary with different cultivars.

The most striking symptom on grape infected with ToRSV is what is referred to as the 'hen-and-chick' grape clusters. These are grape clusters that have a few normal sized berries, but the majority are small and tasteless (Fig. 29.3). If the vine has more than one main trunk the hen-and-chick clusters may initially be present only on one side of the vine but eventually the entire vine will be infected. Infected vines may appear somewhat stunted but otherwise look healthy and survive for several years.

Biology and life cycle

Two species of *Xiphinema* are commonly found in Pennsylvanian orchards and vineyards. These are *X. americanum* and *X. revesi*. They can

Fig. 29.1. Spread of tomato ringspot virus in a peach orchard. Stunted tree in the foreground shows early symptoms of PSP and will die next season. Author's own photograph.

Fig. 29.2. Sudden collapse of prune stem pitting infected peach tree in mid-summer. Foliage will be lost but the fruit will remain hanging on the dead tree. Author's own photograph.

Fig. 29.3. Healthy grape cluster (A) and symptoms of tomato ringspot virus infection transmitted by *Xiphinema americanum* referred to as the 'hen-and-chick' grape cluster (B). Author's own photographs.

be distinguished based on subtle morphometric differences but there does not appear to be any significant difference in their biology, host range or virus vectoring capability. Therefore, for practical purposes they are considered together as *X. americanum* s.l. There is some debate over splitting dagger nematodes into numerous species based on subtle morphometric differences. However, one clear difference that sets these nematodes apart from other species is that they only pass through three juvenile stages before becoming adult instead of the usual four stages (Halbrendt and Brown, 1992). The nematodes are almost exclusively female, reproducing by parthenogenesis. It is extremely rare to find a male specimen (personal observation).

Interactions with other nematodes and pathogens

In order to develop an effective strategy to prevent virus infection, it is important to understand the interactions between the nematode, virus and host plants.

ToRSV cannot survive outside of a host or vector. Nevertheless, the virus can be found throughout south-central Pennsylvania in many broadleaf weeds and woody plants. Some herbaceous plants pass the virus on through seed, and this can be a mechanism for long-distance dispersal. However, ToRSV can only pass from one plant species to another with the aid of a vector. Two vectors of ToRSV have been identified, *X. americanum* s.l. and the parasitic dodder (*Cuscuta gronovii*).

Dodder is relatively common throughout south-central Pennsylvania. It apparently does not transmit the virus to woody plants but may serve to build up a large virus reservoir in nearby weed populations (Welliver and Halbrendt, 1992).

Xiphinema americanum s.l. is the only vector known to transmit ToRSV to woody plants. As it feeds, the nematode introduces the virus to a living cell which is essential for virus replication. Evidence suggests that adult nematodes are capable of transmitting ToRSV throughout their life which is estimated to be several years (Jaffee *et al.*, 1987). The long life cycle of *X. americanum* s.l. contributes to the difficulty of controlling virus spread in perennial cropping systems.

Recommended integrated nematode management (INM)

Nematode management is warranted when there is a potential for crop damage. Damage threshold levels have been established for many plant parasitic nematodes to aid growers in determining when and to what extent nematode management should be employed. However, establishing damage threshold values for *X. americanum* s.l. as a virus vector presents unique challenges. The nematode is only a problem when it transmits ToRSV to a susceptible crop. Without the virus, even high population levels do not cause disease problems in orchards and vineyards (personal observation).

Nematode control measures delay planting and increase the cost of orchard establishment. Growers prefer not to add nematode management to site preparation if it is not necessary. However, because of the risk of ToRSV transmission, nematode testing services often recommended control of *X. americanum* s.l. even at low population levels. This may be unwarranted if ToRSV is not present. Ideally the decision to employ nematode control measures should be made in combination with results of virus testing that confirms the presence of ToRSV. Although this information would be useful, there is relatively little demand for this type of testing and there are no commercial virus testing labs that provide this service for growers.

Nevertheless, understanding the interaction of *X. americanum* s.l., ToRSV and weeds serving as virus reservoirs in nature can aid in determining whether or not nematode management practices are justified. Three examples follow:

- Monocots do not serve as reservoirs of ToRSV and there is very little risk of virus transmission to new orchards or vineyards that are planted on sites with a cropping history of maize, wheat or other grains. Providing that good broadleaf weed control has been practiced, such sites tend to be free of ToRSV even if the dagger nematode population is relatively high.
- Commercial apple orchards are typically not affected by ToRSV but broadleaf weeds within the orchard frequently harbour the virus. Therefore, stone fruit orchards planted on old apple orchard sites are at high risk of virus infection even if the dagger nematode population is very low.
- Although ToRSV cannot survive outside of a host or vector, it is very successful in infecting new hosts. The virus can be distributed over long distances in the seed of some plants and the dagger nematode transmits the virus to new hosts. Fallow fields that are unmanaged for any length of time are very likely to harbour ToRSV infected plants due to invading weeds.

For many years soil fumigation with methyl bromide was a standard practice for preparing a peach orchard in Pennsylvania. Fumigation added extra cost but was very effective for controlling *X. americanum* s.l. and other soil-borne diseases. Orchards on fumigated soil grew well and had a long productive life with little tree mortality. Despite its effectiveness, soil fumigation is rarely, if ever, utilized today. Custom fumigation companies have gone out of business and growers cannot justify the cost, special equipment, licensing and legal requirements of applying fumigants themselves. Post-plant nematicides have not been very effective for preventing virus transmission likely because surviving nematodes continue to feed on weed reservoirs harbouring ToRSV.

Biofumigation is an alternative approach to managing nematode populations and may help renovate a new orchard or vineyard site, but it requires further testing for efficacy in orchards (Dutta *et al.*, 2019). Biofumigation is based on the natural chemical defences that some rapeseed and other *Brassica* spp. use to deter insect feeding. When the plant is injured, glucosinolates present in the tissues are quickly converted to toxic isothiocyanates by hydrolysis. For biofumigation to be effective, large quantities of isothiocyanate must be generated. This is accomplished by planting a cover crop of rapeseed known to produce high levels of glucosinolate and allowing it to grow almost to maturity to maximize biomass. While still green, the rapeseed is chopped with a flail mower and quickly tilled into the soil. This will release a flush of isothiocyanate into the soil to reduce the nematode population (Matthiessen and Kirkegaard, 2006).

Current recommendations for preparing new and replant orchard and vineyard sites that test positive for dagger nematodes includes a 2-year rotation with biofumigation and green manure crops such as sudangrass. The addition of sudangrass in the rotation will suppress the broadleaf weed population and help eliminate reservoirs of ToRSV. Incorporation of the sudangrass into the soil at the end of the season will also add organic matter and improve the soil (Fiola, 2020). In addition, a 2-year rotation will improve replant sites by providing the grower an opportunity to make adjustments to soil pH and optimize nutrient levels.

Optimization of nematode management

Previously, soil fumigation was a quick and very effective tool to prevent virus infection. However, due to environmental concerns it appears that fumigation will no longer be an option. The recommended 2-year rotation with biofumigation and green manure crops is effective if done properly, but it does require postponing planting and there is no income during the renovation period.

A few seed companies have acknowledged the benefit of biofumigation and are attempting to breed rapeseed with higher levels of glucosinolate to improve the process. A 1-year biofumigation rotation would be more acceptable to growers but this may not be sufficient to make the site safe for replanting.

Outlook and future research requirements

Research to stop ToRSV spread in stone fruit and grape has focused on management practices the grower can employ to prevent infection. Reducing the dagger nematode population is important but eliminating the virus reservoirs is also key. Because of the widespread distribution and broad host range of dagger nematodes and the naturally occurring reservoirs of ToRSV, it appears that the risk of virus transmission will always be a concern in Pennsylvanian orchards and vineyards.

The ultimate solution to the ToRSV problem would appear to be incorporating virus resistance or tolerance into the crop. For example, it is known that grafting apple cultivars resistant to ToRSV onto rootstocks susceptible to ToRSV will result in tree death, but resistant or susceptible apple cultivars can be grafted to resistant rootstocks with no problem. This knowledge permits the establishment of apple orchards on dagger nematode infested soil without concern.

In order to develop virus resistant crops with commercially desirable traits, a source of virus resistance must first be identified. Native American grapes are not affected by ToRSV and would appear to be a potential source of resistance. Over the years, breeders have crossed *Vitus lambrusca* with *Vitus vinifera* to develop acceptable hybrid wine grapes, but generally have not tested for virus resistance in the process. After going into commercial production, some hybrid grapes have proven to be susceptible to ToRSV while others appear to be resistant. This could have resulted from virus resistance being unknowingly carried over in the breeding programme.

Peaches present a somewhat greater challenge for developing virus resistant trees since only the plum rootstock Marianna 2624 has been reported to have resistance. Nevertheless, great progress is being made in the field of genetics with the development of designer genes and it may one day be possible to engineer a resistant rootstock for peaches.

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30 A multi-pronged approach for the management of plant parasitic nematodes in vineyards in South Africa

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Introduction

In South Africa, grapes are grown for wine production and table grapes, with a small percentage grown for raisins. Vineyards are grown using dryland farming or irrigated with drip- or micro-irrigation. Of the approximately 120,000 ha planted to vines, the vast majority (85%) is under wine grapes (Table 30.1). However, the economic value of the table grape production primarily destined for the export market exceeds that of the wine production of grapes. South Africa is also the fifth largest producer of raisins worldwide, with an estimated 65,589 tonnes of raisins produced in 2017.

The total hectares of vineyards in South Africa for 2019 stood at 92,067 hectares. This has decreased steadily by a total of 9.1% since 2009. As of 2019, Chenin Blanc is the most planted cultivar, followed by Colombar, with Cabernet Sauvignon and Shiraz being the most planted red cultivars (Table 30.2). Viticulture stretches across ten regions in South Africa, with 90% of the production in the Western Cape province (Fig. 30.1). This wide distribution covers an extensive range of soils and climates. The Western Cape has a Mediterranean climate whereas the table grape production area along the Lower Orange River is in a semi-desert region, with very high summer temperatures.

The most common pests and diseases of grapes in South Africa include the banded fruit weevil, the pear leafroller, the grapevine mealybug, flower thrips, the African bollworm, grape berry moth, powdery mildew, downy mildew, bacterial leaf spot, black rot and plant parasitic nematodes.

Economic importance and distribution

The nematodes that parasitize grapevines include root-knot nematode (*Meloidogyne* spp.) including *M. javanica*, *M. incognita* and *M. arenaria* (Fig. 30.2).

Root lesion nematodes (*Pratylenchus* spp.) are also very common. Within the dagger nematode group, *Xiphinema americanum* and *X. elongatum* are the most prevalent species. The grapevine fanleaf virus, which is transmitted by *X. index*, is becoming an ever-increasing problem. In South Africa, *Criconemoides xenoplax* (identified by molecular probes) is the most commonly found

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Table 30.1. Grape production figures of the 2014–2015 growing season.

	Wine grapes[a]	Table and dried grapes[b]
Planted area (hectares)	99,463	18,212
Production (metric tonnes)	1,519,708	291,442
Rand value (ZAR in billions)	4.7	5
Export percentage	44	90
Rand value of export (ZAR in billions)	8	7.4

[a]J. Lombardt, SA Wine Industry Information and Systems NPC, Stellenbosch, 2016, personal communication.
[b]C. Whitehead, South Africa Table Grape Industry, Stellenbosch, 2016, personal communication.

Table 30.2. Surface areas of most planted vineyard cultivars planted in South Africa in 2019.

Cultivar	Hectares	% of total
Chenin Blanc	17,103	18.6
Colombar	10,601	11.5
Cabernet Sauvignon	10,087	11.0
Sauvignon Blanc	9,654	10.5
Shiraz (Syrah)	9,178	10.0
Chardonnay	6,685	7.3
Pinotage	6,662	7.2
Merlot	5,371	5.8
Ruby Cabernet	2,009	2.2
Cinsaut	1,659	1.8
Muscat d'Alexandrie	1,606	1.7
Pinot Noir	1,201	1.3
Semillon	1,023	1.1
Other	9,227	10.0
Total	92,067	100

nematode in vineyards and is considered to be the nematode responsible for most of the damage to grapevines (Figs 30.3 and 30.4).

To a lesser degree, stubby root nematodes (*Paratrichodorus* spp.), the citrus nematode (*Tylenchulus semipenetrans*) and pin nematodes (*Paratylenchus* spp.) are also present in the grapevine production soils.

Recommended integrated nematode management

The vine growers in South Africa are familiar with nematode problems. The growers are familiar with the different genera as well as the rootstock resistance which is available to them. They have access to information regarding the host status of a limited number of cover crops against ring and root-knot nematode. They use diagnostic laboratories, extension services and private companies to take measures to control nematodes as outlined below.

The success of an integrated pest management programme in grapes in South Africa is based on six principles developed by the extension service and other research organizations and includes: prevention; monitoring; risk determination; decision making; intervention; and evaluation.

Prevention

The old adage 'prevention is better than cure' certainly holds true for nematodes. Once a vineyard's soil is infested with a specific nematode species, it is extremely difficult, if not impossible, to eradicate that species from the soil.

Regulatory requirements

Various national plant certification schemes reside under the South African Plant Improvement Act. This is to ensure that healthy plant material is provided to growers. In South Africa, the Vine Improvement Scheme requires that all vine nurseries are tested free of the presence of the dagger nematode, *Xiphinema index*, which transmits grapevine fanleaf virus.

Nursery material must also be visually free of root-knot nematode symptoms when lifted from the nursery. This measure does not guarantee absolute nematode-free plant material, but it does give the producers a measure of reassurance. Any rooted vine material showing root-knot nematode symptoms (galling) is dipped in a bath of hot water at 50°C for 30 minutes to kill root-knot nematodes (Fig. 30.5).

Resistance

Vine rootstocks resistant to root-knot nematode, and in particular *M. javanica* and *M. incognita*, are widely used in South Africa. The rootstock

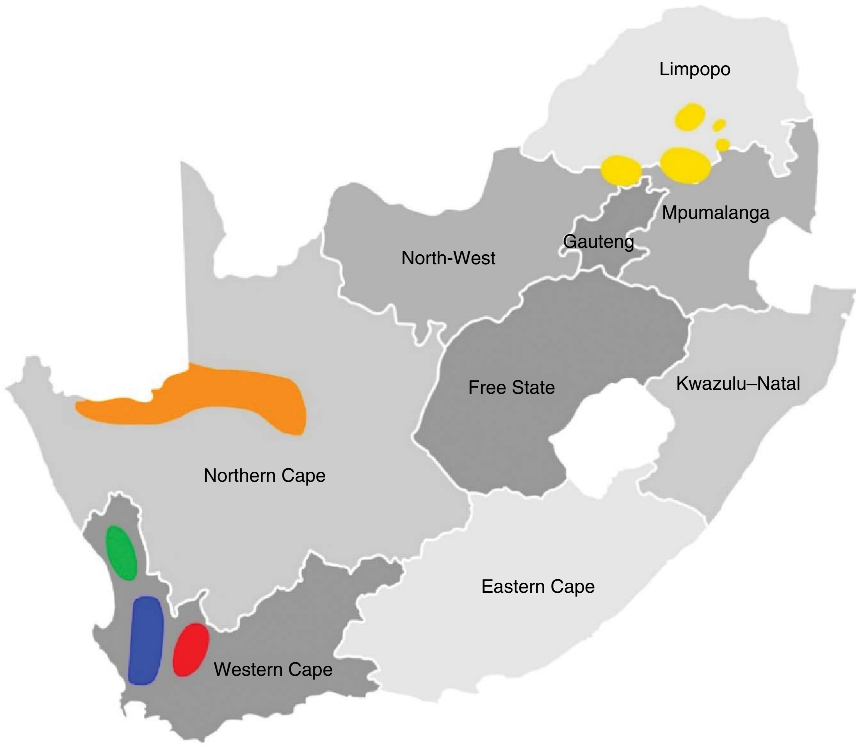

Fig. 30.1. Table grape growing regions in South Africa. Figure courtesy of South African Table Grape Industry – SATI.

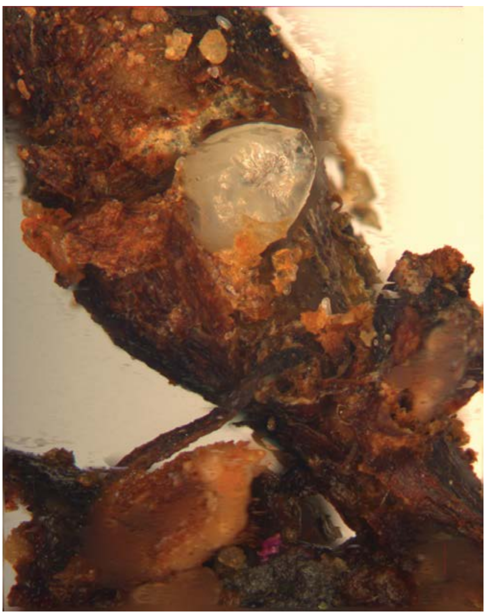

Fig. 30.2. Anterior of root-knot nematode female visible in a vine root. Photograph courtesy of Welma Pieterse.

resistance and level of resistance is shown in Table 30.4. However, observations by various nematologists suggest that in the case of the rootstock Ramsey the resistance to root-knot nematode has broken down. No rootstock resistance to other nematode genera is known.

Monitoring

Monitoring is by way of visual scanning of the symptoms, deductions and/or by means of a laboratory analysis. In the case of grapevines, the majority of the current rootstocks are resistant or moderately resistant so galls are seldom seen. Producers look at patches of poor growth, reduced berry or bunch with size, short internodes and poor potassium uptake. The visual scanning is then confirmed by a laboratory analysis. Some of the larger production units run tests on a regular basis. Laboratory assays using the Jenkins centrifugal flotation, modified Baermann or Flegg techniques will show which nematodes are present in the soil and/or roots and also the

Fig. 30.3. Area of poor growth in a vineyard as a result of *Criconemoides xenoplax* infection. Author's own photograph.

Fig. 30.4. Deformed roots caused by *Criconemoides xenoplax* damage on grape roots. Author's own photograph.

Table 30.3. Plant parasitic nematode genera occurrence in vineyards in the Western Cape Province of South Africa (Smith, 1977).

Nematode genus	Frequency of occurrence (percentage) of genera in 100 soil samples
Pratylenchus	86
Meloidogyne	77
Xiphinema	70
Criconemoides	48
Nanidorus and *Paratrichodorus*	41
Paratylenchus	30
Helicotylenchus	27
Longidorus	27
Scutellonema	20
Tylenchorhynchus s.l.	13
Rotylenchus	12
Tylenchulus	10
Hoplolaimus	9
Hemicycliophora	6
Criconema	3

population levels of each. In 2019, almost half of the samples tested at Nemlab were vine samples (Table 30.5).

Risk determination

Once the species or genera present and their population levels are known, the risk can be determined. Various factors are taken into consideration when determining the risk. These include the host status of the rootstock, the life cycle of the nematode and reproduction rate with due consideration given to the many factors influencing the reproduction rate, such as soil type, temperature (seasonal), water source and vine age. The risk is divided into three levels: (i) high; (ii) intermediate; and (iii) low. The level of risk will influence the decision (Table 30.6).

Fig. 30.5. Samples in a hot-water bath to treat root-knot nematode infestation. Photograph courtesy of Rinus Knoetze.

Decision making

Once the risk has been determined, a decision must be made about which management strategies can be considered. The strategy can be either long term or short term.

Intervention

The type of intervention is determined by factors such as the value of the crop, cost, risk type and time available until planting. Intervention can be divided into: (i) fumigation; (ii) nematicides; (iii) management; (iv) biology; and (v) root and soil health.

Fumigation

Given the high cost of land in the Western Cape and prevailing economic conditions, leaving soil fallow for 2 to 3 years is no longer economically viable. Fumigation is therefore a solution when extreme population levels exist, but even then, fumigation should only be done after the soil has been left fallow for a year, otherwise endoparasites such as *Meloidogyne* and *Pratylenchus* survive inside the old vine roots. In addition, the prerequisites for successful soil fumigation, such as the correct soil temperature for the specific product; the depth, moisture, organic material

Table 30.4. Grapevine rootstock resistance to *Meloidogyne* spp.

Rootstock	Resistance level	Rootstock	Resistance level
Ramsey	Resistant	110 Richter	Moderately susceptible
99 Richter	Moderately resistant	US 1-9	Moderately susceptible
101-14 Mgt	Moderately resistant	US 8-7	Moderately susceptible
143 B Mgt	Moderately resistant	Jacquez	Susceptible
1103 Paulsen	Moderately resistant	140 Ruggeri	Susceptible
US 2-1	Moderately resistant	C Metallica	Susceptible
3306 C	Moderately resistant	1202 C	Susceptible

content, condition of the tilth; and sealing of the soil, makes fumigation an unpopular practice for farmers. Also, recent studies have shown that *C. xenoplax* occur at depths of 60 cm and deeper, causing visible above- and below-ground damage to the vines (see Figs 30.4 and 30.5), rendering the use of fumigants less effective in such vineyards. It might be more effective against *Xiphinema* spp. and *Longidorus* spp., which can occur at depths of 10–30 cm. According to South African law, the majority of the fumigants, 1,3-dichloropropene and metham sodium, may only be applied by a registered pest control officer. Due to the toxicity profile of the fumigants and difficulty to apply them, the use of fumigants has dwindled.

Table 30.5. Sample types tested at Nemlab in 2019.

Sample type	% of tests done
Vines	47
Citrus	11
Apple	9
Plum	5
Peach	4
Potato	3
Maize	3
Pear	2
Triticale	2
Tomato	2
Pecan	1
Other	12

Nematicides

Nematicides remain the most popular form of intervention. The timing of a nematicide application is the key to a successful treatment. In the case of vines, it coincides with the root flushes in autumn and spring. Apart from correct timing, another requirement is dispersal of the chemical through the root zone. Given the requirement of water for the adequate dispersal of nematicides through the root zone, effective control is not always achieved. The active ingredients registered in South Africa for use against nematodes in vineyards are: cadusafos, fenamiphos and furfural. DiTera is a biological contact nematicide registered for the control of nematodes in table grapes. It has as an active ingredient referred to as killed *Myrothecium verrucaria* solids and solubles. The fungus is produced in a fermenter and then killed. The by-product is nematicidal.

Failure of control is linked to a lack of residual activity in the soil, which is influenced by:

- leaching by means of too much irrigation or rainfall;
- chemical degradation, e.g. a soil pH >7;
- ultraviolet light – nematicide exposed to sunlight;
- organic material present in soil or as a mulch; and/or
- accelerated microbial degradation.

Table 30.6 Nematode density threshold levels in California vineyards. Source: Grape Pest Management, University of California, Berkeley, California.

	Nematodes present in 1 kg of soil					
	Low population		Medium population		High population	
Nematode	Oct–Mar	Mar–Oct	Oct–Mar	Mar–Oct	Oct–Mar	Mar–Oct
Meloidogyne spp.	<75	<25	75–500	25–200	>500	>200
Xiphinema americanum	<20		20–200	20–100	>200	>100
Pratylenchus vulnus	<20		20–100		>100	
Tylenchulus semipenetrans	<50		50–500		>500	
Stubby root nematodes	<20		20–200		>200	
Ring nematodes	<50		50–500		>500	
Paratylenchus spp.	<100		100–1000		>1000	
Xiphinema index	<20		20–200		>200	
Longidorus spp.	<20		20–200		>200	
Helicotylenchus spp.	<50		50–500		>500	

Management

There is a definite move away from the expensive and mostly harmful nematicides to more sustainable practices. More and more winemakers are producing organic wines, which prescribes no chemical nematicides.

Fallow

Fallow as a control measure is the temporary removal of all vines from a vineyard to deny pathogens their host. The soil must, however, be kept free of weeds and volunteer plants which may act as hosts in this period. At least 1 year of fallow is a prerequisite for successful soil fumigation of vineyards and fruit orchards, otherwise endoparasitic nematodes such as root-knot nematodes will survive the fumigation. Fallow is not a feasible option under the climatic conditions of the Mediterranean Western Cape. The dry, windy summer months and wet winters can lead to water or soil erosion.

Cover crops

Rotation requires knowledge of the nematode species and in some cases also the race present. The host range of the nematode(s) present must also be known. The host range can take on various agronomic characteristics, i.e. resistance, tolerance or susceptibility. The terms used are non-hosts, poor or weak hosts, and good hosts. Care must be taken with the crop selection because a particular crop may reduce the population of one nematode but increase the level of another. Cover crops used in vineyards in South Africa are shown in Fig. 30.6.

Oats are the most commonly used cover crop in South African vineyards. Contrary to popular belief, oats are a good host to *M. javanica* (Hugo, 2009), but as the oat roots do not show galling it has been overlooked as a host. For three consecutive seasons, canola (cv. Jade) and yellow mustard (cv. Caliente 199) showed a constant reduction in the *C. xenoplax* population in the vine row (Kruger *et al*., 2015). However, canola is a very good host for two root lesion nematode species (*Pratylenchus neglectus* and *P. quasitereroides*). When considering a cover crop in the vine row, careful consideration must be given to the most important nematode present and the host status of the cover crop.

Biological control

Possible biocontrol agents include fungi and bacteria. Biological control agents are generally highly specific and provide some level of disease management, but only if used together with other disease control strategies. Their effectiveness can also be limited by certain soil conditions e.g. soil temperature. There are currently no registered biological products available to producers.

Novel chemistry

The current focus on nematicides is focused on finding plant or tree extracts that have nematicidal properties. Examples of such extracts include neem, quillaja, garlic extract and sesame oil. On the local front, researchers are looking at indigenous plants with known pest suppressive properties. However, some of these plants also carry molecules that are harmful to humans.

Root and soil health

Nematodes are known as 'stress pathogens'. This refers to their ability to attack compromised roots as a result of drought or poor growing conditions. Any amendment or treatment that improves or stimulates root growth will lessen the possibility of the nematode parasitizing the roots. The amendments or treatments used can include:

- Plant growth promoting rhizobacteria such as the various *Bacillus* spp. which stimulate root growth, preventing or delaying invasion by the pathogen.
- Mycorrhizae are beneficial fungi that act as an extension of the host plant's root system and greatly facilitate the uptake of nutrients, such as phosphorus. In this association they act as bioregulators and protectors against some pathogens.
- Some endophytes trigger a broad-spectrum defence response of the plant, either through systemic acquired resistance (SAR) or induced systemic resistance (ISR), e.g. salicylic acid, flavonoids and harpin protein.

Fig. 30.6. Cover crops, mainly cereals, used in two vineyards of the Cape Winelands region in the Western Cape, South Africa. Author's own photographs.

- Antagonists, particularly the fungi *Trichoderma*, are antagonistic to a number of soil-borne pathogens including nematodes. The latest research suggests their action is based on a form of SAR and/or ISR.
- Products containing auxins and cytokinins, which act as root stimulants, go a long way in providing healthy roots. The locally produced seaweed extract, Kelpak, is an excellent example of such a product.
- Carbon supplements in the form of compost, mulches, compost tea, animal manures, bark, etc., are often added to the soil to improve soil structure. These organic amendments must adhere to quality control standards to prevent contamination with unwanted by-products. Successful organic amendments generally require large amounts of material to be added to the soil. Their use is localized, being limited by available raw materials and transportation costs.

A very important aspect of any integrated pest management programme is monitoring and evaluation. Throughout the growing season observations must be logged. At the appropriate times in the growing season samples must be analysed and the results interpreted to measure the success of the control strategy. The decision taken prior to planting or to application should be re-visited and a new measure decided on if the previous treatment was unsuccessful.

Conclusion

Nematode control in a perennial crop such as grapevine poses tremendous challenges to both researchers and extension officers. However, much progress has been made during the last 20 years and with the continuous development of more environmentally friendly practices, a number of more sustainable viticultural practices are now available to growers.

Outlook: anticipating future research requirements

Vine production in South Africa is on the decline. However, quality grapes are now sought for quality wine. This will mean a definite decrease in the use of nematicides. Future research into the use of novel control measures which include biocontrol, novel biology and soil health will be required.

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31 Litchi and guava nematode challenges in South Africa: Can we change nematode communities and minimize the problems?

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Introduction

Lychee (*Litchi chinensis*) and guava (*Psidium guajava*) are both subtropical crops planted in limited areas of South Africa. Lychee cultivation is estimated at around 1800 ha, with many of the orchards being more than 25 years old. This has brought about its own problems, including nematode and fungal infections that together may result in poor vigour, yield losses and, eventually, tree death.

Guava was cultivated on 1200 ha until recently when, unfortunately, guava production was drastically reduced due to guava wilt disease (GWD) caused by the fungus *Nalanthamala psidii*, and nematode problems throughout most of the production areas in the north-eastern parts of South Africa (Schoeman and Labuschagne, 2014). When GWD disease was originally observed, no nematode problems had been recorded on guava. However, when a new guava wilt resistant cultivar TS-G2 was released by the end of the 1990s, nematodes suddenly became a major problem on guava (Willers, 1997), damaging large parts of the guava industry in the north-eastern part of South Africa.

Economic importance

In lychee, damage is not linked to one specific nematode species but rather a combination of species of *Criconema, Helicotylenchus, Hemicycliophora, Meloidogyne, Pratylenchus, Xiphinema*, as well as *Hemicriconemoides strictathecatus*. Nematode damage can cause lychee slow decline, which has a significant impact on growth potential and yield. Yield losses due to nematode damage have not been determined but are estimated to be considerable. In addition, these trees are prone to attack by a range of root-rot fungi (including *Pythium, Phytophthora, Cylindrocladium, Fusarium, Rhizoctonia* and *Armillaria*) responsible for litchi die-back, causing specific branches or, in severe cases, the entire tree to succumb to the disease (Daneel *et al*., 2010; Manicom, 2010). The farmers struggle to distinguish between both sets of symptoms but are very aware of declining or dying trees especially in old orchards, with problems observed throughout the lychee producing areas.

Nematode damage in guava is mainly caused by *Meloidogyne enterolobii*, although *M. incognita* and *M. javanica* have also been found

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infecting guava (Visagie *et al.*, 2018). The damage done to the root system is severe, causing the entire tree to succumb within a few years. When newly planted trees are attacked, the trees never reach adult age and produce no marketable fruit. In the guava producing areas in the eastern and northern parts of South Africa, nematode damage is a severe problem and can reduce guava production to a non-profitable scale and farmers are acutely aware of the nematode problem.

Host range

Although a combination of nematode species has been recovered from lychee, only *H. strictathecatus* was recovered from all the lychee producing areas. This species is found on lychee and mango trees in South Africa (Van den Berg *et al.*, 2015). The other nematodes recovered from lychee have been found on a variety of crops. It is worth mentioning that although both *Meloidogyne* and *Pratylenchus* are found on lychee, lychee does not seem to be a major host for these genera.

In South Africa, *M. enterolobii* is more widely distributed than anticipated as it has been recovered from green peppers (*Capsicum annum*), maize (*Zea mays*), potato (*Solanum tuberosum*), soybean (*Glycine max*), tomato (*Solanum lycopersicum*), weeds and all guava orchards consisting of the cultivar TS-G2 (in the eastern and northern parts) sampled were found to be infected by this species (Visagie *et al.*, 2018; Rashidifard *et al.*, 2019). In the Western Cape Province, the problem is insignificant due to the usage of another cultivar 'Fan Retief', that seems to be resistant to *M. enterolobii* and other *Meloidogyne* spp. infections. A recent screening confirmed this statement (W Steyn, Mbombela, 2020, personal communication). The cultivar TS-G2 proved to be highly susceptible to *Meloidogyne*, since it was released in 1990s to replace Fan Retief, a selection that was highly susceptible to GWD and this has resulted in orchards being destroyed by root-knot nematodes.

Distribution

The species complex differs between the production areas (which vary in climatic conditions, but all have a range of soil types), with *Hemicycliophora* and *Meloidogyne* only being found in the Kwazulu-Natal Province whereas *Pratylenchus* and *Xiphinema* were only found in the Limpopo Province. The most abundant species recovered from all samples throughout the lychee production areas is *H. strictathecatus* with numbers being as high as 4950 individuals per 250 ml soil (Daneel, 2017).

For guava, *M. enterolobii* and, to a lesser degree, other thermophilic *Meloidogyne* species are widely distributed in the northern and eastern parts of South Africa, while root-knot nematodes seem to be absent or much less abundant in the guava producing areas in the south-western parts, due to the use of a tolerant cultivar Fan Retief. Recently, several studies were conducted on distribution and recovery of *M. enterolobii*, with the recovery rate on guava in the northern and eastern parts of South Africa being 100% (Visagie *et al.*, 2018; Rashidifard *et al.*, 2019).

Symptoms of damage

Besides having a tap root, lychee has lateral absorptive roots that consist of an entirely shallow lateral branching root system with most roots being concentrated in the top layer of soil to a depth of about 20 cm. Below-ground damage of the root system is noticeable in a less dense root system and while healthy roots are mostly whitish, damaged roots have stubby, darkened appearance with limited development of feeder roots. Galls are seldom visible. Nematode damage triggers excessive leaf drop, resulting in bare twigs and branches ending in a tree with a more open canopy where weeds grow underneath the tree canopy, in contrast to healthy trees where the canopy is very dense and no sunlight is able to reach the soil (Fig. 31.1). Furthermore, infected trees experience poor flowering and bear small, less abundant fruits compared to healthy trees. The nematode complex is, however, not able to kill the entire tree, even over a period of several years, and the symptoms are known as litchi slow decline in South Africa. When combined with fungi such *Pythium, Phytophthora, Cylindrocladium, Fusarium* and *Rhizoctonia* that are often isolated from roots of affected trees, dying back of branches and eventually the death of trees are experienced since these fungi prevent nutrients and fluids from being translocated to the upper part of the trees (Fig. 31.2).

Nematode damage on guava trees typically resembles below-ground symptoms caused by root-knot nematodes, ranging from galls on the root system with eventual rotting of the roots due to infection by soil pathogens. Above-ground symptoms appear as reduced tree growth and vigour, smaller leaves, leaves turning yellow or reddish, reduced fruit set with smaller fruit and eventually death of the entire tree (Fig. 31.3). It is important to note that nematodes cause the complete tree to die over a period of one to several years in contrast to GWD where the whole

Fig. 31.1. Symptoms of nematode damage on lychee tree showing an open canopy, bare twigs and branches and weeds growing underneath the canopy compared to the healthy tree on the left. Author's own photograph.

Fig 31.2. Symptoms of lychee die-back. Author's own photograph.

tree or parts of the tree can die within a few weeks while the dead tree still carries dried leaves and fruit.

Biology and life cycle

Most of the nematodes associated with lychee are ectoparasites, with the exception of *Meloidogyne* and *Pratylenchus*, which are recovered from root and soil rhizosphere samples but have never been recognized to be really important as damaging species of lychee. Several generations per year can occur with a shorter life cycle during summer and considerably lower activity during the colder winter months.

For guava, *M. enterolobii* is the main culprit and the life cycle of *Meloidogyne* spp. can be found described in various literature. It is interesting to note that in a glasshouse study conducted by Collet (2020) on maize, soybean and tomato, it was observed that the life cycle of *M. enterolobii* had shorter degree days than *M. incognita* and *M. javanica* for the three crops tested. This could possibly partly explain why the guava industry struggles to contain the root-knot problem, as the usual nematode control strategies are not sufficient.

Interactions with other nematodes and pathogens

Nematodes and environmental factors, including drought, soil compaction, poor watering regime and incorrect fertilization, enhance stress to the tree, increasing its susceptibility to root-rot fungi. While these organisms are present in soils throughout the year, they are able to switch from saprophytic to pathogenic when such stress signals are detected. The fungi subsequently enter the roots, obstruct nutrient and fluid movement, resulting in branch and tree death. This is in contrast to damage caused by nematodes which cannot kill the tree.

For guava, no interaction has been detected between nematode damage and GWD, except for the observation that highly susceptible cultivars and selection to GWD might show tolerance to nematodes and vice versa. This was highlighted by cultivar Fan Retief and seems to be true for other more recent selections and cultivars. Cultivar Lucknow, imported from India, seems to possess some tolerance to both *M. enterolobii* and GWD (Maritha Schoeman, Mbombela, 2020, personal communication).

Fig. 31.3. Symptoms of nematode damage on guava tree compared to a healthy tree on the right. Author's own photograph.

Recommended integrated nematode management (INM)

In the lychee industry, INM includes application of selective chemical products such as cadusaphus or fenamiphos together with compost, manures and organic material as the latter amendments enhance root growth and soil health.

Because of large amounts of mulch and decomposed material found underneath the canopy area, biological control could be a possible alternative in nematode control. Several years ago, trials were conducted to test the efficacy of *Purpureocillium lilacinum* (previously known as *Paecilomyces lilacinus*) and the results showed good nematode control. However, product registration was never completed, mainly because lychee production areas are too limited in South Africa and it was therefore not economically sustainable to register products on such a minor crop (H Botha, Mbombela, 2005, personal communication).

Selections from the guava breeding programme are screened continuously for resistance to root-knot nematodes and GWD, which remains one of the major pillars in a successful INM programme to reduce the nematode problem on guava in South Africa. Furthermore, cover crops, addition of organic material, the use of resistant/tolerant plant material and biological control in combination with chemical nematode control are part of the INM strategy. Chemical control on its own has not been successful in controlling root-knot nematodes in guava, causing orchards to be abandoned as trees eventually died.

Optimization of nematode management

Lychee trees are mainly propagated using air layering, where the stem is wrapped with damp moss to encourage roots to form. This technique is effective and fast but results in trees with shallow root systems. Seedlings and grafted trees form taproots, resulting in deeper and more extended root systems that provide a more stable root environment which is most likely more tolerant to attack by fungi and nematodes. It is important to optimize farming practices that include optimal soil preparation, fertilization, irrigation and other practices that can reduce stress in the soil rhizosphere and increase tolerance to nematode infections.

Although breeding programmes form part of the lychee research programme, screenings do not include testing for tolerance/resistance to the nematode community. A major problem is that lychee is attacked by a combination of nematode genera of which most are difficult to rear, making screenings very difficult.

One of the aims should be to diversify nematode communities whereby the most pathogenic species are replaced, at least partially, with plant parasitic species that are present in the soil rhizosphere but do not seem to cause damage compared to pathogenic species, otherwise called mitigating species. This could be achieved by planting other crops such as grasses, under or around the canopy, that are favoured by these mitigating species (including *Helicotylenchus dihystera* and *Tylenchorhynchus*), especially when lychee trees are young.

For guava, the use of tolerant or resistant cultivars to both nematodes and GWD must be developed continuously. Registration of greener chemical and biological control agents will help in the fight against nematodes. Cultural practices need be fine-tuned and used more consistently and effectively. These will include the use of mixtures of cover crops and the addition of organic amendments such as mulches, manures and compost.

Mixtures of cover crops can be established at planting to increase biodiversity. Such mixtures should include combinations of grasses, nitrogen-fixing plants and Brassicaceae, while it will have to include plants that are resistant to *M. enterolobii* and preferably also to *M. incognita* and *M. javanica* as both these species have been found on guava. Presently, such screening studies are being conducted (H Fourie, Mbombela, 2020, personal communication). Cover crops will not only reduce nematode and disease problems by direct action, but they will reduce weed problems, erosion and water loss through evapotranspiration resulting in an increase in nutrient availability and provision of higher biodiversity in the soil environment. It is anticipated that due to increased biodiversity, nematode communities can be manipulated and highly pathogenic ones be replaced by less pathogenic species; thereby further reducing nematode problems.

If no action is taken to manage nematodes and GWD, this will result in the complete disappearance of the guava industry in the north-eastern parts of South Africa.

Tools available in other industries such as infra-red photographs should be integrated in management programmes to predict, anticipate and manage nematode problems.

Future research requirements

For minor crops to be cultivated successfully in the future, more interest from the government will be necessary. This will include resources for additional research and a more efficient registration process of chemicals (including biological control products) which will make it more appealing to the chemical industry especially for minor crops. This will ensure that new safer nematicides are also registered in minor crops.

More focus needs to be directed to alternative nematode control strategies. Breeding and subsequent screenings will always be a very important factor in nematode control. However, research is necessary on improving biodiversity in orchards to decrease pest and disease problems. Biodiversity can be enhanced by planting a variety of cultivars/selections together in one orchard, by planting combinations of cover crops maybe even as a permanent cover in orchards. This above-ground biodiversity will most likely influence and increase biodiversity below ground. Additional methods for improving biodiversity below ground include the addition of composts, manures and organic amendments. There is a wealth of organic materials that need to be evaluated and might provide excellent nematode control.

Another important aspect is manipulation of nematode communities by providing feeding material for mitigating species. If pathogenic species can be replaced this way, nematode problems will be considerably reduced.

Outlook: anticipating future development

Going forward towards 2050 and thereafter, it is anticipated that no currently known class I chemical control products will be available for nematode control and that producers will use a completely new strategy in controlling nematodes. A major aspect will be the use of resistant or tolerant cultivars. In addition, a variety of commercial biological control agents and softer chemical control agents will hopefully be available for use on the majority of crops produced in South Africa. These chemicals can manage specific problems such as *M. enterolobii* on guava.

We also anticipate that orchards will be planted using a variety of cultivars/selections of one or more crops to increase biodiversity. This will inherently reduce pest and disease pressure but will require a change of attitude by the producer because it will entail a different production system since harvesting, irrigation and fertilization will have to be carried out according to precision agricultural principles. Monitoring will also be a key activity in reducing pest and disease problems.

Climate change will put pressure on resources such as water, but subtropical crops will mostly be produced in the warmer areas and we do not foresee significant changes in nematode communities attacking the above-described crops.

Future developments will entail precision farming even in perennial crops such as fruit trees.

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32 *Pratylenchus vulnus* going nuts in California

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Introduction

Nut crop production in California is on a strongly expanding trajectory. For almond, world market shares above 80% are reported. Walnut and pistachio production expand at more moderate rates, but nut crops occupy increasing percentages of the agricultural lands. In the highly productive California Central Valley, soil-borne pests and diseases find a highly conducive soil environment for their parasitic activities. The deep rooting soils either naturally or rendered by heavy duty mechanical deep-loosening tillage to 1.5–2.0 m soil depth are favourable growth environments for tree roots and their nematode parasites. Soil sampling to these depths often reveals the highest population densities below 1 m depth. The expanse of soil volume creates challenges for the mitigation of these soil dwelling nematodes.

Soil fumigation has been an essential tool in dealing with these infestations. After the phase out of the highly effective and versatile methyl bromide in 2005, fumigation tactics rely on 1,3-dichloropropene (1,3-D). Chloropicrin is often added to the fumigant mix if fungal and bacterial diseases are to be reduced simultaneously. These fumigant strategies have been useful to establish healthy orchards and are paramount if orchards follow a planting of the same species. Due to environmental and human health concerns, fumigant uses are under review, and alternative management strategies are urgently needed.

A stellar example of using genetic control for reducing plant parasitic nematodes is the peach rootstock 'Nemaguard' that has highly durable resistance to the southern root-knot nematodes, *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. It has successfully suppressed these root-knot nematode species for more than 50 years, protecting stone fruit and almond. Because of its susceptibility to other soil-borne pathogens and less than optimal anchorage in high wind areas, this rootstock is slowly being replaced by modern peach–almond hybrid rootstocks. There is a significant pool of rootstocks with resistance to root-knot nematodes, but virtually all of the currently available rootstocks for almond production have some susceptibility to *Pratylenchus vulnus*, the walnut root lesion nematode.

In the principal host walnut, challenges with *P. vulnus* are exacerbated. Walnut is frequently and most productively grown on fine-textured soils. After the ban on methyl bromide, these areas face increased challenges when establishing new orchards. The alternative soil fumigants

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require more stringent soil conditions for efficacy in these soil texture compositions. It is often difficult to create the tilth and low moisture content in these fine-textured soils necessary to allow the less volatile 1,3-D to effectively penetrate the soil matrix. The development of rootstocks within *Juglans* has long been hampered by the demands of the propagation. Although the widely used 'Paradox' seedling rootstock presents a hybrid, it has been extremely difficult to fully exploit superior rootstocks of seedling populations. Implementing tissue culture since 2000, namely excising hybrid embryos from directed or random crosses, enabled exploiting the genetic breadth of the *Juglans* gene pool for nematode and other resistances. Tissue culturing from vegetative or embryonic plant material allows for culturing multiple copies of desirable genotypes, enabling novel selection strategies that have led to novel elites of rootstocks. Three clonal rootstocks are presently available commercially.

In pistachio, the third important nut crop, nematode problems, in general, have been discounted. Such is based on surveys of the 1980s where few plant parasitic nematodes were detected in pistachio soils (McKenry and Kretsch, 1984). The role of plant parasitic nematodes in pistachio in California remains suspect, and it remains to be seen how it is ultimately defined. In greenhouse pot trials, high nematode numbers developed on pistachio rootstocks. Under field conditions, numbers were much lower than under *Prunus* and *Juglans*. Increasing production areas of this crop often following crops with known nematode loads call for vigilance in monitoring for potential nematode problems.

Economic importance

Population densities are a key factor when making soil fumigation decisions. Thresholds for fumigation for *P. vulnus* are fluid at the writing of this chapter. Soil fumigation typically allows for a quick and uniform establishing of a new planting of almond or walnut. While predictors for risk of root lesion nematode damage on almond are given in ranges of population densities, the threshold for walnut is reached as soon as one nematode is detected. This extremely low level for damage in walnut makes mitigation methods mandatory because even the highly root lesion nematode-tolerant rootstock (compared to other rootstocks) 'VX211' can be damaged by low and medium population densities compared to cultivation in nematode-free soil when first planted.

Host range

In the absence of sufficient host range studies, *Pratylenchus vulnus* appears fairly limited in its hosts. Examples of woody perennial hosts are *Juglans, Malus* and *Prunus*, and maybe to a lesser extent *Pistacia*. *Vitis,* typically a host for many different plant parasitic nematodes, can be a host, but *P. vulnus* infestations seem to rarely occur under field conditions. In a recent greenhouse screen of potential cover or companion crop species for nut crop orchards, differences among brassica and legume plants were found, but populations supported were lower than under the included nut crop hosts.

Distribution

Pratylenchus vulnus is widely distributed within the Central Valley of California. In a survey in the 1990s, about 85% of the walnut production area tested was found to be infested with this nematode (McKenry, 1997). Current production practices favour its spread. Virtually all equipment that operates in soil and is used on multiple farms can further spread the nematode infestation. For example, expensive equipment for deep loosening the soil could be a vehicle for nematodes to be disseminated because it is difficult to clean it of infected root residues and contaminated soil. Such equipment may introduce initial infestations in a new field that can be further distributed with tillage operations. Such initial infestations may take years, if not decades, before discovery when damaging population densities are reached.

Symptom of damage

Traditionally, above-ground nematode disease symptoms in trees have been described as 'unthriftiness'. This rather complex term summarizes the lack of tree size compared to same aged healthy trees close by. It refers to the lack of new growth or severe stunting as trees come out of dormancy. Plants lack vigour despite proper fertility levels and adequate irrigation practices. A *P. vulnus* affected orchard is characterized by uneven plant growth from tree to tree down a row and across rows. Root symptoms of *P. vulnus* are frequently described as necrotic lesions caused by the nematode's migratory feeding. These are often difficult to confirm, and symptoms alone are not diagnostic. Laboratory procedures that include extracting the nematode from roots or surrounding soil are necessary to identify the nematode.

Biology and life cycle

Pratylenchus vulnus is classified as a migratory endoparasite. Against this over-simplification, a large proportion of its life history is spent in soil surrounding the host roots. This nematode remains in vermiform shape for all its life. The mature female lays eggs within the root tissue. There, the nematodes meander through the cortical tissue while feeding on single cells. The necrotic plant response to this feeding is believed to lead to those name-giving lesions. Noteworthy is that under artificial inoculations in field trials with a *Juglans* breeding population after 1 year, >55% of genotypes harboured no extractable root lesion nematodes in the roots. After 2 years of field cultivation, most genotypes (>90%) had high numbers of nematodes in the roots (Westphal, unpublished). Because there was no additional soil inoculation with the nematode, those root infections are surmised to be the result of populations that sustained themselves in the soil. A pre-infection resistance had been previously reported and may have contributed to these observations. It is unclear what triggers the nematode to enter roots, or what changes in their population allowed such. It is known that they prefer the terminal 15 cm roots (Buzo *et al.*, 2009).

Interaction with other nematodes and pathogens

Pratylenchus vulnus is a challenge for replanting crops of the same species. It is an additional pest that can possibly exacerbate the syndrome of the 'replant problem' of almond or walnut. Hypothetically in this disorder, trees on rootstocks of the same species as cultivated in a field previously are prone to microbially incited lack of performance (Fig. 32.1). The aetiology of this replant problem has not been solved, and it is not clear what key organisms are involved in these detrimental soil characteristics. The lesion nematode can be part of this disorder but does not seem required for growth reductions.

Interactions of *P. vulnus* with other plant parasitic nematodes warrant further investigations. Some observations under greenhouse conditions suggest positive feedback of root lesion and ring nematode infections on pistachio. More detailed studies are required to fully describe these interactions.

Recommended integrated nematode management (INM)

As of this chapter's writing, the management of *P. vulnus* relies heavily on pre-plant soil fumigation (McKenry, 1999). Observations and experiments quantifying the effects of early infections of tree crops currently support this strategy as long as meaningful population densities of *P. vulnus* are found in soil extracts. While thresholds for nematode mitigation vary between almond and walnut, but especially in walnut, the orchard removal protocol includes at least a 1-year process of killing the trees of the pre-crop with a systemic herbicide to destroy feeding sites within the roots before plant removal to reduce contained nematodes. After plant removal, a cover crop should follow to reduce soil moisture to levels conducive to soil fumigation operations (CDFA, 2009). Proper soil fumigation following soil moisture conditions and preparation recommendations can support vigorous early and uniform growth of new plantings.

Currently, few tools are available that would allow foregoing this critical step in orchard establishment. The grower attitude 'I know, I have a *zoo of nematodes* in my orchard soil, but I will just plant and deal with the problem later' seems risky at best. In studies with almond on a nematode susceptible rootstock in heavily infested soil

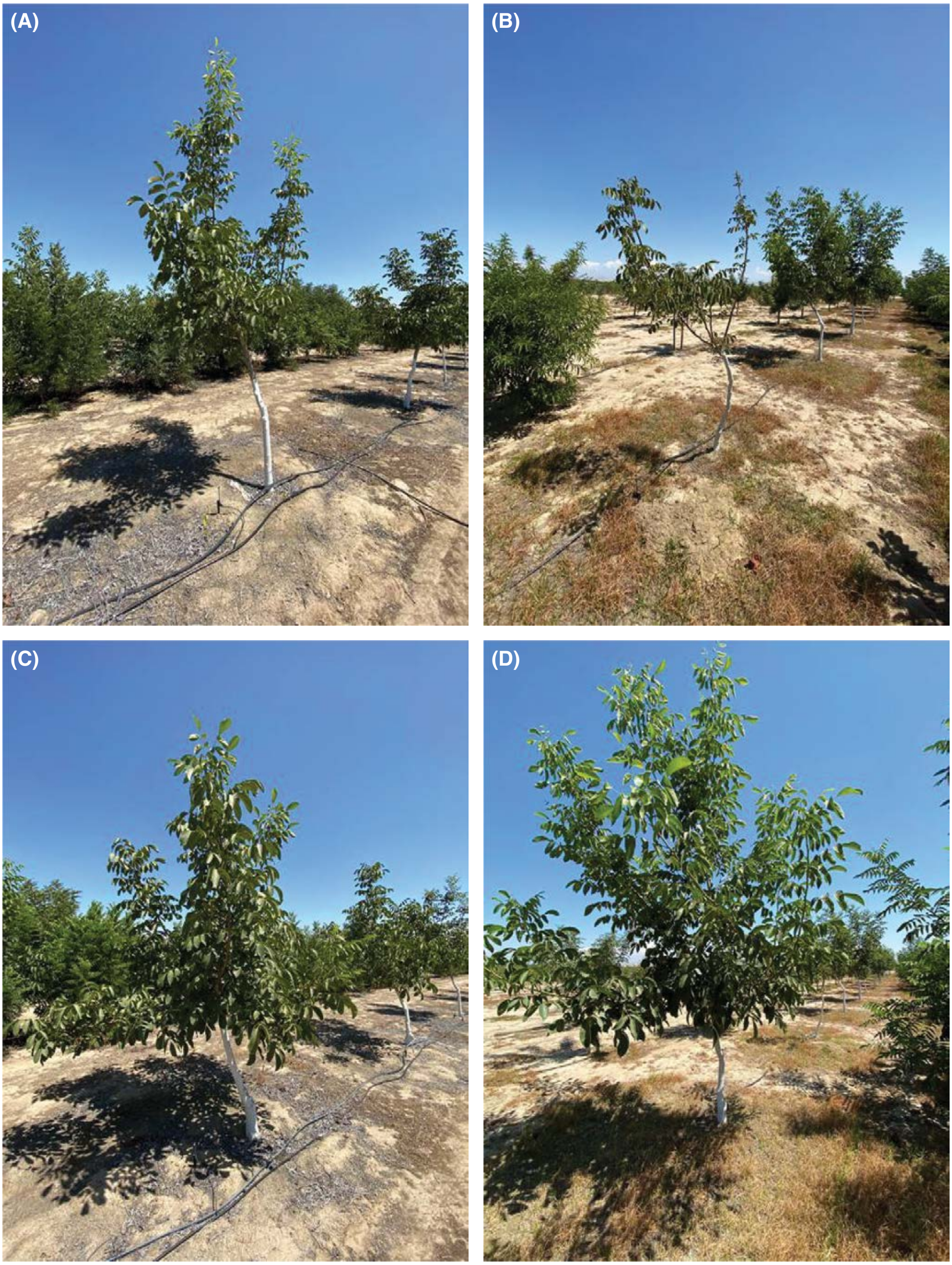

Fig. 32.1. Walnut 'Chandler' planted own-rooted (non-grafted) in **(A)** fumigated or **(B)** non-fumigated soil following walnut; or 'Chandler' grafted on rootstock 'XV211' in **(C)** fumigated or **(D)** non-fumigated soil following walnut. Multiple trees of the same rootstock genetic varied. The figure summarizes the benefit of soil fumigation in protecting non-adapted roots and the benefit of a rootstock with increased tolerance to soil-borne maladies under non-fumigated conditions. Author's own photographs.

without pre-plant treatments, it took multiple years before a somewhat productive orchard was attained. While trees in this orchard after 4 years of treating with chemical post-plant nematicides grew somewhat uniformly and vigorously, they continue to yield below standard and seem to lack the vigour and productivity necessary for making economic returns. Likely, foregoing pre-plant soil treatments will require active nematode management for the life of the orchard. There was insufficient early yield for creating a positive cash flow after absorbing establishment costs. Because of the higher sensitivity of walnut rootstocks, it is even more critical to be pro-active in reducing damaging nematode populations.

Even with soil fumigation, the choice of rootstock is critical. Under commercial conditions, the most comprehensive soil fumigation is unlikely to eradicate nematode problems from a field. If the planting is in an area where *P. vulnus* seems to thrive and has been very damaging in the currently replaced orchard, then the rootstock choice needs to take this into account. In walnut, the clonal rootstock 'VX211' provides some tolerance protection from *P. vulnus* of new plantings. Because of its sensitivities to the microbial replant problem and some apparent susceptibility to root-knot nematodes, care needs to be taken when choosing it. Its tolerance to root lesion nematode should not lead to a risky bypassing of pre-plant soil fumigation.

Studies on the use of more biologically based management approaches are ongoing. For example, anaerobic soil disinfestation (ASD) has shown the potential to reduce the adverse effects of plant parasitic nematodes. It may equal or outperform chemical soil fumigation partially because of the added fertility aspect when done with the proper protocol. Costs and logistics challenge this method where typically large amounts of an easily decomposable substrate (e.g. 20 t ha^{-1} of rice bran) are spread and incorporated in the soil, drip lines are placed on the soil surface, which is covered with a plastic tarp. The soil is initially heavily watered and then kept moist for about 1 month. While highly effective, this method appears currently too expensive to become a replacement for chemical soil fumigation.

Cover cropping has appeal to reduce nematode population densities. In a pre-plant strategy, the direct effects of resistant cover crops could be combined with a biological disinfestation strategy similar to ASD. These techniques range from biofumigation approaches with brassica crops to nematode antagonistic plants that are followed by the bio-disinfestation method. Companion cropping within an orchard will need to allow the nut crop roots and the cover crop to explore similar soil volumes for potential antagonistic activities to express themselves to the benefit of crop growth. Many facets of this strategy still need to be worked out. In existing orchards, risks for phytotoxicity of this additional crop on the main crop need to be considered because materials toxic to pest or weed populations may have negative side effects on the main crop. Cover crop strategies can be complicated for the grower who needs to take care of two crops in the same field. Herbicide strategies and the need for a 'clean floor' for nut harvesting, especially in almond production, need to be considered.

Optimization of nematode management

Nematode management relies heavily on pre-plant soil fumigation. This makes the production systems vulnerable in the dependence on one active ingredient to ensure protection from severe nematode damage. As of today, the availability of 1,3-D is restricted by so-called township caps. This quantitative restriction per 36 square mile area is challenging when multiple crops benefit from its use, and not all demands can be served. Other broad-spectrum material (e.g. metam sodium) are difficult to implement for treating soil layers infested with the nematode. Adding more pre- and post-plant strategies to the mix of options is critical to protecting young (and older) orchards from nematode infections when pre-plant opportunities are foregone because of low levels of nematode infestations. The use of companion crops in nut crop orchards needs to be further optimized as far as candidate crops, including known nematode-suppressive plants radishes, sunn hemp and marigold, and their planting patterns are concerned. Adding these tools to the mix of strategies can reduce reliance on a single method and make the production more sustainable, environmentally safe and economically feasible.

Future research requirements

Genetic control of the long-term plantings of 25 to 45 years has broad appeal for nematode management. Efforts must not tire to develop and deploy superior rootstocks in *Prunus*, *Juglans* and *Pistacia*. The latter crop relies foremost on rootstock genetics from one particular cross because these genotypes withstand Verticillium wilt of pistachio that threatened the industry previously. However, to focus on one disease when choosing a rootstock appears risky when other maladies occur on a crop. Thus, all three trees require constant rootstock development efforts.

The inevitable development of higher levels of salt in irrigation water due to the nature of California production systems demands monitoring for salt tolerance of rootstocks. Nematode evaluations, at the very least, need to go along with these rootstock development efforts to safeguard new rootstock releases. In parallel, the effects of water deficit, either by planned deficit irrigation or by the simple restriction of water availability, on nematode dynamics and damage potential needs to be further monitored.

More environmentally safe post-plant remedies are urgently needed for true integrated pest management tools for managing root lesion and other nematodes on walnut, almond and pistachio. Zonal irrigation set-ups that consider horizontal soil texture composition distribution throughout a field can more accurately deliver water to nut crops. They also offer zonal application capacity for the chemigation approach for nematicide delivery as current experimentals gain registration. *Pratylenchus vulnus* occurs at highly damaging population densities in various soil texture classes. In an integrated pest management approach, such zonal nematicide application strategies should be examined to fully exploit saving opportunities.

Outlook: anticipated future developments

Nut crop production in California faces several obstacles. Critical issues like scarcity of water, and reduced chill hours for proper flowering in the subsequent production cycle are just two of the restrictors that will likely change production patterns in California. Novel regulation for groundwater use restricts available water amounts, especially in the southern part of the central valley greatly. Such regulation may also encumber nut crop production in other parts of the valley. Changing climate and the more favourable water supply conditions in the Sacramento valley and even further north are likely to move significant production areas in this direction. Currently, almonds have been yielding more copiously in the southern valley, so some yield reductions may come into play.

More water-saving strategies during irrigation may further reduce the water percolation to the groundwater layers. One could hypothesize that this process will even accelerate salt increases in the ground water. Because almond and walnut are more sensitive to the salt content of irrigation water and soil, the more salt tolerant pistachio will likely become more important as a nut crop in relation to those two crops in the southern parts of the valley. Root lesion nematode is likely to persevere on its prolific host walnut. Current regulatory developments suggest that soil fumigants may be gone by that time and that truly comprehensive production systems using cover cropped fallow periods, nematode antagonistic companion crops, and highly resistant and tolerant rootstocks will be used. The whole production system will need to become less conducive to nematode diseases.

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33 The root-knot nematode: Importance and impact on coffee in Brazil

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Introduction

Coffee (*Coffea* spp.) is a crop of significant importance for Brazilian agribusiness, which in 2019 generated a gross revenue of US$3.73 billion (CONAB, 2020). As a perennial crop, coffee stays in the field for many years, subjected to nematode parasitism from the seedling stage throughout the economic life of the plantation. In Brazil, it is a challenge for growers to produce coffee in the presence of the root-knot nematodes (RKN). Five species of RKN have been detected in Brazilian coffee plantations: *Meloidogyne exigua*, *M. incognita*, *M. paranaensis*, *M. coffeicola* and *M. izalcoensis.* As the most widespread species, *M. exigua* is probably responsible for the greatest losses. However, *M. paranaensis* and *M. incognita* are the most destructive species and their spread has expanded in recent years.

Economic importance

Most information on the economic importance of RKN on coffee comes from Brazil where for over 100 years the areas of cultivation have migrated across the country due to the pressure of nematode damage. Losses caused by plant parasitic nematodes to coffee in Brazil have been estimated at 20% of yield (Oliveira and Rosa, 2018). In some cases, populations of *M. exigua* have been reported to cause a reduction of up to 45% in coffee production. The amount of damage is greatest in coffee plantations infested with *M. paranaensis* and *M. incognita* since these species can kill plants.

Distribution

RKN are found in all Brazilian coffee-growing regions. *Meloidogyne exigua* is the most widespread species in the state of Minas Gerais, the largest Arabica coffee producer, where it is found in at least 25% of coffee farms (Castro *et al.*, 2008). In recent years, *M. paranaensis* has expanded its spread to other states, notably to Minas Gerais (Terra *et al.*, 2019). In the states of Paraná and São Paulo, the species most frequently found are *M. paranaensis* and *M. incognita*. In addition, in the state of Espírito Santo, the largest producer of conilon coffee (*Coffea canephora*), *M. incognita* is the most widespread species in that state (Barros *et al.*, 2014). Another RKN that has been detected to date in only one coffee plantation is *M. izalcoensis* (Stefanelo *et al.*, 2019). *Meloidogyne*

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coffeicola, which has caused significant damage in the past, has not been detected in coffee plantations for decades. The mechanization of coffee harvesting over the last two decades has contributed to the extensive spread of RKN.

Host ranges

The host range of *M. exigua*, *M. paranaensis* and *M. incognita* is not limited to coffee. Several weed species commonly found in coffee plantations are hosts of *Meloidogyne* spp. For example, *Euphorbia heterophylla*, *Ipomea acuminata*, *Stachys arvensis*, *Cyperus rotundus*, *Solanum americanum*, *Echinochloa colonum*, *Sorghum halepense*, *Eleusine indica*, *Raphanus raphanistrum* and *Galinsoga ciliata* are considered to be good hosts of *M. exigua*, *M. incognita* and *M. paranaensis* (Lima *et al*., 1985; Roese and Oliveira, 2004). These plants allow the rapid increase of the nematode populations in the area and, therefore, the management of these invading plants in coffee areas is essential.

Symptoms of damage

Arabica coffee cvs Catuaí and Mundo Novo, the most planted cultivars in Brazil, are severely affected by *M. paranaensis* and *M. incognita* parasitism (Figs 33.1 and 33.2). Plantations infested with these RKNs show a high rate of plant mortality and severe loss of vigour due to the drastic reduction of the root system.

The roots parasitized by these species rarely exhibit galls. Root symptoms observed in the field are cracking, peeling and intensive necrosis (Fig. 33.3). These plants never recover, even with application of synthetic or biological nematicides.

Meloidogyne exigua causes typical rounded galls, mostly on young roots formed after the first rains in spring and are easy to recognize in the field (Fig. 33.4). The galls are initially white to yellowish-brown and turn dark brown as the root becomes older (Villain *et al*., 2018).

The more drastic damage symptom causes a reduction in the root system by up to 80% in plants damaged by *M. paranaensis*. On the other hand, *M. exigua* does not cause this level of destruction of the root system but forms numerous galls in the new roots with egg masses. This is the main difference between this species and others on susceptible coffee roots. The edaphoclimatic conditions in a coffee-growing region, as well as age and crop cultivar, influence the intensity of parasitism and, consequently, the severity of symptoms and ultimate damage. In addition, there is an intraspecific variability within populations of *M. exigua* and *M. paranaensis* (Muniz *et al*., 2008; Santos *et al*., 2018).

Biology and life cycle

Meloidogyne biology in coffee roots is similar to that of other crops and will not be explained in detail (Karssen and Moens, 2006). On coffee, the females lay hundreds of eggs and remain almost completely inside the roots. From the eggs, another cycle begins with the J2 parasitizing the same plant and even the same root. At optimal temperatures, the life cycle of *M. paranaensis* is completed in 37 days (Alves *et al*., 2019).

Interactions with other nematodes and pathogens

Interactions of RKNs with other phytopathogenic microorganisms are known as *Meloidogyne*-based disease complex (MDC) (Wolfgang *et al*., 2019). Although coffee is parasitized by 19 species of *Meloidogyne* spp., only some of them, such as *M. incognita*, *M. paranaensis* or *M. arabicida* cause coffee MDC called 'corky-root disease'. MDC-diseased coffee shows cracking, peeling and intensive necrosis in the roots. The aboveground parts of infested plants show chlorosis, defoliation, reduced growth and often plant death over extensive areas. MDC is a result of the synergistic interaction between RKN that predispose plants to the root rot fungus, *Fusarium oxysporum*. Under controlled inoculation conditions, the combined inoculation using *M. arabicida* or *M. exigua* with *F. oxysporum* showed that only *M. arabicida* plus fungus produced MDC on *Coffee arabica* cvs Caturra and Catuaí (Bertrand *et al*., 2000). *Meloidogyne arabicida* alone causes gall formation without corky-root symptoms. Some authors speculate that this occurs because the *M. arabicida* females develop frequently close to the surface of the roots causing rupture of the

Fig. 33.1. *Coffea arabica* plants heavily affected by *Meloidogyne paranaensis.* Author's own photograph.

Fig. 33.2. *Coffea arabica* plants heavily affected by *Meloidogyne incognita.* Author's own photograph.

cortex, which allows egg masses to emerge out of the root (different from *M. exigua* parasitism), and which may favour the subsequent invasion of secondary pathogens. Different genera of plant parasitic nematodes, especially species of *Pratylenchus* and *Rotylenchulus*, have been found in coffee roots parasitized by *Meloidogyne* spp. However, the importance of cohabitation has not been well defined (Castro *et al.*, 2008).

Recommended integrated nematode management (INM)

Management of nematodes in perennial crops is more difficult than in annual or herbaceous crops. Nematode management in coffee in Brazil today focuses on the specific RKN species involved. For instance, *M. exigua* is effectively managed

Fig. 33.3. Crack symptoms caused by *Meloidogyne paranaensis*. Author's own photograph.

Fig. 33.4. Coffee roots parasitized by *Meloidogyne exigua*. Author's own photograph.

with granular nematicides, which cannot be done in plantations infested by *M. paranaensis* or *M. incognita*.

As a perennial crop, coffee requires a large financial investment in crop production and plantation maintenance and for the processing of the fruit. Therefore, preventive measures such as the use of healthy seedlings and adequate choice and preparation of the area for planting are mandatory measures.

There are four primary means used to manage RKN in coffee plantations in Brazil: exclusion, growing resistant coffee cultivars, application of nematicides and grafting on resistant cultivars.

Exclusion

Since it is impossible to eradicate nematodes from a coffee field, the ideal management decision is to prevent their entry during the renewal or establishment of new plantations. Nematode management begins with measures to avoid RKN spread by contaminated seedlings, machinery, irrigation or run-off rainwater between and within plantations. The selection of the land to establish a new coffee crop must be very carefully done, avoiding the recently eradicated old coffee plants, proximity of infested trees and never below it, where the risk of contamination from run-off water is high. In Brazil, nurseries must have a certificate issued by an official nematology laboratory stating the absence of RKNs based on processed samples. In addition, management requires the elimination and/or management of RKN weed hosts listed above as well as the elimination of infested coffee plants and their roots. The latter is an expensive undertaking.

Resistant coffee cultivars

The use of resistant cultivars has resulted in an increase in Arabica coffee production in areas infested with RKN. The cultivars Catiguá MG 3 (derived from crossing Catuaí Amarelo IAC 86 × Timor Hybrid), IAPAR 59 (Villa Sarchi CIFC 971/10 × Timor Hybrid) and IAC 125 RN (Villa Sarchi 'CIFC H361/4 × Timor Hybrid) are resistant to *M. exigua* and have been used by coffee growers. In Brazil, IPR-100 was the first registered cultivar of Arabica coffee resistant to *M. paranaensis* and *M. incognita*. Currently, this is the cultivar most used in the management of these nematodes. Its use is followed by cv. IPR-106, also resistant to those nematodes. However, the late fruit ripening of IPR-100 has discouraged many coffee growers from planting this cultivar. The Agricultural Research Company of Minas Gerais state (EPAMIG) has been working on the selection of PPN-resistant genotypes. In 2008 EPAMIG started the genealogical selection of coffee plants resistant to *M. paranaensis* and *M. incognita* in the infested coffee areas. This research allowed the identification of *C. arabica* progenies, resulting from the cross between the cultivars Catuaí and Amphillo, with resistance to *M. incognita* and *M. paranaensis* (Perez *et al.*, 2017). The MG 0185 PL.1R2-11-7-II progeny reduced the *M. paranaensis* population by 78% and increased the productivity of the crop by 308%; MG 0179 PL.3R1-16-6-I increased productivity by 119% and reduced the nematode population by 83%.

Monitoring and application of nematicides

The application of nematicides is an alternative that gives short-term control of nematodes in coffee plantations. Before application, the RKN species and population density as well as the condition of the coffee root system needs to be assessed. The use of nematicides is not recommended for situations where the coffee tree is severely damaged, with intense defoliation and advanced destruction of the root systems since none of the nematicides can induce recovery of a plant in this pre-death stage. Several nematicidal products are available in the market and are used by growers (Table 33.1).

The efficacy of nematicides in INM towards *M. paranaensis* and *M. incognita* depends on the periodic monitoring of the coffee crop, collecting soil samples and roots from the coffee plants, even without apparent symptoms of malnutrition or poor development. The study and use of biological nematicides, composed of bacteria (*Bacillus* spp.) or fungi (*Purpureocillium lilacinum, Pochonia chlamydosporia*), has increased in INM in coffee.

However, their effectiveness in RKN infested coffee plantations is influenced by the plant age, crop management, and soil characteristics (physical, chemical and biological). In Brazil, on-farm production of biological products has intensified in recent years. However, this type of nematode management still needs confirmation of efficacy and under field conditions, which is still scarce.

Grafting on resistant cultivars

In Brazil, grafting *C. arabica* on Apoatã (*C. canephora*) rootstock was the only INM measure that made possible the cultivation of coffee in areas infested with *M. paranaensis* and *M. incognita*. In Brazil, the EPAMIG has developed a progeny of *C. arabica*, called MG 0179 PL.3R1-5-3-IV, resistant to *M. paranaensis*, which has morphophysiological compatibility as a rootstock for the susceptible cv. Catuaí IAC 144 commonly grown by farmers (unpublished data). The grafted seedlings using the *C. arabica* rootstock have the advantage of being obtained by seed, rather than clonal seedling, as is done with *C. canephora*.

Optimization of nematode management

Precision agriculture could have an impact on coffee INM. Field mapping by drones, which

Table 33.1. Nematicides registered for the control of *Meloidogyne exigua* (*Mex*), *M. paranaensis* (*Mpar*) and *M. incognita* (*Minc*) species in coffee cultivation in Brazil. Source: MAPA (2020).

Meloidogyne spp.	Trading names	Active substance	Chemical group
Mex, Minc	Counter 150	Terbuphos	Organophosphorus
Mex, Mpar	Nimitz EC	Fluensulfone	Fluoroalkenyle (-thiother)
Mex	Rugby 200	Cadusafos	Organophosphorus
Mex, Minc	Apache 100	Cadusafos	Organophosphorus
Mex, Minc	Cierto 100	Fosthiazate	Organophosphorus
Mex, Minc	Nemacur	Fenamiphos	Organophosphorus
Mex	Verango Prime	Fluopyram	Benzamide

can localize infested locations for nematicide application can optimize nematode management. This technology is outlined in Chapter 59 in this book.

It is important to highlight that the environment and the type of management adopted on the farm may change the performance of the cultivar. Therefore, the agronomic performance of the coffee cultivar, mainly productivity and maturation period of the fruits, are required by the grower for him to accept the idea to grow RKN resistant cultivars.

In the state of Minas Gerais, EPAMIG continues to conduct many experiments in infested areas to compare the agronomic efficiency of resistant progenies in the control of RKNs, such as the progenies MG 0185 PL.1R2-11-7-II and MG 0179 PL.1R1-10-7-II, with different productivity and plant height.

Optimization of the use of resistance in the control of RKNs has been also achieved with the use of progeny as a rootstock. The resistant progeny MG 0179 PL.3R1-5-3-IV is compatible with the commercial Arabica coffee cultivar most used in the field, cv. Catuaí (unpublished data).

Outlook: anticipating future developments

- The management of RKN in the future is likely to be achieved by doing an accurate RKN identification. The used techniques should be faster and low cost, as is done by loop mediated isothermal DNA amplification (LAMP). The diagnosis of *M. exigua* and *M. paranaensis* by LAMP is being validated for *Meloidogyne* spp. identification in Brazil.
- New strategies for selecting coffee progenies resistant to the main parasitic RKNs of coffee are being developed, such as assisted selection using molecular markers which reduces the time to identify resistant genotypes. In the short term, seedlings of the Arabica coffee cultivar Catuaí grafted on a rootstock of *C. arabica* resistant to *M. paranaensis* will be used for crop renewal in infested areas. This combination of two *C. arabica* plants should boost the use of grafted seedlings due to the possibility of forming seedlings for grafting from seeds.
- Global climate change may intensify the damage caused by RKN on coffee. The potential impact of climate change may modify the spatial distribution of the coffee nematode and thereafter increasing the disease severity. The use of remote sensing technology would be important in following RKN in coffee in fields and across regions.
- Another point to be investigated in the *Meloidogyne*–coffee pathosystem refers to zero harvest, a common practice mainly in mountain coffee farming, in which 'skeleton pruning' of the coffee trees is done to recover their productive capacity. However, the effect of pruning on the root system associated with the RKN population still needs investigation as to how much the nematodes impair the recovering of the root systems.

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SECTION V:

Vegetable Crops

34 A root-knot nematode paradise made in plastic: The case of Florida vegetables

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Introduction

Florida's agricultural industry is only second to tourism, generating more than US$100 billion in annual economic impact and employing more than 500,000 people. Florida's farmers grow nearly 300 commodities and are a major producer of fresh fruits and vegetables. Tomato, pepper, aubergine, strawberry, melons, cucumber, squash, and many specialty vegetables covered >100,000 ha and generated US$1.54 billion in gross sales in 2018 (Table 34.1). Most production occurs in open field and on plastic-mulch raised beds in combination with drip irrigation. This plasticulture system originated in Florida and California in the 1960s and is used on approximately 35,000 ha in Florida. The benefits of this system include earlier and higher yield, reduced weed pressure, higher irrigation and fertilizer efficiency and the ability to grow two or three crops on the same plastic.

Florida has a humid subtropical climate with mostly beach-like soils containing 90–98% sand that lacks organic matter, aggregates and nutrients. Root-knot nematodes (RKN; *Meloidogyne* spp.) are perfectly adapted to these fast-draining and well-aerated soils, and root-knot disease has been known and dreaded as a foe to vegetables in Florida as far back as 1805 (Neal, 1889).

Economic importance

Root-knot nematodes are by far the most important nematodes in Florida vegetables. Yield loss is significant, and damage can occur at any crop stage, often leading to early crop senescence and reducing the number of harvests. In severe cases plant death can occur, especially when other soil pathogens like *Fusarium* wilt are present. When populations are high and conditions conducive, crop losses of more than 50% are not uncommon. Yield loss due to nematodes is difficult to determine, especially under tropical conditions like in Florida. The heat and humidity favour many other yield-limiting pests and diseases, confounding direct identification of damage caused by nematodes. Fumigation is seen as the most reliable way to protect the high cost of plasticulture production systems (~ US$30,000/ha for tomatoes), and almost always soil is fumigated prior to planting. While fumigation is also done out of concern for weeds and diseases, RKN are often the primary target. Since the ban on methyl bromide, usage of the fumigant 1,3-D, which is primarily a nematicide, has increased sevenfold in vegetables (US EPA, 2020).

Important regional differences on the importance of RKN exist within the state's vegetable area. For example, in the Everglades agricultural

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Table 34.1. Major vegetable crops in Florida. Source: United States Department of Agriculture, National Agricultural Statistics Service (USDA-NASS) (2019).

Crop	Harvested acreage (ha)	Value million US$	Rank in US
Tomato	27,000 (12,140)	260.0	2 (#1 fresh)
Strawberry	9,800 (4,371)	336.9	2 (#1 winter production)
Bell Pepper	11,900 (5,463)	206.3	2
Sweet corn	39,700 (15,216)	158.3	2 (fresh)
Snap beans	31,500 (12,748)	68.8	2 (fresh)
Watermelon	22,500 (9,105)	135.6	2
Cucumber	24,900 (10,077)	97.0	1 (processing)
Spring potatoes	20,800 (8,417)	122.0	2
Cabbage	8,900 (3,602)	42.0	3 (fresh)

region of southern Florida, which has mostly organic 'muck' soils and a naturally high water table, RKN is much less of a problem than in the drier deep sand soils.

Host range

RKN have wide plant host ranges, and all vegetables grown on plasticulture, as well as many associated weeds, are good hosts to most RKN species. Pepper seems to be immune to certain populations of RKN (e.g. *M. javanica*), and differences have also been observed among certain cucurbits, but few studies have been done. *Meloidogyne hapla* is the only root-knot species that affects strawberry in Florida, causing late season damage to strawberries, and early season damage to vegetables that are double-cropped after strawberries, such as cantaloupes, squash and pepper. It is possible that significant differences in host range and damage potential exist not just among species, but also among different populations across Florida. The multitude of RKN species and crops in Florida means that significant research will be required to investigate their distributions.

Distribution

RKN are found throughout Florida and it is assumed that soil anywhere in the state contains RKN. In one of the first reports on root-knot nematodes, Neal in 1889 wrote that while the disease can be found throughout the southeastern USA, it seems to 'reach a climax in Florida which seems to possess the requisite soil, humidity and warmth for proper environment of root-knot'. More than fifteen RKN species have been identified in Florida, of which at least seven have been found in vegetable fields. The most common species are the pantropical *Meloidogyne incognita*, *M. javanica*, and *M. arenaria*. Other species, such as *M. haplanaria, M. enterolobii, M. floridensis* and the more cryophilic *M. hapla* have also been reported but less frequently. Very few data are available on the relative importance and distribution of the different RKN species in Florida. Recent sampling indicated that *M. hapla* is common in strawberry fields and was possibly introduced with transplants from Canada over the years, while *M. enterolobii* was commonly found in Asian vegetable farms.

Symptoms of damage

Above-ground symptoms of RKN are generally unthrifty plants, that are stunted, yellowish and may show wilting during the hottest time of the day. Tomato and pepper plants often turn chlorotic later in the season (Fig. 34.1), while cucurbits may have malformed and dry fruit (Fig. 34.2).

Above-ground damage can look more severe when other pathogens, especially *Fusarium* wilt or crown rot, are present, in which case plants can die rapidly 'as if struck by lightning' (Neal, 1889).

Root galls can show up within a few weeks after planting, and especially roots of tomato and cucurbits can enlarge enormously (Fig. 34.2 and Fig. 34.3), before turning into masses of decaying tissue. Root gall size will depend on RKN species and crop and cultivar, and galls are typically smaller and more discrete on pepper, sweet corn

Fig. 34.1. A field showing **(A)** yellowing of peppers and **(B)** heavily galled tap root due to severe soil infestation by mixed population of *Meloidogyne incognita* and *M. enterolobii*, the latter known to induce large-sized galls. Author's own photographs.

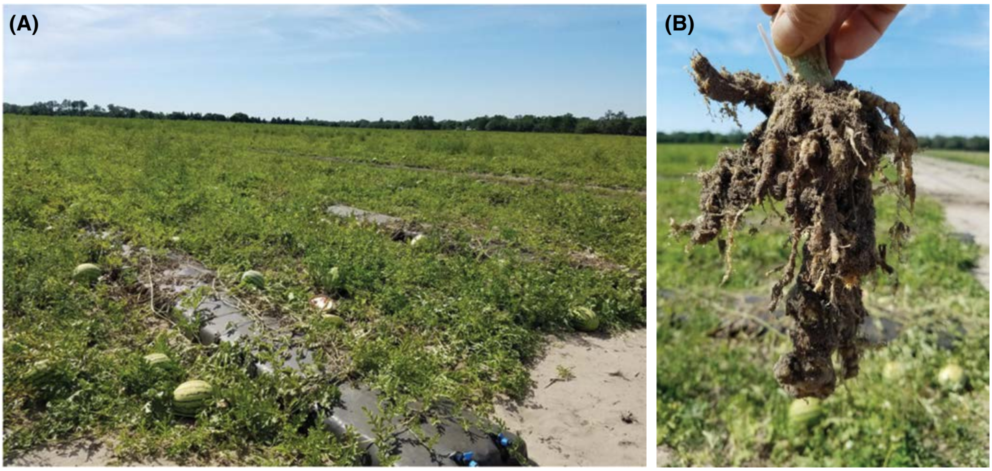

Fig. 34.2. A field showing **(A)** dying watermelon plants and **(B)** massive root galling due to *Meloidogyne javanica*. Author's own photographs.

and strawberries. Also, *M. hapla* will cause smaller galls as compared to the thermophilic RKN, especially *M. enterolobii* and *M. arenaria*.

Despite its prevalence, RKN damage is often not recognized as such, and is frequently confused with soil disease, poor fertilization, too much or not enough water, plugged drip tapes or heat stress.

Biology and life cycle

Root-knot nematodes thrive in the warm sandy soils of Florida with average soil temperatures between 20°C and 35°C. Life cycles from egg to egg are typically 3 weeks with minor differences among tropical RKN species. This allows for multiple generations, often three to four per growing season. As successive crops of vegetables are often cultivated year-round, many more generations per year can be produced. Essentially, RKN can reproduce 12 months of the year in Florida and build up rapidly and continuously.

Root-knot damage can occur any time of the year, but yield loss tends to be more severe in autumn when at plant soil temperatures are high, nematode reproduction rapid, and young plants

Fig. 34.3. Interactions of root-knot with *Fusarium* are common in tomato fields in Florida. **(A)** Wilted plants showing symptoms of vascular infection with *Fusarium* and **(B)** associated severe *Meloidogyne* root galling in Myakka, Florida, 2019. Author's own photographs.

more susceptible. The exception is *M. hapla*, which prefers cooler soils and will mostly cause damage to vegetables in early spring. RKN damage is always more likely when a second crop is planted on the same bed, and this is true for all RKN species.

Between the spring and autumn growing seasons or during the hot and rainy summer months, RKN tend to move downwards into the soil. This seasonal vertical movement is not very well documented, but probably should be considered when predictive nematode soil sampling is done.

Interactions with other nematodes and pathogens

Interactions of RKN with *Fusarium* wilt (*Fusarium oxysporum* f.sp. *lycopersici* race 3, FOL) are common in tomato fields in Florida (Fig. 34.3). Often wilted tomato plants showing vascular infection with FOL are heavily galled, and together RKN and FOL have a synergistic effect on plant damage. FOL race 3 is the predominant race in Florida, and while most commercial tomato cultivars have resistance against FOL race 1 and 2, this is not the case for race 3. The few cultivars that have resistance are not popular among growers because of the increased susceptibility to blossom-end rot and bacterial spot, and issues with reduced fruit size (Hutton *et al.*, 2014). Most likely other interactions with pathogens are important, like *Fusarium* crown rot for tomato and *Pythium* for cucumber, but little is known about this complex. In addition to occurring in complexes with plant pathogens, RKN can also be difficult to control because they are commonly found on weed hosts in the production system, including yellow and purple nutsedge, nightshade, goosegrass and portulaca.

Recommended integrated nematode management (INM)

The prevalence and severity of root-knot in Florida vegetables is so high that it is often the number one overall pest or disease problem. Preventing infestations is the main objective, and it is almost hopeless to attempt to combat RKN once they become established. The best way to deal with infested fields is by combining different strategies and following a continuous and diverse approach.

Pre-plant fumigation and fumigant alternatives

Florida's porous sandy soils are ideally suited for fumigation, which has been the standard practice in vegetables since the 1950s and remains the preferred nematode management choice to this day. Although fumigation is expensive (US$1000–2000/ha) and cumbersome, the cost of plasticulture and the risk of soil-borne–related crop loss is so great that growers have been reluctant to give up on fumigants. 1,3-D, chloropicrin and metam are the most common fumigants and their use has significantly increased since the ban on methyl bromide about 10 years ago. 1,3-D and chloropicrin are mostly applied as mixtures, with higher amounts of 1,3-D recommended when nematodes are the main target. Growers may also drip apply metam as an end-of-season ('crop destruct') treatment when nematode populations are high and easier to kill.

Non-chemical fumigant alternatives are uncommon and mostly limited to organic fields. Organic vegetable production in Florida is still in its infancy, but demand is growing and the area is increasing. Organic growers will often apply crab meal, compost and chicken litter before planting, but no data is available on how these impact RKN. Molasses from the roughly 180,000 ha of sugarcane in south Florida have been studied as a carbon source for anaerobic soil disinfestation (ASD). When this combination is used with solarization it can reduce RKN (Butler *et al.*, 2014). However, the large amount of material required is a problem, and for ASD to become a realistic option for growers it has to become easier and more affordable. Temporary flooding of fields is practiced in the Everglades agricultural region, and probably one of the reasons why RKN is not a major problem in this area.

Non-fumigant nematicides

For decades oxamyl was the only non-fumigant nematicide available to vegetable growers in Florida. With the recent registration of less toxic fluorine nematicides like fluensulfone and fluopyram, and others such as fluazaindolizine and cyclobutrifluram to follow, growers have some new options. While these new nematicides have generally shown good potential to reduce RKN damage, they do not provide disease or weed control. Therefore, if they are to replace fumigants, they will have to be integrated with a weed and soil disease management programme, and such a strategy will have to provide comparable control at a similar cost to the fumigant programme.

Several biological products are available in Florida, but their use is limited. They typically require multiple applications and are mostly used as part of a programme. Products are often toxins derived from plants (thyme oil, mustard oil, neem oil and other essential oils), or bacteria (*Burkholderia*) and fungi (*Myrothecium*), while others are biocontrol organisms such as *Bacillus* and *Pasteuria* spp. and the fungus *Purpureocillium lilicanus*. Limited field data is available on their efficacy.

Crop rotation and cover crops

All plasticulture vegetables in Florida are good hosts and susceptible to RKN, and it is practically impossible to introduce commercial crop rotation schemes to manage RKN. The best option is to include cover crops in between vegetable crops. Cover crops were very common in the early days of Florida agriculture and their benefits in terms of soil fertility and nematode management was well recognized. However, with the introduction of synthetic fertilizers and soil fumigants in the 1950s, they were largely abandoned. Since the ban on methyl bromide, cover crops have made somewhat of a comeback. Sunn hemp (*Crotalaria juncea*), sorghum-sudangrass (*Sorghum sudanense*) and cowpeas (*Vigna unguiculata*) are the most common summer cover crops, while rye is mostly planted in winter. Sunn hemp and sorghum-sudangrass are poor hosts to most root-knot nematodes, but sorghum-sudangrass is a good host for sting (*Belonolaimus longicaudatus*) and stubby root (Trichodorids) nematodes, and sunn hemp for lesion nematodes (*Pratylenchus*). While these nematodes may also damage vegetables, they are much less harmful than RKN. Some growers started experimenting with cover crop mixtures, which is something the author has studied previously in smallholder farms in Kenya. Planting cover crop mixtures there resulted in a more diverse plant parasitic nematode soil population, and reduced RKN damage to a subsequent susceptible crop (Desaeger and Rao, 2001).

Crop resistance

Ideally, if all a grower had to do was turn to using a RKN resistant cultivar that would solve a major problem. Unfortunately, it is not quite that simple. While RKN resistant tomato cultivars having *Mi* gene have been available for decades, they are rarely planted in Florida. Even after the ban on methyl bromide, when it was thought *Mi* cultivars would increase, this did not happen. Concerns about heat sensitivity of the *Mi* gene are largely unfounded, as several trials by the author and others have shown that the resistance holds up quite well in Florida. The main reasons why *Mi* tomatoes are not grown in Florida are the continued reliance on fumigants, the lack of horticulturally desirable cultivars acceptable to the fresh fruit market and the focus on other pests and diseases for introduction of resistance genes, such as viruses and diseases. If resistant plant material is to become a primary foundation of root-knot management in Florida, it will require that RKN become a priority within vegetable breeding programmes.

Vegetable grafting in which a resistant rootstock is paired with a horticulturally desirable scion has had some limited success for pepper and tomato and has been principally utilized in organic production systems for the production of heirloom tomatoes (old traditional cultivars) that have no resistance to RKN or diseases.

There is no resistance as of now for cucurbit vegetables, but we have seen considerable variation in susceptibility, with often higher root gall damage on cucumber than on squash. More research is needed to determine whether this is RKN species dependent or not.

Soil management and soil health

Florida's sandy soils (>90% sand) are relatively young and to say they are not the best in the world is an understatement. The general advice to improve these weak sandy soils should be to embark on a programme to routinely add organic material. A good practice employed by some growers in addition to growing cover crops, is to build on-site composting facilities to turn yard waste into nutrients, thereby increasing soil quality and resiliency to pests. Soil health has been defined in many ways, but a key feature in agricultural soils should be reduced damage from soil pests. Soil organic matter is regarded as a key component of soil health, and this is demonstrated by the organic 'muck' soils of south Florida, which seem to have high natural suppressiveness to nematodes. In Florida's sandy soils, nematode suppressiveness seems to be associated with fungal diversity, which may explain the often-rapid resurgence of RKN following fumigation with chloropicrin, a very effective soil fungicide. Interactions between plant root exudates, soil microorganisms and nematode suppression are an intriguing subject, and understanding these microbial consortia and the mechanisms underlying disease suppression may one day help to manage or even develop microbial communities for biocontrol of PPN (Topalovic *et al.*, 2020).

Optimization of nematode management

RKN problems in Florida vegetables have been on the rise since the ban on methyl bromide, despite significant efforts to optimize efficacy of replacement fumigants (see Chapter 26). RKN are elusive and difficult to detect in Florida's deep sands, showing significant vertical movement throughout the year (up to depths of >100 cm). Ideally, nematode soil samples should be collected according to this seasonal movement, or at greater depths than the standard 20–30 cm depth. This would create more accurate nematode maps and allow for more targeted nematicide applications (see Chapter 59).

Better estimates of nematode yield loss in relation to the economics of treatments is needed but difficult, due to many confounding production factors. Yield loss due to nematodes is often more severe in autumn than in spring, so more aggressive management tactics could be reserved for autumn. This could include the use of nematode resistant tomatoes, but for that to happen, cultivars are needed that not only have nematode resistance, but also other major disease resistance traits important for Florida, such as FOL race 3.

The next generation of chemical and biological nematicides may help to reduce fumigant dependency, but they need to be affordable and will have to be supplemented with non-fumigant solutions for other soil-borne pests like nutsedge and *Fusarium*. Another practice gaining interest among tomato growers in Florida is the use of narrower and taller beds. Such configuration requires less fumigant, without compromising nematode control, and offers greater protection against hurricanes and flooding.

Planting non-host cover crops such as sunn hemp in between crop seasons will continue to be an important nematode management component, besides providing other soil benefits. To increase its effectiveness, cover crop cultivars should be routinely screened against the different RKN species important in Florida, as well against other potentially damaging nematodes, such as sting *Belonolaimus*, stubby root *Trichodoridae* and lesion *Pratylenchus*. This is tedious work that takes time, but such information would help growers to select appropriate cover crops, including cover crop mixtures that are tailored according to the resident nematode population of a field.

Future research and outlook

Nematodes are only one of the many obstacles that Florida vegetable growers face, including hurricanes, hail, freezes, labour and increasingly competitive and fluctuating markets. The recent COVID-19 pandemic amplified the dependency on migrant labour. To reduce this reliance, significant research is ongoing in mechanical harvesting, both in terms of equipment and plant breeding. Commercial harvesters will likely become available within the next decade, and precision agriculture and artificial intelligence, including the use of drones and robotic sprayers, will see a rapid growth. It remains to be seen how this will impact nematode management, which will largely depend on how we can more accurately determine nematode hot spots in a field.

With the expected increase in organic vegetable production, non-chemical practices will become more important. Improving the health of the inherently weak sandy soils in Florida by increasing organic inputs will be especially important, as well as any other practice that will stimulate natural suppressiveness.

The long-term sustainability of the plasticulture system is another issue. While it has allowed crops to be grown productively on what would otherwise be marginal land, it is not without problems. Farmers spend thousands of dollars to buy plastic mulch and drip tape, which are often used for only one season, producing a lot of waste and being a source of microplastics in soils and water. Biodegradable mulches are available but cannot be used with fumigants, which has limited their adoption in Florida. It is a vicious cycle, with the lack of alternatives reinforcing the need for fumigants, and the continued use of fumigants reinforcing the notion that alternative options are not necessary. While new and safer nematicides may reduce fumigant use, this will ultimately depend on the total cost of non-fumigant programmes.

Host-plant resistance to RKN is the best management tool and development of RKN resistant vegetable cultivars or rootstocks should be among the top research priorities in Florida. Sadly, nematodes are rarely topping the priority lists of companies or funding agencies. There is some cautious optimism that interest in RKN resistance is increasing among vegetable seed companies and federal agencies, but a much more concerted effort will be needed for RKN resistant vegetable cultivars to become a major factor in Florida.

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35 Managing root-knot nematode in open-field and protected tomatoes in India

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Introduction

India ranks second (next to China) in tomato production, with 18.4 million tonnes (10.4% share) harvested in an area of 0.76 million ha and productivity of 24.21 t/ha. During 2017–2018, 47,446.09 MT of tomato was exported (Anonymous, 2018). The major tomato growing states include Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh and Telangana.

The main cultivated varieties/hybrids of tomato are both determinate and indeterminate; in greenhouses, mostly indeterminate hybrids are grown. The varieties/hybrids developed by premier institutes such as the Indian Institute of Horticultural Research (Arka series) and Indian Agricultural Research Institute (Pusa series) are very popular, besides those developed by state agricultural universities. The hybrids introduced by private companies also have a sizeable market share. Among all the tomato varieties, *Nema Mukt* (SL 120) developed by the Indian Agricultural Research Institute, *Punjab Chhuara* (PNR 7) developed by the Punjab Agricultural University, and *Hisar Lalit* developed by Haryana Agricultural University were specifically bred for resistance to root-knot nematodes. However, with the introduction of hybrids these are no longer in use.

Tomato is grown throughout India and all year round. In the southern parts tomatoes are grown in three cycles: (i) December to January; (ii) June to July; and (iii) September to October. In the northern plains the planting schedule is: (i) July; (ii) October to November; and (iii) February.

The protected structures used for vegetable cultivation are generally naturally ventilated polyhouses or nethouses. The polyhouses have foggers and shade nets but are not equipped with cooling pads and exhaust fans. While protected structures invariably have drip irrigation systems, the open-field tomatoes generally use flood irrigation. Most of the small and marginal farmers raise tomato seedlings in nurseries inside their field plots. Greenhouse tomato nurseries are mostly raised in protrays using sterilized growth media.

The major diseases affecting tomato production in India are damping off, early blight, tomato wilt, septoria leaf spot, bacterial wilt, bacterial leaf spot, tomato mosaic virus and leaf curl. Among nematode problems, only root-knot nematodes (*Meloidogyne* spp.) figure prominently. Others include reniform nematode (*Rotylenchulus reniformis*) and a few less damaging ectoparasitic groups like spiral and stunt nematodes.

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Economic importance

It is difficult to assess the per cent of nematode infected area. However, based on random surveys it is estimated that about 10% of the area may harbour root-knot nematode populations above economic threshold levels. The economic losses are also calculated on this premise. According to the latest estimates (Kumar *et al.*, 2020) made by ICAR-All India Coordinated Research Project -Nematodes (Table 35.1), fruit yield losses in tomato due to *Meloidogyne* spp. accrue up to 23%, which in monetary terms amounts to Rs. 6035.20 million per year (equivalent to US$80.47 million at current rate of 1 USD = 75 Indian Rupees).

The greenhouse tomato growers and farmers with large acreages are aware of nematodes and attribute about 25–30% losses to nematode damage. Small and marginal farmers are by and large unaware of nematodes.

Host range

Major root-knot nematode species attacking vegetable crops, i.e. *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, are polyphagous and most of the vegetable crops are good to very good hosts of these species. Among vegetables, only onion and garlic are poor hosts or non-hosts.

Distribution

M. incognita is widespread all across the country, followed by *M. javanica* which is more common in the arid/semi-arid zones, and among *M. arenaria* and *M. hapla*, the latter is encountered only in high altitude areas. The sandy soils of Rajasthan and porous red soils prevalent in many parts of south India are more conducive for nematode damage.

Symptoms of damage

In the nurseries within field plots, such as those owned by small and marginal farmers, nematode infected seedlings are common. General symptoms of poor establishment upon transplanting and stagnant growth appear initially, which then turn into pale foliage. The yellowing of leaves intensifies with signs of epinasty (temporary wilting). Such farmers are unaware of the problem or diagnose the nematode problem very late; at this stage there is no remedy. More often than not, towards the later part of growth, the nematode infected plants are usually attacked by secondary pathogens and the above-ground symptoms are manifested in the form of wilt and subsequent death of the plants (Fig. 35.1). Gradual increase in root galling can be noticed and becomes more prominent when the crop reaches the 2- to 3-month stage. The galled roots provide attractive avenues for the rot-causing pathogens. Once rotting sets in, the plant succumbs. This 'disease complex' situation is quite common, with typical symptoms shown in Fig. 35.1 (right).

Biology and life cycle

Temperature plays a major role and regulates the number of generations completed by the nematode in open-field and greenhouse conditions. Under north Indian conditions, in the winter the night temperature drops substantially, while in polyhouses it remains significantly higher. Consequently, the nematode completes a higher number of generations in polyhouses as compared to open-field conditions.

Table 35.1. Estimated yield losses (%) due to *Meloidogyne incognita* at hot spots in different states of India (Singh, 2015).

State	Losses	State	Losses	State	Losses
Bihar	27.8–40	Karnataka	10–21.5	Rajasthan	19–22
Gujarat	18.6	Madhya Pradesh	19.82	Tamil Nadu	11–26
Haryana	27–40	Maharashtra	27.5–30	Uttar Pradesh	32–49
Himachal Pradesh	18–33	Odisha	10–20	West Bengal	18.6–31

Fig. 35.1. Above-ground symptoms and progression of disease from left to right. Photographs courtesy of Bayer CropScience.

Interactions with other nematodes and pathogens

Two major interactions prevail; one involving the wilt fungus, *Fusarium* spp., and the other involves the bacterium, *Ralstonia solanacearum*. Root-knot nematode often predisposes the infected plants to enhanced levels of infection by these organisms. Interaction between root-knot nematode and wilt-causing pathogens or rot-causing pathogens is common. The galled tissues are hard and whitish to creamish in the beginning; however, as the crop growth progresses, the galled tissues start turning brownish and soft (rotting), which finally results in plant death.

Recommended integrated nematode management (INM)

Summer solarization

In many parts of India, summers are very hot and temperatures often exceed 40°C. Usually the vegetable growers do not like to keep their fields free, but in areas having chronic nematode infection, they may discontinue growing vegetable crops for a few weeks during summer months (May/June). Farmers are advised to plough the fields two to three times at an interval of 10–15 days during this period.

In India, most of the farmers that grow in open fields still have their own nurseries in the fields themselves, which is often a source of nematode infection (Fig. 35.2). Soil solarization of the nursery beds using linear low-density polyethylene transparent film for 15 days is very effective in such conditions (Fig. 35.3A).

In greenhouses solarization using transparent polythene sheets (25 μm) during May/June for about 3–4 weeks gives very good results (Fig. 35.3B). The temperature in the upper soil layer (about 15 cm) is raised up to 62°C, killing most of the J2 population in the rhizospheric zone along with other soil-borne pathogens. However, root fragments in deeper soil layers still harbour a residual population of nematode eggs that multiplies gradually. This practice is gaining popularity among polyhouse growers.

Nematicidal application

Nursery bed treatment with carbofuran at 0.3 g/m^2 at the time of seeding along with bare root dip treatment of seedlings with carbosulfan at 500 ppm at transplanting is recommended. Seedling root dip is not favoured by growers as it involves keeping the planting materials dipped for several hours before transplanting. Therefore, application of carbofuran at 0.3 g/m^2 in the solarized nursery beds is advocated.

Use of carbofuran in polyhouses is very common, but many growers express reservation on the efficacy and resurgence of nematode pest after a few weeks. Use of phorate is already banned, and other carbamates and organophosphates are being phased out.

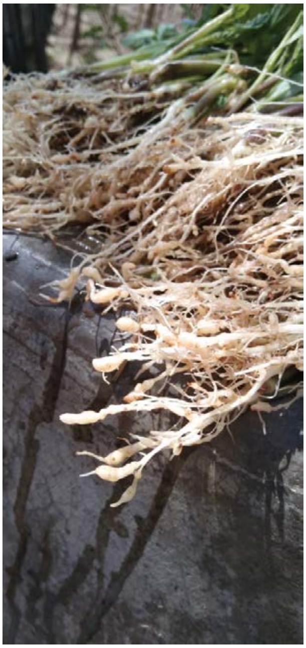

Fig. 35.2. Severe galling on roots of tomato seedlings raised in infested field plots. Author's own photograph.

Crop rotation

Commercial vegetable growers usually are not inclined to deviate from vegetable crops most of which are good hosts of root-knot nematode. They are advised to include onion and/or garlic in their vegetable cropping systems; these being vegetable crops, they fit well in their growing scheme. However, under severe and chronic root-knot infections, the growers are recommended to shift to a cereal-based cropping system for a couple of seasons. Though it is not as remunerative to them as the vegetable crops, if they comply with the recommendation they can then resume vegetable cultivation for profitable cultivation. Use of marigold (*Tagetes* sp.) as an intercrop or as a rotational crop is also recommended in peri-urban areas, where the flowers can be marketed.

In polyhouses, farmers have little choice except for cucumber, tomato and capsicum. They are recommended to grow capsicum in between cucumber and tomato as it is a less favoured host of root-knot nematode. However, the comparatively stable and assured price, and short duration of cucumber, are lucrative for them to grow cucumber after cucumber.

Resistant varieties

The old varieties of tomato developed by conventional breeding methods (Hisar Lalit, SL 120,

Fig. 35.3. **(A)** Polythene mulch on a tomato nursery bed. **(B)** Soil solarization in a polyhouse. Author's own photographs.

Punjab NR-7 etc.) for resistance against root-knot nematode are no longer preferred by growers. As such, no nematode resistant varieties/hybrids are currently available, though some seed companies claim otherwise.

Organic amendments

Incorporation of organic materials is an age-old practice. Farm-yard manure (FYM) prepared from animal dung mixed with crop residues like wheat straw is readily available since most of the farming community rear cattle. It is routinely done before planting. However, commercially produced vermicompost is also recommended and being used. De-oiled cakes of neem, castor, mustard, groundnut, etc. are also recommended. Application of poultry manure at 2–3 t/ha 15 days prior to seeding is recommended to obtain more transplantable seedlings and may also help to manage root-knot nematodes. FYM, vermicompost and neem cake fortified with bioagents is advocated in polyhouses in particular.

Rabbing

Dried residues of pearl millet or paddy husk are placed over root-knot nematode infested tomato nursery beds at 7 kg/m^2 and burned a week prior to seeding. This is recommended in Gujarat state. The slow simmering heat emitted during the process kills nematodes along with other soil-borne pathogens and weeds, but the practice is not eco-friendly as it also destroys the beneficial biota.

Bioagents

Many farmers use *Purpureocillium lilacinum* (= *Paecilomyces lilacinus*) and it is fairly popular among vegetable growers both in open-field and polyhouses; however, the results are mixed. The optimum conditions (temperature 25–30°C) prerequisite for the efficacy of *P. lilacinum* are prevalent for a brief period only. Sub-optimum thermal conditions result in reduced efficacy of the fungus. Moreover, as the compound galling sets in, most of the egg laying is inside the galls, and as a result the fungal hyphae are unable to reach the eggs for their colonization.

Other bioagents like *Trichoderma harzianum*/*T. viride* and *Pseudomonas fluorescens* are also recommended. In polyhouses liquid formulations are used through drip irrigation systems. The organic base is augmented in the beds at the time of transplanting/seeding.

INM examples for open-field vegetable cropping systems

INM systems are region specific. Figure 35.4 is a typical example for root-knot nematode management under north Indian conditions.

INM example for protected cultivation system

Though the area under protected cultivation systems (polyhouses, nethouses) is only about 40,000 ha, these structures are owned by relatively affluent people who are ready to invest almost anything to protect their crops. Nematodes are a serious constraint in these systems and the farmers are well aware of this menace. Sometimes the problem starts in the first crop that is planted in a newly constructed polyhouse. This happens when the polyhouse is constructed on root-knot nematode infested land. It has been recommended that construction of new polyhouses/nethouses be allowed on root-knot/reniform nematode-free land after soil testing in a designated laboratory (Fig. 35.5). This recommendation is being followed by Haryana state and an effort is being made for other states to follow suit.

Optimization of nematode management

Removal of infected plant roots

More or less complete removal of infected roots after the crop is harvested is strongly advocated. This simple and practical method can

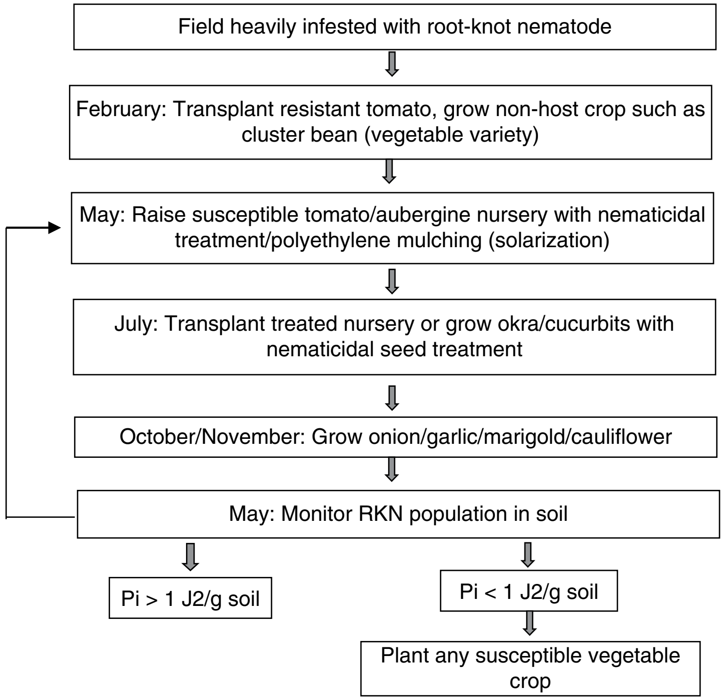

Fig. 35.4. Management of root-knot nematode (*Meloidogyne* spp.) in an open-field vegetable cropping system (north Indian conditions). Pi, nematode density at planting. Author's own figure.

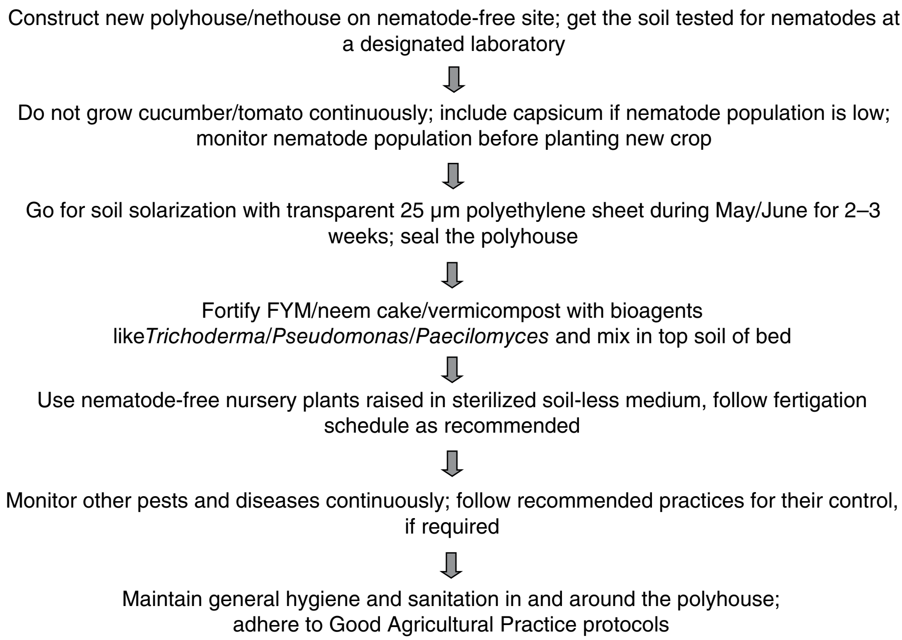

Fig. 35.5. Management of root-knot nematode (*Meloidogyne* spp.) in a protected cultivation system. Author's own figure.

remove billions of nematode eggs that are likely to constitute the potential inoculum for the next crops. Such roots can be piled up outside, dried and burnt.

Raising nursery plants in protrays

Many small and medium farmers have tomato nurseries in their actual fields, which often results in infected seedlings. A switch to protrays for raising seedlings in sterilized media like cocopeat is strongly advocated. A little cost escalation can easily be offset by returns on yields.

Use of organic matter fortified with bioagents

Well-rotted FYM (about 1 tonne) can be mixed thoroughly with 2 kg or 2 l of *Trichoderma viride*/*T. harzianum* or *Pseudomonas fluorescens* or *P. lilacinum* and moistened lightly. FYM should be stacked on a cemented floor in a shady and cool place, protected from sunlight and rain. The heap is turned every week for 3-4 weeks and moistened. The bioagents multiply in the organic matter and give better results upon application in the field.

New greener molecules

Two new greener molecules, namely fluopyram and fluensulfone, have been granted label claims on tomato against root-knot nematodes by the Indian Government. The application protocols for both the products have been developed and validated. However, the institutional support is awaited for including these products in the 'Package of Practices' of respective state governments/institutions. Fluopyram has been launched as a liquid formulation that facilitates its application via drip irrigation systems both in open fields and greenhouses. Fluensulfone is available in granular formulation. These molecules are safer to the environment and give longer protection against root-knot nematode. Fluopyram, in particular, has fungicidal properties as well; therefore, it may serve as an ideal product to manage disease complexes.

Combinations of chemicals and bioagents

Bioagents need a strong organic base that can be incorporated in the beds at the time of transplanting. Initial protection with chemicals is usually required. Liquid formulations of bioagents like *P. lilacinum* and *Pochonia chlamydosporia* (egg parasites) can be applied through drip irrigation after about one month to coincide with the laying of the first batch of eggs (usually laid on the root surface). Favourable temperatures for the establishment and optimum activity of *P. lilacinum* are more likely under south Indian conditions, where temperature fluctuations are less divergent.

Interculture/rotation with nematode antagonistic crops

Growing onion or garlic or marigold as intercultural or as rotational crops is recommended in infested fields, should the agronomic and economic parameters qualify for the same. Recent reports of root-knot infection on onion and garlic, however, warrant a cautious approach.

Future research requirements

- RNAi is a promising tool to understand virulence traits in the nematode and resistance pathways in the host. Basic research in molecular plant nematology is expanding the knowledge that can be applied to provide crop resistance to parasitic nematodes in an economically and environmentally benign manner.
- Disease complexes are common, therefore developing and synthesizing dual action new greener chemicals that target nematodes as well as wilt problems, will be desirable.

- The successful *in vitro* cultivation and subsequent commercialization of bacterial strains of *Pasteuria* spp. parasitizing sting and cyst nematodes, did not lead to much awaited commercialization of *Pasteuria penetrans* attacking root-knot nematodes. However, until then *in vivo* techniques developed for small-scale production could be taken up for greenhouse applications.
- Commercial nursery production systems (public/private) should be developed to provide disease-free seedlings to small farmers.
- Information and communications technology-enabled smart nematode population monitoring systems may be developed for taking effective and timely region-specific integrated pest management modules.
- Use of grafting technology using nematode resistant rootstocks should be perfected at least for use in greenhouses.

Outlook: anticipating future developments

The amenability of fresh tomatoes for food processing will continue to enhance the area under tomato farming, both in open-field and protected systems in India. The acreage under protected cultivation system will increase exponentially. Higher minimum temperatures during winter seasons in polyhouses are likely to increase the number of generations of root-knot nematodes as compared to open-field conditions. Water scarcity is driving farming towards drip/sprinkler irrigation systems, which ensure the availability of continued moisture in the rhizosphere.

In this foreseeable scenario, an explosive nematode-centric biotic stress is envisioned. The ultimate solution is embedded in genetic management; either using techniques like RNAi or developing robotic interventions for enabling commercial-scale grafting using nematode resistant rootstocks.

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36 Sustainable control of root-knot nematodes in protected tomatoes in Italy

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Introduction

Peninsular Italy enjoys warm weather from late spring to mid-autumn and mild temperatures in the remaining months. These conditions favour growing of vegetables all the year round. In 2019, 33,614 ha of greenhouses were used for vegetable and strawberry production, of which 7614 ha (22.6%) grew tomatoes that yielded 578,824 tonnes of fruit valued at about €500 million, making Italy the second largest producer of tomatoes in Europe after Spain and the fifth largest exporter. The greatest areas of greenhouse tomatoes are in Sicily (3100 ha), Lazio (1700 ha) and Campania (900 ha), where the average yield is around 70 tonnes/ha. Tomato cycle can be short (5–6 months) (70%), from midsummer to December or from January/February to June/July, or long (8–9 months) from October/November to May/June (30%).

Economic importance

In Italian protected tomatoes, the only nematode problem is caused by the root-knot nematodes *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. The extent of damage they cause varies according to the virulence of the nematode population and the tomato cultivar.

Between 2015 and 2017 a survey was conducted to determine the importance of root-knot nematodes in Italian greenhouses (N. Greco *et al.*, Italy, 2017, personal communication; Greco *et al.*, 2020). For this purpose, the infestation level was classified as causing low (<20%), medium (21–50%) or high (>50%) yield loss. These losses generally correlate with nematode infestation levels at planting of <2, 3–5, and >5 eggs and juveniles/cm^3 of soil, respectively (Greco *et al.*, 2020). In Italy, 30%, 50% and 20% of the greenhouses have small, medium and large infestations of *Meloidogyne* spp., respectively. Often, *Meloidogyne* spp. are the only soil-borne problem and are so common that tomato cropping cannot be undertaken without controlling them.

According to the survey, the total cost of producing tomatoes is €41,500/ha. The gross margin in a non-infested or treated with effective fumigant nematicide greenhouse is €21,006, but it would be reduced to €9,641, €7,847 and become negative, −€14,154, in lightly, medium and highly infested soil, respectively. If nematode control is not performed effectively, great losses of jobs and yield are to be expected, with the risk of making Italy no longer self-sufficient in tomato supply.

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Host range

These *Meloidogyne* species can damage hundreds of plant species and reproduce also in wild plants. All plants grown in greenhouses, especially solanaceous and cucurbitaceous species that are rotated with tomato, are susceptible to one or more *Meloidogyne* species.

Symptoms of damage

The mentioned root-knot nematodes reproduce and infect crop plants better and are more damaging during warm seasons, with optimal conditions of 25–28°C, and in sandy soils. The most obvious symptom of nematode attack are the galls on the roots that can be a few and small (1–2 mm diameter) in lightly infested soil and large (about 10 mm diameter), covering the entire root apparatus (Fig. 36.1) in medium to highly infested soils, coupled with yellowing, stunting and poorly yielding plants (Fig. 36.2). *Meloidogyne* spp. may interact with the wilting fungus *Fusarium oxysporum* or the corky root agent *Pyrenochaeta lycopersici* (Perry *et al.*, 2009). Generally, when root-knot nematodes are the major problem other nematodes have low soil density.

Biology and life cycle

Meloidogyne spp. may develop three generations per crop cycle in the field and more in greenhouses, thus resulting in reproduction rates as great as 1000 times the nematode level at planting (Perry *et al.*, 2009). However, in Italy, after harvest of a host crop in September, the nematode soil populations decline to 50% in two weeks, to 20% after 1 month and to 5–6% by the following spring (Greco and Di Vito, 2009).

Following frequent planting of resistant cultivars or rootstocks, nematode populations that are virulent toward resistance genes may be selected.

Fig. 36.1. Tomato root severely galled by root-knot nematodes. Photograph courtesy of Nicola Greco.

Recommended integrated nematode management (INM)

The sustainable use of pesticides must rely on integrated pest management (IPM). Based on guidelines prepared by Italian regional phytosanitary services, the control of root-knot nematodes in tomatoes can be by: (i) agronomic measures, such as crop rotation with less susceptible crops, destruction of residues of the preceding crop, use of tolerant/resistant rootstocks and cultivars, avoidance of water stagnation, incorporation of brassica seed pellets in the soil at 2.5 tonnes/ha, 7–10 days before transplanting; (ii) physical method: soil solarization for 45-60 days during June to August; (iii) biological agent products; (iv) plant extracts; and (v) chemicals containing, abamectin, azadirachtin, fenamiphos, fluopyram, fosthiazate and oxamyl.

However, these guidelines often are not followed because the results are not always satisfactory.

Optimization of nematode management

The most used control methods are chemical fumigation, resistant rootstocks and cultivars, plant extracts, soil solarization, non-fumigant nematicides, bionematicides, soil amendments and biofumigation. In 2017, about 10,600 ha of vegetables in greenhouses were grown in fumigated soil, of which 4894 ha were tomatoes. Among the fumigants registered in Italy are products containing dazomet, metam sodium or metam potassium. Some not registered active substances, such as chloropicrin, 1,3-D (now

Fig. 36.2. Plastic-house showing yellowing and stunted tomatoes in soil infested by root-knot nematodes. Photograph courtesy of Nicola Greco.

under re-evaluation) and DMDS (under European evaluation for inclusion in Annex 1), have received derogation grants for use on tomato.

As soil treatments are costly and may impact the environment, control measures must be used only when nematodes are present and must take into consideration any impact on the environment, efficacy, level of nematode infestation and economic benefit.

Therefore, farmers must know the presence and level of nematode soil infestation. Several Italian laboratories are accredited by the Ministry of Agriculture to perform nematological analysis, and farmers can use their services.

To estimate economic thresholds, information is necessary on the relationship between nematode population densities at planting (*Pi*) and yield of the host crop. In microplots, the tolerance limit (*T*) of tomato to *M. incognita* was 0.55 egg and/or second-stage juveniles per cm^3 soil at transplanting [$T = 0.55$ and m (minimum yield) = 0 at very large *Pi*] (Di Vito *et al.*, 1991). Sasanelli (1994), based on these data and their logarithmic calculation, produced tables of nematode pathogenicity and derived the curve in Fig. 36.3 relating yield of tomato to *Pi*, expressed as % of yield in the absence of nematodes (left axis) and absolute yield (right axis), assuming an average tomato yield of 70 tonnes/ha. This relationship is useful for the estimation of yield loss if *Pi* has been determined.

Greco *et al.* (2020) surveyed the available scientific literature and compared the efficacy of different methods of control used mainly in greenhouses (Table 36.1). This table reports, for each group of control methods, the percentage of nematode control at harvest in soil and roots, and also percentage yield increases. In general, good performance, in both nematode control and yield increase is given by soil fumigants (1,3-D, DMDS, dazomet, metam sodium and metam potassium), resistant cultivars and rootstocks, and soil solarization. The other control methods were less effective. The differences in yield among different control methods increase, moving from lightly infested soils to medium and heavily infested soils, where yield increases of 336%, 289% and 203% were achieved with DMDS, 1,3-D and resistant cultivars and rootstocks, respectively. Thus, the impacts of fumigants and alternative methods of control on yield of tomato vary greatly (Table 36.2).

To avoid spread of nematodes, farmers must transplant healthy plants. Farming machines and tools, boots and shoes must be sanitized before moving to another greenhouse. If, when checking for root galling, a few galled plants are found in a small area, this area must be isolated, treated with a fumigant and left

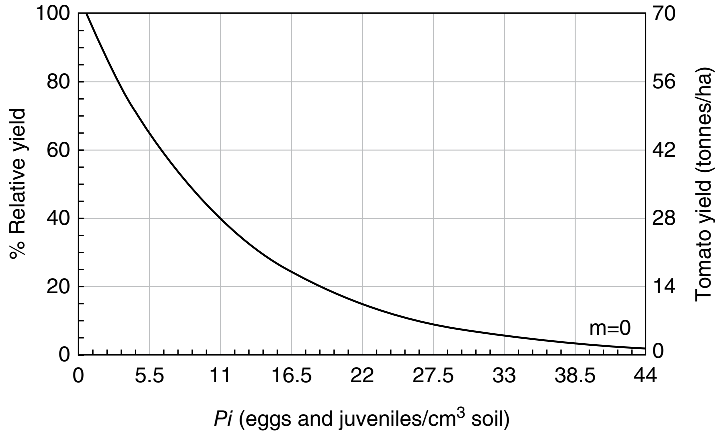

Fig. 36.3. Curve relating population densities of *Meloidogyne incognita* at planting (*Pi*) with yield of tomato, according to Di Vito *et al.* (1991) and Sasanelli (1994). Image courtesy of Nicola Sasanelli.

uncropped and free of weeds until the nematodes disappear.

To predict the effect of a treatment on yield, rather than considering the proportion of nematodes killed, we have to consider the number of nematodes escaping the treatment. For instance, if the nematode population before treating (*Pi*) is 20 eggs and juveniles/cm^3 soil and the control method is expected to kill 60% of the nematodes, 8 nematodes may still remain in the soil and cause about 52% yield loss (Fig. 36.3). If, instead, *Pi* is 2, only 0.8 nematode would escape the treatment with no noticeable yield loss of the following crop. Therefore, to use the control methods recommended by the Italian IPM guidelines, it is necessary to reduce soil infestation. Our suggestions for appropriate and practical procedures are given below.

Sampling for infection intensity

An easy way to assess and predict plant and soil infestation levels is as follows. Soon after harvest, plants must be uprooted and checked for root galling to assess uniformity and intensity of infection. The root gall degree can be evaluated according to 0–5 or 0–10 scales (Ambrogioni *et al.*, 2014). The expected soil infestation would be low if the gall index is 1–2 (0–5 scale) or 1–3 (0–10 scale), medium if root gall index is 2–3 (0–5 scale) or 3–5 (0–10 scale), and high if root gall index is greater than these figures.

Infested root removal and soil drying: All plants must be removed, put in a safe place and burned, and the greenhouses must be kept free of weeds. A severely infected root may contain more than 100,000 eggs. The soil should then be left humid for 1–2 weeks to favour the hatching of eggs remaining in the soil and ploughed two or three times to a depth of 30–40 cm at 2- to 3-week intervals. If possible, the greenhouse must meantime be kept closed; dry conditions and high soil temperatures will further reduce the nematode population. Just before planting, a soil sample must be collected, mixed thoroughly and a minimum of 1000 cm^3 in a plastic bag taken to a laboratory to have nematodes extracted and counted (as eggs and juveniles/cm^3 soil). Identification of the nematode to species level may also be useful. Based on the results of soil analysis, data in Fig. 36.3 on expected yield loss and Table 36.1 on the effectiveness of different control methods, the most appropriate nematode control measure can be selected.

Nematicides and resistant cultivars

If the nematode population is rather high, select the best available fumigant. An effective treatment with a fumigant could be useful for two consecutive crops. Nematicides containing one of the metam products are not very effective against nematodes at the permitted rates – almost a double rate would be necessary for satisfactory control

Table 36.1. Efficacy of methods to control root-knot nematodes on solanaceous and cucurbitaceous crops in greenhouses infested with low, medium and high levels of the nematodes at planting (from Greco *et al*., 2020).

	Infestation levels								
	Low			Medium			High		
	% reduction of nematode infestation at end of crop			% reduction of nematode infestation at end of crop			% reduction of nematode infestation at end of crop		
Control means	Soil	Roots	% yield increase	Soil	Roots	% yield increase	Soil	Roots	% yield increase
Fumigants:									
1,3-D	60.5	66.1	45.2	82.2	65.7	88.7	85.6	74.3	289.2
DMDS	61.7	73.4	42.3	81.0	82.3	79.2	89.3	70.6	336.6
Dazomet, metam sodium, metam potassium	47.0	36.9	61.1	74.8	57.5	53.9	46.5	24.7	54.7
Non- fumigant nematicides	60.5	42.8	30.8	50.9	45.0	48.5	23.1	19.7	34.1
Soil solarization	66.5	60.2	36.4	98.1	68.6	56.7	66.3	65.5	69.1
Soil solarization + nematicides or bioagents	63.8	71.1	21.5	na	na	na	na	na	na
Resistant cultivars or rootstocks	90.2	95.7	136.3	85.3	81.4	43.3	na	76.1	203.3
Bioagents	38.5	25.2	3.5	29.8	21.6	58.2	40.7	21.7	34.8
Plant extracts	26.9	39.0	23.4	na	na	na	na	na	na
Soil amendments	58.9	54.9	65.3	73.6	35.7	33.4	58.3	64.9	72.0

na, Data not available.

Table 36.2. Effect of different groups of control methods on yield of tomatoes grown in greenhouses in Italy at low, medium and high levels of *Meloidogyne* spp. infestation (from Greco *et al*., 2017, unpublished).

		Infestation level		
Yield	Treatment group	Low	Medium	High
Tonne/ha	Non-treated	42.1	36.5	17.4
	Chemical fumigation (with 1,3-D or DMDS)[a]	69.4	69.4	69.4
	Other control means	56.8	54.8	30.4
	Losses by other means *vs* chemical fumigation	12.6	14.6	39.0
% increase *vs* non-treated	Chemical fumigation	65	90	300
	Other control means	35	50	75

[a]Here as average yield obtained with soil fumigation at any infestation level was considered 69.4 tonne/ha, as estimated through interviews.

in heavily infested soils. If tomato has to be planted in July–September, the use of resistant cultivars or rootstocks must be avoided. The *Mi-1* gene, conferring resistance in these materials, loses resistance at soil temperatures above 28°C. On the long tomato cycles planted in October/November, the combination of soil fumigation with resistant cultivars/rootstocks would be a sound approach to control. Also, some nematode populations may have developed virulence toward the *Mi-1* gene. Therefore, farmers are advised to perform a biological test. Resistant materials must be planted only once in 3–4 years. However, a nematode population virulent to *Mi-1* gene may not be virulent to resistance genes incorporated in other crop plants, such as pepper and aubergine rootstocks and, therefore, these plants could be rotated with tomato.

Biofumigation

If the management choice is biofumigation, the selected plant species (cultivars of *Brassica napus*, *Eruca sativa*, *Raphanus sativus*) can be sown directly in the greenhouse or in a nearby field and, when at flowering stage, should be chopped and spread evenly in the greenhouse, incorporated into the soil and irrigated (Ambrogioni *et al*., 2014). Instead of growing selected plant species, their pellets or those of defatted seed meals can be used.

Solarization

For a better performance, the soil can also be covered with transparent 30–50 μm thick polyethylene film to solarize the soil jointly with the biofumigation treatment. Soil solarization can be effective in the top 20–25 cm soil but both ploughing and soil solarization during cool periods are useless; this would be the right time for planting resistant material.

Treatment rotation

Before each crop cycle, monitoring nematode soil infestation is strongly recommended to provide the right basis for making the best decision. Farmers are advised not to use continuously the same control measures but to rotate and integrate them. In general, all treatments should be applied 2–3 weeks before planting to kill most of the nematodes in the soil prior to crop planting. If necessary, the same or another treatment can be applied 2–3 weeks after planting to protect roots for a longer period.

Future research requirement and outlook

Effective means and combined strategies of control should be made available to farmers and research into more effective methods with less impact on the environment should be fostered. The lack of effective means of control would encourage a shift to soil-less cropping, with the problem of getting rid of exhausted substrates, increased import of production means and, eventually, the abandonment of heavily infested soil, with the risk for Italy of turning from a positive to a negative import/export balance.

If global warming continues, nematodes may complete more generations per crop cycle such that larger population densities of the nematodes develop by the end of each crop cycle, making damage more severe and control on the following crop more difficult. Control measures must be used at times and rates that are effective and as directed. The ideal control method should be cheap, easy to handle, effective, not leave toxic by-products in soil and edible plant parts, and possibly be effective against concomitant soil-borne problems. So far, such a method does not exist but progress has been made with effective strategies using more technical solutions.

Further research is needed to identify new and effective and less impacting chemical nematicides and bionematicides and breed for new genes able to confer resistance to populations virulent to the *Mi-1* gene and at high temperatures. This requires information and investigation on nematode virulence occurring in different tomato areas and their virulence toward known resistance genes. New genes have already been identified in wild *Solanum* species that are not currently compatible with *S. lycopersicum*. Efforts should be made to incorporate these genes into new hybrids/cultivars of tomato, even if that requires the use of non-conventional genetic techniques. Moreover, seed companies should indicate the resistance genes incorporated in their cultivars; the indication of intermediate resistance as done currently is too vague and confusing. Finally, an effective extension service is needed for transfer information of new technologies as soon as they are discovered to farmers.

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37 Integrated management of root-knot nematodes for cucurbit crops in Southern Europe

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Introduction

The family Cucurbitaceae includes vegetable crops cultivated worldwide such as bottle gourds or calabashes (*Lagenaria*), cucumbers (*Cucumis sativus*), luffas (*Luffa*), melons (*C. melo*, *C. metuliferus*, *Momordica charantia*), watermelons (*Citrullus lanatus*) and gourds, marrows, pumpkins, squashes and zucchinis (*Cucurbita*). In Spain, cucumber, melon, watermelon and zucchini are frequent rotational crops with tomato or pepper under polyethylene greenhouses. The most common cropping cycles are: (i) a spring cycle from December–February until April–June; and (ii) an autumn/winter cycle, from late July–September until January–March. In many cases, both cycles are combined using different rotational sequences of solanaceous and cucurbitaceous plants (Talavera *et al.*, 2012). Transplants of peat-block seedlings from specialized nurseries are generally used for greenhouse cucurbit production.

Plant parasitic nematodes associated with cucurbits include numerous genera, but *Meloidogyne* spp. (root-knot nematodes; RKN) are by far the most important due to their worldwide distribution, potential damage and economic importance.

Economic importance

The damage caused by RKN is directly related to nematode soil densities at planting (*Pi*), but the extent of yield losses depends on RKN prevalence and abundance in the cropping area, crop susceptibility tolerance, soil type, temperature, crop management and the length of the growth period. Greco *et al.* (2020) estimated the impact of RKN on cucurbits in Southern Europe as extremely high since cultivation of these crops under high nematode pressure and without soil fumigation is not profitable. They estimated the total revenue lost by farmers from solanaceous and cucurbitaceous crops together to be about €800 million per year. In southern Spain, economic losses due to RKN in 17,500 ha of greenhouse grown cucurbits were estimated at €2.3 million per cropping cycle, which represents 5% of the market value received by farmers (Talavera *et al.*, 2012). These values, however, could be interpreted as a lower boundary of the economic losses incurred since they were estimated in fields treated with nematicides and loss would therefore be higher without nematode control. Tolerance limits (*Pi* up to which no measurable yield occurs) for RKN in cucurbits may range

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from below 0.5 to more than 50 nematodes/100 cm^3 soil, and yield losses from 10% up to 75% (Krishnaveni and Subramanian, 2005). We have estimated tolerance limits of 0.01, 20, and 402 *M. javanica*/100 cm^3 soil for cucumber, watermelon and zucchini, respectively, which indicates different tolerance levels of cucurbits to RKN damage. Maximum yield losses due to RKN in protected cultivation in Spain were estimated to be 88% in cucumber, 53% in zucchini and 35% in watermelon (Verdejo-Lucas and Talavera, 2019).

Host range

The most common RKN species associated with cucurbits are *M. arenaria*, *M. incognita* and *M. javanica*. In addition, some cucurbits are hosts for *M. enterolobii*, *M. floridensis*, *M. hapla* and *M. hispanica*.

The suitability of a host plant for parasitic nematodes is expressed as the ability of the plant to reproduce the nematode, and it is measured by its reproduction factor ($Rf = Pf/Pi$), *Pi* being pre-planting population densities and *Pf* is population densities at the end of the crop. As a rule, susceptible host plants show a $Rf > 1$, whereas resistant or non-host crops register a $Rf < 1$. Cucurbit crops are all hosts to RKN, but they differ in host suitability levels. Nonetheless, low *Pi* levels will result in a high *Rf*, and high *Pi* levels, in a low *Rf* which is often due to severe root damage. Bottle gourd and cucumber are better hosts for *M. incognita* than muskmelon, bitter melon, zucchini and watermelon in this order. Besides, cucumber and melon are better hosts for *M. arenaria* and *M. javanica* than watermelon and zucchini (Verdejo-Lucas and Talavera, 2019). Furthermore, nematode infection depends on the RKN species; *Meloidogyne incognita* showed lower reproduction than *M. javanica* on zucchini. By contrast, *M. javanica* had less reproductive success than *M. incognita* on bottle gourd and watermelon, whereas both RKN species reproduced similarly on cucumber (Verdejo-Lucas and Talavera, 2018).

Distribution

RKN are widely distributed in cucurbitaceous growing areas worldwide. In the intensive protected cultivation areas of southern Spain, about 30–40% of the fields were infested by RKN at levels that caused economic problems or even made the cultivation of cucurbits on certain plots impossible (Talavera *et al.*, 2012).

Symptoms of damage

RKN are polyphagous obligate sedentary endoparasites that disrupt the vascular system of the host plant and interfere with physiological processes involved in water and nutrient uptake. Consequently, the nutrient balance is upset, resulting in stunted plants, retarded plant growth, leaf chlorosis (Fig. 37.1A), abnormal wilting even when the soil is wet, early senescence, small leaves, few flowers, poor fruit quality and yield losses. Nematode infected plants are usually located in patches or rows, reflecting the typical spatial pattern aggregation of plant parasitic nematodes. Under heavy nematode infestation, crop seedlings may fail to develop (Fig. 37.1B).

Regarding below-ground symptoms, the presence of galls in the roots is the main sign associated with RKN infection. Galls are formed by the hyperplasia of the root cortical cells and vary in form, size and number, depending on the RKN species but also on the host status of the plant. On watermelon, RKN display profuse root galling but low population increases, this suggests the hypersensitivity of watermelon to RKN. *Meloidogyne incognita* induced larger galls than *M. javanica* on zucchini despite similar gall numbers. Root galling severity is inversely related to zucchini yield. Therefore, *M. incognita* has higher pathogenic potential than *M. javanica* on zucchini.

Biology and life cycle

RKN are poikilothermic organisms and therefore temperature influences their life cycle. The *Rf* is strongly influenced by temperature and related to the accumulated degree-days over a base temperature during the cropping cycle. Information on the thermal requirements of *Meloidogyne* spp. are useful to estimate the number of generations per cropping cycle. RKN have shown similar thermal requirements in zucchini, melon, cucumber and pumpkin, with life cycles from 28 days at constant soil temperatures of 28°C to 85

Fig. 37.1. Above-ground symptoms of root-knot nematode in cucurbit crops. **(A)** Stunted zucchini plant with leaf chlorosis. **(B)** Patches of poor growth in watermelon crop. Author's own photographs.

days at 17°C. Accordingly, one or two nematode generations can be completed on most cucurbits in a single cropping cycle of three months. Thus, *M. incognita* reproduction in zucchini was greater in autumn than spring cropping cycles, despite similar growth periods (105 days), but zucchini yields were lower in autumn than spring. These differences were probably due to the progressive decline in soil temperatures occurring in autumn (from 25°C to 16°C) in contrast to their progressive increase in spring (from 16°C to 26°C) which produced optimal and suboptimal conditions for RKN development, respectively (Verdejo-Lucas and Talavera, 2019).

Interactions with other nematodes and pathogens

There are few studies on the interrelations of microbial pathogens and plant parasitic nematodes causing disease complexes in cucurbits. *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani* have been reported to interact synergistically with RKN and cause greater disease in cucumber, pumpkin, melon and other cucurbits.

Recommended integrated nematode management (INM)

Integrated nematode management starts with preventive measures meant to exclude nematodes from areas where they have not existed before, and it is accomplished by using nematode-free seedlings and substrates. Methods to suppress the RKN disease on cucurbits include chemical control, plant resistance, biosolarization, biological control, biopesticides and cultural management. All these methods can be applied alone, in combination or sequentially, and their effects should be considered on a short- and long-term basis as actions taken in one crop may affect the subsequent crop in the rotation. Growers in Spain are familiar with RKN and use different combinations of INM.

Chemical control

Chemical fumigation is the first option for Spanish farmers wherever RKN is a limiting factor; 95% of farmers disinfest soils, annually or biannually after the autumn/winter crop, using a combination of soil solarization and nematicides (mainly 1,3-dichloropropene [1,3-D]) under plastic sheets (Talavera *et al.*, 2012). Even though the use of most chemical fumigants is currently banned or restricted within the European Union, member states can allow temporary authorizations in exceptional cases. This exceptional use has been granted for 1,3-dichloropropene+chloropicrin in protected vegetables in several European countries. As an alternative, a new fumigant, dimethyl-disulphide, is under registration process in Europe and it has been tested successfully as an effective nematicide (Greco *et al.*, 2020).

Improvements in fumigant application have been developed to reduce dosages and environmental risks. For instance, low rates of 1,3-dichloropropene (84–168 l/ha) were sufficient for satisfactory RKN management in a short-season crop of squash, and chisel-applications were more effective in terms of RKN control than drip applications (Desaeger *et al.*, 2008).

Cucurbits are generally cultivated as short cycle crops for 3–4 months under protected cultivation. Therefore, nematicides must be applied as pre-planting treatments to prevent accumulation of any toxic residue in the fruits. Numerous chemicals and non-chemical methods were tested in a 12-year period to determine their efficacies in reducing RKN soil densities in experimental plots in southern Spain. Fumigation with 1,3-dichloropropene+chloropicrin, was the most efficient treatment against RKN, which reduced RKN soil populations by 87% on average. Dimethyl-disulphide ranked second in efficacy (78%), but it is not yet registered for use in Europe. The group of non-fumigant nematicides followed in the third place (50–65%) with no differences among them (Fig. 37.2). Currently, only fenamiphos, fosthiazate, fluopyram and oxamyl are authorized against RKN in cucurbitaceous crops in Spain. Dazomet, ethoprophos, metam sodium and metam potassium were deregistered in 2020.

Plant resistance

Plant resistance is an effective, safe and economical method to control RKN, although gene-mediated resistance to RKN has not been identified so far in most cucurbits. Zucchini has shown intermediate resistance to *M. incognita*. The resistance mechanisms include malfunction of the giant cells that caused 74% of the feeding sites to deteriorate prematurely, which prevented the transition of fourth stage juveniles to adult females. Consequently, only 26% of the *M. incognita* within the roots attained the egg-laying female stage but they showed low fecundity (Verdejo-Lucas and Talavera, 2019). In contrast, zucchini was highly susceptible to *M. javanica.*

Grafting susceptible high-yielding cucurbit scions onto resistant or less susceptible rootstocks could be an option to circumvent the lack of resistance in some cucurbits. Most rootstocks used for cucurbits are hybrids of *Cucurbita maxima* × *Cucurbita moschata*, which are good hosts for RKN and cucurbit scions grafted on them suffer yield losses under field conditions (López-Gómez *et al.*, 2016). Poor or non-host rootstocks to RKN such as *Cucumis metuliferus*, some *Citrullus amarus* and *Luffa cylindrica* have been tested as rootstocks for cucurbits (Verdejo-Lucas and Talavera, 2018; García-Mendívil *et al.*, 2019). Grafting prevented growth reduction and lowered the build-up of nematode populations, making the grafted plants tolerant to nematodes. This technique is commonly used for watermelon in Spain, since farmers can purchase watermelon grafted on *C. amarus* from commercial nurseries. Other RKN resistant rootstocks are not yet commercially available.

Biosolarization

Soil solarization reduces RKN populations effectively when soil temperatures reach 40–45°C at the 0–30 cm soil layer for at least 6 weeks. When cucurbits are grown in locations where sunlight can provide enough irradiation to heat the soil up to these temperatures, soil solarization is an alternative to soil disinfestation with chemicals. The efficacy of soil solarization is highly improved when it is complemented with the addition of organic manures under the plastic sheets (biosolarization). Thus, biosolarization can reach efficacies in reducing RKN populations close to chemical fumigation (see Fig. 37.2). In addition, amending soil with organic matter stimulates the growth of soil microorganisms antagonistic to nematodes and can increase cucurbit crop yields, indicating an increase in plant tolerance against RKN infection (Krishnaveni and Subramanian, 2005).

Biological control

Several biological control agents (*Arthrobotrys oligospora*, *Bacillus firmus*, *Purpureocillium lilacinus*, *Pseudomonas fluorescens*, *Trichoderma* spp.)

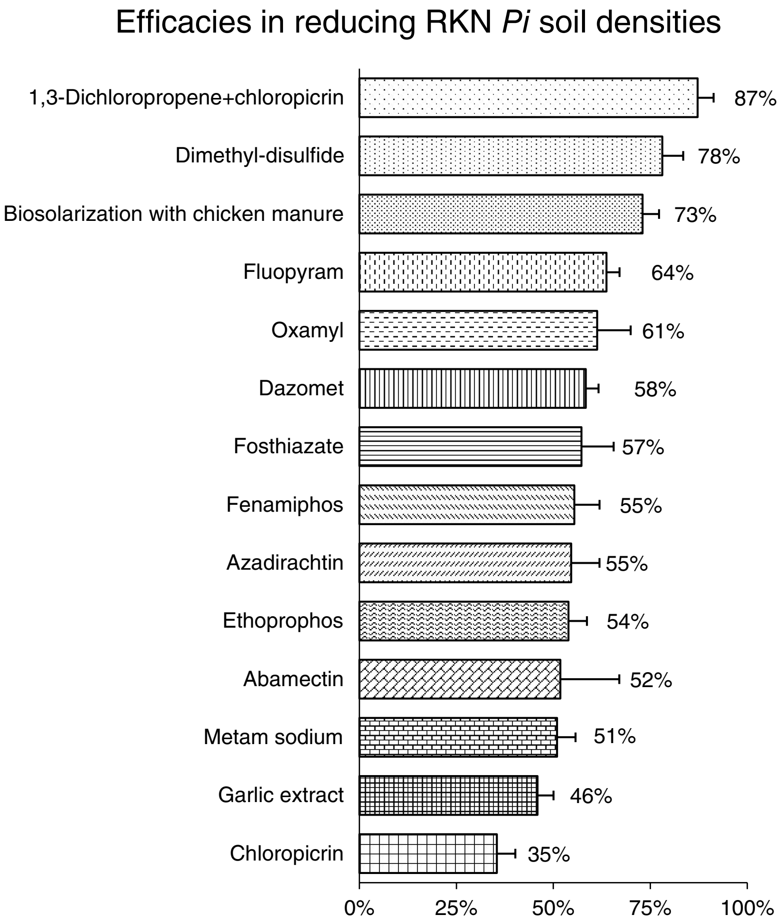

Fig. 37.2. Efficacies of several chemical and non-chemical treatments in reducing pre-plant soil densities (*Pi*) of root-knot nematodes in protected vegetable plots. Author's own figure.

have been tested in cucurbit crops (Krishnaveni and Subramanian, 2005) and they increased cucurbit growth and yield, and reduced root galling. Currently only *B. firmus* and *P. lilacinus* are registered for use against RKN in cucurbits in Spain, but they are used only as support in INM combined with other control measures when no chemical fumigation is used.

Biopesticides

Some products derived from microorganisms or plants (azadirachtin, abamectin, garlic extract) are also registered for use against RKN in cucurbits in Spain and have shown efficacies of 45–55% on average in reducing RKN populations (see Fig. 37.2). Such efficacies can be sufficient in cases of low or medium RKN infestation levels but would not be efficient when RKN *Pi* levels are high.

Cultural management

Crops started from transplants will be more tolerant to higher RKN *Pi* levels and subsequent nematode damage than when directly seeded because they have already a well-developed root system at the time of transplanting. Rotation in Spain with resistant plants (Mi-tomatoes or Me-peppers), prior planting cucurbits has proven effective to reduce plant damage and increase yield of cucumber and zucchini because soil population densities after a resistant or non-host crop were lower than after a susceptible one. This tactic will reduce nematicide use although nematode densities will increase after growing a susceptible cucurbit.

Optimization of nematode management

Nematode management still does strongly rely on the use of nematicidal fumigants, but a shift from chemical to non-chemical control is required to ensure environmentally safer measures. A combination of non-chemical methods is needed to reduce RKN population levels, but this approach would be profitable only for low or medium RKN infestation levels. Modifying planting time, a careful selection of cultivars, including resistant solanaceous crops in rotations, and adjusting the length of the cropping cycle could mitigate yield losses. Nonetheless, when RKN host crops are cultivated intensively, twice or three times a year for profitability, and no tools are used to reduce RKN soil populations, *Pi* will reach high levels and yield losses will occur in subsequent crops because none of the non-chemical methods have enough efficacy for nematode control on cucurbits (Greco *et al.*, 2020). Therefore, nematicides are still necessary to maintain intensive cucurbit production, and their use will be justified by high infestation levels of RKN in the field. In any case, fumigant nematicides should be applied by certified applicators to reduce risks for human health.

Management decisions will depend on the RKN–cucurbit crop combination due to the differential host suitability of cucurbits to RKN species. The poorer host suitability of zucchini to *M. incognita* and watermelon to *M. javanica* on numerous genotypes can be used to limit population build-up and subsequent yield losses providing that the *Meloidogyne* species infesting the field is known (Verdejo-Lucas and Talavera, 2018). Growers have an ample choice of genotypes for nematode management particularly useful for sustainable agricultural systems. Zucchini shows a range of tolerance to low and medium RKN population densities despite its susceptibility to the nematode. Although zucchini is less susceptible to *M. incognita* (reduced population build-up), it might suffer greater damage due to greater root galling. By contrast, zucchini would be more tolerant to *M. javanica* damage because of less root galling and thus would stand higher *Pi* before affecting crop growth and yield. Watermelon genotypes show less root galling and RKN reproduction when infected by *M. javanica* than *M. incognita*, suggesting that watermelon would tolerate higher *M. javanica Pi* levels before showing yield losses. Cucumber and melons have low tolerance limits to all RKN, and therefore, highly effective control methods such as soil fumigation or grafting onto resistant rootstocks are needed to prevent yield losses. Grafting cucurbitaceous crops on *L. siceraria* could be an alternative method to grow susceptible cucurbits in *M. javanica* infested soils.

Future research requirements

Breeding cucurbit rootstocks that are resistant or tolerant to RKN would provide a helpful tool in INM against RKN, especially if they offer resistance against two or more key pathogens (i.e. *Fusarium* + RKN). Additional studies are needed to elucidate the effect of wider scion–rootstock combinations on fruit quality and yield. Host range studies have shown large variation in host status within RKN species and among genotypes due to their genetic background which may provide tolerance to the nematode. These differences in host status could be exploited to regulate population increases in the absence of resistance genes. The use of databases as Best4soil or Nemaplex could be helpful when determining the host status of a cultivar, subspecies, or plant species for a RKN species and would facilitate INM decisions. Knowledge on the economic threshold for different cucurbits to different RKN species is essential to design INM strategies suitable to specific cropping systems in different agroenvironments.

Outlook: anticipating future developments

The ban on most chemical fumigants for intensive cucurbit production has left farmers with very few alternatives for RKN control. A smart choice of cultivars, rootstocks and non-chemical control methods will be necessary to keep production in low- or medium infestation plots, but in cases of high infestation levels, farmers will have to still use chemical control or change to soil-less cultivation.

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38 The northern root-knot nematode: A forking problem of carrots in Germany

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Introduction

The northern root-knot nematode *Meloidogyne hapla* is one of the most damaging plant parasitic nematodes on vegetables in temperate regions. But *M. hapla* can also infect several other crops, in temperate regions as well as at higher altitudes in the tropics. In Germany, *M. hapla* is a major problem in organic farming, particular on carrots. During a survey conducted in 2005, *M. hapla* was found in about 50% of the organic fields investigated (Hallmann *et al.*, 2007). The average population density of *M. hapla* was 109 nematodes/100 ml soil, but maximum numbers reached up to 3312 nematodes/100 ml soil. Those numbers show the enormous potential of this species to build up to high infestation levels when conditions are favourable.

Economic importance

When organic farmers in Germany were asked about the crops that were most damaged by plant parasitic nematodes, carrots were mentioned first with 64% of all incidences, followed by celery and onion with 15% and 6%, respectively (Hallmann *et al.*, 2007). Asking further about what nematode species were damaging carrots, *M. hapla* was by far the economically most important species mentioned, whereas *Pratylenchus penetrans* and other plant parasitic nematode species were considered of much lower relevance. The economic damage caused by *M. hapla* is mainly due to poor crop quality such as taproot and root deformation and less to reduced yield, because poor root quality reduces marketable yield. In addition to the losses in marketable yield there are also higher costs for sorting out deformed carrot taproots. If carrots become infested in the early seedling stage, roots become stunted and forked. Such poor-quality carrots need to be hand sorted from the rest, which is time consuming and costly. If carrot batches reach a certain level of total deformed taproots, harvest might be terminated resulting in complete failure of the crop. The crop quality accepted by the market has no fixed value but is rather a function of supply and demand as well as of the production type (fresh market versus processed). In some years, 10% culls will result in rejection of the produce, in other years the cut-off level can be 50%.

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Host range

Meloidogyne hapla is an extremely polyphagous species attacking mainly dicotyledonous crops and weeds of herbaceous and woody origin. However, monocotyledonous plants can also be damaged, such as onion, although onion is reported to be a poor host for *M. hapla*. Goodey *et al.* (1965) listed over 550 host plants of *M. hapla*, but many more have been identified since then. The main crops affected by *M. hapla* besides carrot and onion are potato, sugar beet, tomato, celery, pea, lucerne, strawberry and roses. At this point it should be stressed that a high nematode reproduction does not necessarily lead to high levels of plant damage. For example, lupins, white clover or phacelia are good to excellent hosts for *M. hapla*, allowing high nematode reproduction, but the crops are not negatively affected by the nematode. These crops seem to be tolerant to nematode infestation. Tolerance causes confusion among the farmers growing those crops. Those farmers who observe good crop performance believe they have a healthy soil but are unaware of the high numbers of *M. hapla* that are propagated under these tolerant crops. When a susceptible crop such as carrot is sown afterwards, complete failure of the crop can occur. Thus, it is important for the farmer to have a good understanding of the reproduction potential of a given crop for *M. hapla* as well as of the performance of the crop under nematode pressure. Information on both aspects is required for managing *M. hapla* by crop rotation. This type of nematological information has been made available through Best4Soil (https://www.best4soil.eu/, accessed 11 October 2020) – a network of practitioners that share knowledge on prevention and management of soil-borne diseases. Finally, it is worth mentioning that *M. hapla* does not reproduce on grasses and cereals, which makes those plants good choices for the management of *M. hapla*.

Distribution

Besides being very polyphagous, *M. hapla* is also widely distributed. It has been reported from all continents except Antarctica. *Meloidogyne hapla* is the most common root-knot nematode in temperate regions, but also occurs in the cooler, higher altitude areas of the tropics. For example, *M. hapla* has been reported from cut flowers grown in Ethiopia at altitudes up to 2300 m (Meressa *et al.*, 2015). Despite their wide distribution, populations of *M. hapla* from around the world are quite similar regarding their morphological and molecular characteristics (Meressa *et al.*, 2015). In Germany, *M. hapla* is found in almost any production system where host plants are grown and soils have a light texture ranging from loamy sand to sandy loam. In general, *M. hapla* is more common in vegetable-dominated rotations than in cereal-driven rotations, and also in organic farming over conventional farming. The lower infestation of *M. hapla* in conventional farming compared to organic farming is best explained by the lower weed pressure and therefore lack of alternative hosts. In addition, organic farmers tend to grow more leguminous crops for nitrogen fixation. These are good hosts for *M. hapla*.

Symptoms of damage

Typical symptoms of *M. hapla* infestations are root galls (Fig. 38.1). Root galls are relatively small and subspherical in comparison to the large root galls caused by tropical *Meloidogyne* species. Root galls of *M. hapla* also often show proliferation of lateral roots, which does not occur on root galls of other species. Early infestation of the main root will inhibit further root growth and the initiation of lateral roots. In the case of carrot, this will result in stunting and bi-forking of the taproot (Fig. 38.2). With increasing nematode numbers, root function is impaired and plant growth inhibited. At this stage, *M. hapla* infestation becomes visible above ground as a heterogeneous plant stand that is unevenly distributed over the field forming a patchy pattern (Fig. 38.3). When above-ground symptoms are first visible, the below-ground quality damage can already reach levels where harvest of the crop is no longer economical.

Biology and life cycle

M. hapla is an obligate sedentary endoparasite. The second-stage juveniles penetrate the root near the root tip, migrate upwards within the root cortex and finally settle close to the conducting elements where they initiate a feeding site. In response to secretions of the juveniles, the plant forms a giant cell system from which the nematode feeds for the rest of its life. The juveniles undergo three moults and with each moult, the nematode becomes more obese. The female swells enormously to become melon-shaped (hence the genus name!) and produces 200–400 eggs that are laid on the root surface in a protective gelatinous matrix. The main reproduction is by parthenogenesis, but sexual reproduction might occur towards the end of the season when nutritional conditions for the nematode become worse. Males are vermiform and leave the root in search of mating females. Unlike tropical root-knot nematode species, *M. hapla* can withstand freezing conditions, but conversely is less tolerant to high temperatures. Under favourable conditions, *M. hapla* produces two to three generations per year.

Fig. 38.1. Roots of carrot heavily galled after infestation with *Meloidogyne hapla*. Photograph courtesy of Julius Kühn Institute.

Interactions with other nematodes and pathogens

M. hapla causes wounding of plant roots during root penetration and feeding, as seen with all plant parasitic nematodes. Such wounds are used by soil-borne pathogens to infect the plants and might result in synergistic yield losses. Interactions of *M. hapla* with soil-borne pathogens haven been described for the fungal pathogens *Fusarium oxysporum*, *Rhizoctonia solani* and *Verticillium dahlia* (LaMondia, 1992), but this list is probably not complete considering the manifold interactions of other root-knot nematodes with soil-borne fungal and bacterial pathogens (Monzanilla-López and Starr, 2009).

Recommended integrated nematode management (INM)

Rotation

Major components for integrated management of *M. hapla* include crop rotation and

Fig. 38.2. Deformation of carrots after infestation with *Meloidogyne hapla*. Photograph courtesy of Julius Kühn Institute.

Fig. 38.3. Carrot field infested with *Meloidogyne hapla*. Note the irregular growth of the plants and missing plants. Photograph courtesy of Julius Kühn Institute.

use of resistant cover crops. The fact that *M. hapla* primarily attacks dicotyledonous crops and populations rapidly decline in the absence of a host plant, make this species an ideal candidate for control by crop rotation. Growing a non-host for a full season will reduce population densities of *M. hapla* by 80% and more, depending on the crop and local environmental conditions. Excellent non-hosts are cereals like barley, wheat and oat, or grasses like annual ryegrass or Italian ryegrass. However, good control efficacy requires a clean stand to avoid dicotyledonous weeds, which in general are good hosts. These weed hosts serve as a green bridge for *M. hapla*, supporting survival and reproduction from one host crop to the next host crop. In conventional farming systems, weed control by herbicide application is highly effective. However, in organic farming systems where synthetic herbicides are not available, weed control is a major challenge and in most cases not satisfactory. Therefore, different approaches are required for effective management of *M. hapla* as outlined below.

Sanitation year

In a sanitation year a crop is grown only for the purpose of reducing *M. hapla* below the economic threshold level of the following high value market crop. To be economic, the costs for such a year without marketable yield must be compensated by the higher yield of the following cash crop. Such a system is successfully applied in organic farming in Germany to control *M. hapla* in carrots and onions. The sanitation year starts with an overwintering clover–grass mix in September of the previous year, where the clover serves as a trap crop and the grasses as non-hosts for *M. hapla* with both crops binding and conserving soil nutrients for the following season. The clover–grass mixture is then chopped and incorporated in late May/early June of the following year. Besides the trap crop effect, clover further benefit by acting as a green manure crop for nitrogen fixation in addition to improving soil structure, organic matter content and water retention. Following incorporation of the clover–grass mix, the field is kept bare for 1–2 months allowing weeds to germinate, which are then destroyed during seedbed preparation of the next crop thereby breaking the nematode life cycle. This approach is a true form of trap-cropping. In early August, black oat (*Avena strigosa*) is planted. Black oat is a non-host for *M. hapla* – it successfully suppresses weeds and conserves the soil nutrients for the following crop. Since black oat is not hardy, plants will degenerate over winter and can easily be incorporated in early spring of the next year to prepare the seedbed for the following high value crop, e.g. carrots or onions. Long-term farmer experience has shown that a sanitation year can increase total yield up to 30% but even more important, significantly improves the quality of the product. As a result, total marketable yield is increased up to 50%. Economic analysis of such a carrot production system in sandy soils in northern Germany indicated that the sanitation year was already economic at initial densities of 24 *M. hapla*/100 ml soil.

Cover crops

Cover crops have several benefits. They increase soil organic matter content, stimulate soil health and protect the soil against wind and water erosion. In terms of *M. hapla* control, they can be used as a non-host crop, a trap crop, for the purpose of biofumigation or as a resistant crop. For optimum nematode control, the cover crop should have early and rapid establishment for efficient weed suppression and an intensive rooting system.

Non-hosts

Good non-host candidates for cover cropping are black oat and French marigold (*Tagetes patula*). Most populations of *M. hapla* cannot reproduce on those crops and the population declines over time.

Trap crops

The ideal trap crop combines an excellent host status, an extensive root system that develops quickly after planting and low seed costs. All those aspects are provided by fodder radish, but other cover crops are also suitable. The trap crop stimulates the hatching of *M. hapla* and juveniles enter the newly emerged roots. With initiation of a feeding site in the root, the nematode becomes sedentary and thus is trapped in the root. The better the rooting in the soil the more nematodes will be attracted and trapped. This explains why excellent preparation of the seedbed is so important for the overall success of such a measure. The cover crop then needs to be destroyed before *M. hapla* starts its reproduction, which is the case at about 350-degree days to the basis of 8°C when first eggs are laid. The decision for the optimum time of trap crop destruction can be made according to the temperature sum or according to visual inspection of the growth stage of the cover crop. Decision support tools are available to help determine timing (see Chapter 60 in this volume). The time for plant destruction is reached in the spring about 4–5 weeks after the plants have emerged and in the summer after 3–4 weeks. In terms of plant phenology, this refers on the BBCH scale to values between 3 (main shoot has reached 50% of its expected height) and 4 (lateral buds begin to develop). Plant destruction can be done mechanically or chemically.

Biofumigation

This process describes the agronomic practice of growing plants rich in organic compounds exhibiting nematicidal mechanisms that are finely chopped and incorporated into the soil. The organic compounds are released into the soil where they control certain soil-borne pathogens, plant parasitic nematodes and weeds (Matthiessen and Kirkegaard, 2006). Plants in the Brassicaceae family with high levels of glucosinolates are especially adaptable for use, such as white mustard, Indian mustard or fodder radish. Following cell disruption, the glucosinolates are enzymatically transformed into volatile isothiocyanates that have nematicidal potential. Re-compacting or covering the soil after burial of the crop helps to reduce volatilization of the compounds and thus improve control efficacy. However, it is still unknown if the observed effect is due to the isothiocyanates, the decomposing plant material or a combination of both (Vervoort *et al.*, 2014;

Sikora and Roberts, 2018). Other crops that are of interest for biofumigation are plants from the grass family, such as forage sorghum, which releases dhurrin that degrades to a hydrocyanic acid, a volatile toxic to nematodes.

Resistant cover crops

Individual plants within a given fodder radish cultivar were shown to vary from highly susceptible to highly resistant towards *M. hapla*. This observation allowed the selection and breeding of cultivars towards increased resistance. Continuous selection of the resistant plants finally led to fodder radish cultivars with a high degree of *M. hapla* resistance, such as Angus, Amigo or Defender. Other crops are probably similarly suited for such resistance breeding.

Optimization of nematode management

The aforementioned tools for nematode management all have enormous potential for improvement, either by optimizing agronomic practices and resistance breeding or by incorporating new non-host plants into the rotation. Modern techniques like satellite remote sensing will help determine plant stress by nematodes before visual symptoms occur and allow determination of the optimum harvest date of the crop as well as the best time for incorporating trap crops. At a regional level, additional tools might be available, such as solarization, an approach used in the light-intensive warmer regions of the Mediterranean.

Future research requirements

Future research strategies should be directed to improve both soil health and plant health. Regarding soil health, production systems have to become more sustainable. They need to ensure good yields even in the presence of the nematode. Sustainability may be improved by reduced tillage and different kind of measures that increase organic matter content of the soil, like green manure crops, compost treatments or living mulch applications. Under ideal conditions, the soil will reach the status of nematode suppressiveness. To get there, we need to better understand the mechanisms causing sustainability and/or suppressiveness and how to manage them by agronomic practices. Besides, farmers need quick and reliable tools that describe the status of sustainability/suppressiveness to be aware of the success of the measures taken.

The main challenge on the plant side is to develop carrots that are resistant to the nematode and at the same time tolerant to the initial damage caused by nematode penetration before resistance mechanisms are activated. Current cultivars of the domesticated carrot *Daucus carota* subsp. *sativus* are all susceptible to *M. hapla*. However, reduced susceptibility to *M. hapla* has been demonstrated, among others, for the subspecies *D. carota* subsp. *azoricus*, *D. carota* subsp. *halophilus* and *D. carota* subsp. *hispanicus*, and the wild carrot species *D. commutatus* (Frese, 1983; Nothnagel *et al*., 2019). Backcrossings of such material with the domesticated carrot resulted in individual plants free of any galls or egg masses. Those promising results are pursued with the aim to develop resistant cultivars.

Outlook: anticipating future developments

The main challenges for the future are seen in worldwide population growth, global warming and soil degradation. Since the global agricultural production area is limited or even declining, either productivity per hectare has to be increased or soil-independent food production systems need to be developed to meet the demand. In the first case, higher productivity per hectare will most likely increase plant stress and plants might become more vulnerable to nematode damage. Global warming resulting in higher annual temperatures will shorten the life cycle of *M. hapla* and most likely result in more generations per year. However, if temperature increases above the optimum for *M. hapla*, the living conditions for the nematode worsen and the damage decreases. Global warming resulting in drier summer conditions will enhance yield losses since *M. hapla* is competing with the plant for water. Finally, increasing soil degradation will limit the natural defensive capacity of soil by decreasing antagonistic microorganisms. All those scenarios clearly show the importance of improving sustainability and plant health.

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39 Mitigating a galling problem in California's carrot production

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Introduction

Based on a survey of shoppers in 2019, carrots (*Daucus carota* L. subsp. *sativus*) were the fourth most popular vegetable in the US. California growers have not only been the leading producers but industry innovators that created and expanded the current US fresh carrot market. In the 1950s, they introduced washed carrots in cellophane bags. Those 'cello' carrots, with broad shoulders and tapered tips, were about 15 cm long. A substantial portion of the harvest was deemed non-marketable because consumers rejected forked, distorted, and galled roots. These disfigurations are mainly caused by root-knot nematodes (RKN; *Meloidogyne* spp.). *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* are the primary disease challenges in California's carrot production (Nuñez *et al.*, 2016). All species are considered C-rated pests, indicating these nematodes are not subject to state-enforced action outside of nurseries except to reduce dissemination. *Meloidogyne incognita* is economically the most important species and is widespread in the lighter soil types throughout central and southern California.

In the mid-1980s, grower Mike Yurosek invented what became known as 'cut and peel' or baby carrots. He often lost a large part of his harvest, sometimes up to 70% of a truckload, because of disfigured carrots, which had to be culled or used for animal feed. To find a better use for these carrots, he started experimenting by chopping taproots, first by hand then with a green bean cutting machine, into short segments. A potato peeler smoothed them into perfect looking baby carrots (Stolarczyk, 2020). Within a decade, per capita consumption of US carrots doubled. Today, baby carrots account for about 80% sales of fresh carrots consumed in the US. Selective plant breeding has resulted in carrots with extended taproot length as well as reduced bitterness and woodiness. Imperator hybrids are the preferred cultivars with almost uniform cylindrical roots of approximately 1.6 cm diameter, 22–28 cm in length and a small core. They are smooth skinned with a deep orange colour. By planting them at higher density than cellos, the roots stay thin, which reduces waste when the carrots are cut into bite-sized pieces. Grimmway Farms, Wm. Bolthouse Farms Inc., and Kern Ridge Growers produce almost all California's carrots. Their headquarters and processing facilities are located in or near Bakersfield, Kern County, located at the south end of the San Joachim Valley. In the first two companies' highly mechanized processing facilities, the carrots are thoroughly washed, rinsed in chlorinated

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water, and chilled to about 3˚C. They are then sorted into several thickness classes, chopped into 5-cm segments, peeled, smoothed, packed, and stored cold until shipped. Misshapen or broken carrots are sliced into sticks, chips, shredded for salads or juiced into beverages. Grimmway Farms, the world's largest producer, asserts that 99.2% of the harvested root is utilized for human consumption. The remainder is used as animal feed or is composted.

Economic importance

California's carrot production accounts for about 88% of the 32,200 harvested US hectares, and almost all are grown for the fresh market. In 2019, carrots generated US$827 million in cash receipts (NASS USDA, 2019). More than two decades earlier, production loss due to plant parasitic nematodes was conservatively estimated at 5–8% despite the use of various soil fumigants (Koenning *et al.*, 1999). Current RKN disease pressure is estimated to result in more than US$50 million in lost income annually.

In addition to carrot-related products, the three leading producers have diversified into several other vegetables and related goods. With more than 9000 employees at peak season, the carrot industry is an important economic factor in the county.

The mentioned companies produce both conventional and organic carrots. In the 2019 US organic vegetable market, carrots were leading in retail volume. Among vegetables, carrots had by far the lowest price premium; on average, organic carrots were only 33% more expensive than conventionally produced ones. Total organic acreage was 6573 ha, half of it located in Kern County, about a quarter in Imperial County, most of the rest along the central coast (CDFA, 2019).

Host range

The southern root-knot nematode *Meloidogyne incognita* and the Javanese root-knot nematode *M. javanica* parasitize and reproduce on numerous crops and weeds with an estimated host range of 2000 plant species. The broad host range of RKN makes crop rotation especially challenging, with few economically viable choices. Some resistant cultivars of otherwise susceptible species or relatively poor hosts can serve as rotation crops, particularly in organic production where one carrot crop is often followed by three alternative crops. In the case of *M. incognita*, certain crops or cultivars are useful to reduce the nematode population, e.g. Mi resistant tomatoes (*Solanum lycopersicum*), resistant cowpea cultivars (*Vigna unguiculata*), and specific cole crops cultivars (*Brassica oleracea*). Cover crops may also be used, such as sorghum-sudangrass (*Sorghum bicolor* × *S. sudanense*), sunn hemp (*Crotalaria juncea*), and small grains rotations. Cultivar selection and proper planting timing can be essential.

Distribution

The geographical diversity and agronomical intensity of carrot production allow for year-round production, essential to keep the processing facilities in operation all year. Carrots thrive in the state's deep sandy loam soils and at mostly perfect climatic conditions (daytime 24°C to 30°C, nights 10°C to 16°C). California's carrots are grown primarily in four regions (Nuñez *et al.*, 2008):

- Southern San Joaquin Valley, Tehachapi, Antilope and Cuyama Valley (Kern, Santa Barbara Counties): carrots are sown from December to March for harvest from May to July and from July to September for harvest from November to February.
- Southern inland deserts (Imperial and Riverside Counties): carrots are planted from August to February for harvest from December to June.
- High desert region (Los Angeles County): carrots are sown from April to July for harvest from August to December.
- Central California coast (Monterey County): carrots are planted from December to August for harvest from April to January.

Most of California's carrot fields are infested with RKN. This is reflected in the decades-long need for the use of soil fumigants. In 2017, about 10,360 ha or 43.7% of the total planted carrot area was treated with the fumigants 1,3-dichloropropene (1,3-D), metam-sodium or metam-potassium (DPR, 2020).

Symptoms of damage

Root-knot nematodes are named for the characteristic root-knots (galls) they induce on many hosts. In carrot production, non-specific above-ground symptoms include stand reductions, stunted growth and a predisposition to wilting. Typical root symptoms develop when the infectious second stage juveniles (J2) interfere with healthy development by penetrating the root. They induce galls both on the main taproot, which results in a bumpy (uneven) surface, and on the smaller feeder roots (Figs 39.1 and 39.2). The root-knots are diagnostic symptoms. Forking, stubbing and twisting of the primary root may also be caused by other biotic or abiotic agents affecting the root tip, such as compaction, rocks or *Pythium* root dieback. If many J2 infect a root close to each other, larger galls and more severe forking may develop. Disfigured roots pick up excess soil that increases the weight of the harvest transported to the processing factory. The additional cleaning effort increases processing expenses.

Biology and life cycle

Second stage juveniles of *M. incognita* hatch from eggs and migrate toward growing root tips in response to signals from root exudates. They penetrate the roots behind the tips and move intercellularly to a site near the differentiating vascular tissue. There they initiate permanent feeding sites, the so-called giant cells. Cortex cells surrounding the giant cells multiply and increase in size to create a gall. The now sedentary juveniles undergo three moults to develop into adults. During this development, the juveniles progressively change their appearance from vermiform to globose. The adult female, reproducing parthenogenically, lays several hundred eggs in a gelatinous matrix on the root surface or embedded in the galled plant tissue. The eggs

Fig. 39.1. Young carrot seedlings stunted due to galling caused by root-knot nematodes (*Meloidogyne incognita*). Author's own photograph.

Fig. 39.2. Non-marketable carrots at harvest with severe symptoms caused by *Meloidogyne incognita*. Author's own photograph.

yield the next generation of J2 to re-infest the same crop if soil temperatures are conducive and the host is still present. At harvest, the galled feeder roots remain behind in the soil, and their attached eggs serve as inoculum for the next crop. The duration of the life cycle is primarily a function of the host status and ambient soil temperature. With *M. incognita* parasitizing tomato, estimates for the base temperature and the required heat sum were 10.1°C and 400°C day, respectively (Ploeg and Maris, 1999).

Interactions with other nematodes and pathogens

Seedling diseases of carrots caused by *Pythium* spp. and *Rhizoctonia solani* include damping-off, root dieback, and forking. Whether the severity of disease is intensified by RKN, as it is in many other crops, has not been reported in California. Suppression of those diseases is considered a side benefit of metam soil fumigation. Under disease-conducive conditions, antimicrobial seed treatments or in-furrow sprays against oomycetes are applied.

Recommended integrated nematode management (INM)

In general, the damage caused by *M. incognita* depends on its population density and activity at seeding, its reproductive potential, the host tolerance, and the accumulation of degree-days. Pre-season soil sampling for nematode detection and enumeration is recommended, with a damage threshold for *M. incognita* of <1 per 250 cm^3 soil. The University of California Carrot Pest Management Guidelines suggest if the environmental conditions are conducive to parasitic nematode activity (sandy loam, soil temperature >18˚C), treatment is warranted whenever RKN are detected (Nuñez *et al.*, 2016). Infection early in the season is the primary cause of losses due to forking and root galling. No damage is expected as long as the soil temperatures stay below the threshold for *M. incognita* to penetrate its host (Roberts *et al.*, 1981). For example, in the Cuyama Valley, soil temperature at 15 cm depth drops at the beginning of November to less than 18˚C. In spring, the soil temperature passes the nematode's activity threshold by mid-April.

The extreme sensitivity to early season root damage by root-knot nematodes, the lack of available resistant cultivars or registered contact nematicides, and no effective biological control agents have led the industry to rely primarily on soil fumigants. Although this practice has been useful for decades, it is increasingly challenged by regulatory, environmental and economic issues. In 2017, 1,3-D was applied at 121 kg/ha to about 14% of the total carrot acreage. Both metam-sodium (209 kg/ha) and metam-potassium (310 kg/ha) were used in 12% and 18% of the carrot fields, respectively (DPR, 2020). Although their efficacy is typically less for nematode control than 1,3-D, they provide for additional microbial disease and pest mitigation, particularly against *Pythium* spp. and weeds. However, as soil fumigants are potential environmental and human health hazards, their use will be increasingly limited by regulatory restrictions. Soil fumigants contain volatile organic compounds (VOC) that combine with nitrogen oxides to produce health-hazardous ozone. Carrots are one of the crops with the highest agricultural contribution to VOC in the San Joaquin and Imperial Valleys. Concerns about air quality drive township limits on 1,3-D use and other regulatory requirements such as fumigant management plans, large buffer zones, increased monitoring times, personal protection equipment, limited applicator working time, etc.

Optimization of nematode management

The future for an optimized nematode management system in carrots is to integrate stable resistance against the main RKN species in commercial cultivars with other desirable properties that the consumer has come to expect. During decades-long breeding and genetics projects, mainly funded by the California Fresh Carrot Advisory Board, UC Riverside Nematologist Phillip Roberts and USDA-ARS/UW Madison Geneticist Phillip Simon identified excellent *M. incognita* and *M. javanica* resistance in carrot germplasm, as well as gene markers essential for advanced selection (Parsons *et al.*, 2015). Bred into fresh market carrot breeder lines, they have been available to carrot seed companies for several years. These seed companies have crossed the resistance into their premier carrot cultivars and are tantalizingly close to market release. However, experience from other crops has taught us not to rely on genetic resistance to nematodes as a sole means of control. The hope is that the need for soil fumigation may decrease as the use of RKN resistant carrot cultivars combined with cultural practices and several new, more environmentally benign non-fumigant nematicides increases.

Several new chemistries with nematicidal properties are already US Environment Protection Agency registered or in late-stage development for crop protection against plant parasitic nematodes in conventional production systems. Each product is expected to be registered with only 'Caution' as the label's signal word. A multi-year study conducted in southern California carrot fields with heavily *M. incognita* infested sandy loam, fluazaindolizine and fluensulfone showed excellent protective activity. It considerably increased the carrot crop's marketable yield compared to the untreated control (Becker *et al.*, 2019). Also, seed coating with a combination of plant disease protection products against *Pythium* spp., soil-borne fungi, and RKN can significantly mitigate early seedling damage during the most susceptible period (Becker *et al.*, unpublished). Microbial inoculants (e.g. *Pasteuria penetrans*, *Pochonia chlamydosporia*), still in product development, may further extend the protection period.

Several additional nematode management strategies have been evaluated. Still, they are usually not effective enough as stand-alone practices against RKN. Soil solarization in southern California's inland deserts can temporarily reduce many soil-borne pathogens and pests, including plant parasitic nematodes and weeds. It requires at least a 4-week treatment during the year's hottest season and moist soil maintenance under a tarp. However, the technique's efficacy is brief and limited to about 30 cm of soil depth. Biofumigation with mustard seed meal has shown similar effectiveness as solarization in reducing RKN populations. Volatile compounds with biocidal properties are released in the soil during decomposition of the seed meal. However, the food oil or biofuel industry's former waste product has become in short supply due to its popularity in organic agriculture. Biosolarization is a combination of solarization and biofumigation. The solarization efficacy is increased by providing easily degradable carbon sources to the thermophile microbial community under a clear or black plastic tarp. Similarly, anaerobic soil disinfestation requires large amounts of crop residues incorporated into the soil (e.g. 10–20 tonnes of rice bran (dry weight)/ha), saturated with water and covered for several weeks with tarpaulin. Facultative anaerobes utilize the carbon sources and deplete the soil of oxygen for a short period. The availability of the substrates is challenging unless they are produced locally. The success of these techniques might be enhanced in combination with resistant cultivars, particularly for organic production.

Future research requirements

The introduction of the next generation of carrots with resistance to *M. incognita* and *M. javanica* will require careful evaluation against other soil-borne diseases and pests. The latest non-fumigant nematicides with new chemistries and mode-of-actions will need to be investigated in combination with RKN resistant cultivars and as stand-alone treatments under a broad spectrum of environmental conditions.

Outlook: anticipating future developments

Rural California is becoming increasingly urbanized. More people are moving to where crops are grown, often opposing the use of soil fumigants. The anticipated transition to RKN resistant cultivars and the availability of effective non-fumigant, soil-applied and seed-delivered nematicides will reduce and eventually replace soil fumigants for nematode management.

The long-term sustainability of a sufficient water supply will continue to be critically important, particularly with increasing temperatures associated with climate change. In the past, groundwater was utilized as a virtually free resource; approximately 50% of carrots are watered with groundwater. The water table in some parts of the San Joachim Valley has dropped as much as 2 m/year due to aquifers' over-drafting, sometimes associated with land subsidence. California's 2014 Sustainable Groundwater Management

Act requires local agencies to address how they will sustainably reduce their reliance on groundwater by the year 2040. Ending the overdraft in the valley might require fallowing tens of thousands of hectares. However, the hope is that the crisis will stimulate creativity in addressing the water shortage. While growers cannot rely on additional surface water availability or increased precipitation, the use needs to be reduced by regulations and economic incentives. Obvious approaches target the phase-out of small value water-thirsty crops, investing in low-volume and smart monitoring irrigation technologies. The 'cut and peel' sector could increase their production efficacy by developing longer carrots that yield an additional 'baby' per root.

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40 Integrated nematode management of *Pratylenchus penetrans* in onion: A versatile approach to control a versatile nematode

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Introduction

Pratylenchus penetrans is a common species on sandy and peaty soils in the Netherlands and is also found to a lesser extent in more clayey soil. On sandy and peaty soils, population densities above damage threshold levels are causing yield depressions of important arable crops, including onions. Originally, most onions in the Netherlands were grown on clay soils. However, because new cultivars have become available suitable for growing on sandy soils, the acreage cultivated on sand and peat is increasing rapidly. With this expansion the nematode problem has become more evident.

Economic importance

About 27,500 ha of onions, representing an average economic value of €150 million are grown in the Netherlands, of which up to 15% is grown on *P. penetrans* preferred sandy, peaty and light marine clay soils. Over the past 10 years the total acreage of onions has increased by an average of 2.5% per year, mainly in regions with sandy or peaty soils. Accurate data are not available, but a conservative estimate is that the financial loss exceeds €1.5 million per year.

Host range

P. penetrans has a very broad host range that includes major cash crops, cover crops and many weed species (Belair, 2007). Potato, maize, wheat, barley, rye, onion, carrot, beans, broccoli and ryegrasses are known as good host plants. Sugar beet is one of the few economic crops that is a poor host for *P. penetrans*. Ornamental plants like tulip, lily, daffodil and rose are also known to be good hosts for *P. penetrans*. Important cover crops like fodder radish, yellow mustard, clovers and vetch are also hosts for *P. penetrans*. *Tagetes patula* (marigold) is known to be a very effective catch/trap crop (Evenhuis *et al.*, 2004). Detailed information can be found in the databases of the EU Best4Soil project (www.Best4Soil.eu/database, accessed 2 February 2021).

Distribution

In the Netherlands, *P. penetrans* is found in all regions but almost all reports of damage come from sandy soils in the south-east part of the country in the province of Noord Holland (light marine clay) and the sandy and peaty soils in the

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north-east part of the Netherlands. A survey in 2005 and 2006 assessed the distribution of *Pratylenchus* spp. in the Netherlands. *Pratylenchus penetrans* was present in all regions; however, it was found in less than 8% of the samples taken from more clayey soil compared to approximately 40% of the samples from sandy and peaty soil. Numbers of *P. penetrans* in more clayey soils were always very low (<5 juveniles/100 ml soil), levels at which no damage to important crops is expected, whereas high numbers of *P. penetrans* were found in sandy and peaty soils.

Symptoms of damage

Pratylenchus penetrans causes reductions in yield of important crops like potato, onion, chicory, peas and strawberry. The yield and quality of bulbs (e.g. lily, daffodils and tulip) and ornamentals (e.g. roses) can be limited by *P. penetrans*.

Infestations of *P. penetrans* can lead to severe quality losses in carrot and black salsify due to forking or stunting of the taproot. Onion is also rather intolerant to *P. penetrans* and infested roots show typical elongated (brow) necrotic spots (Fig. 40.1). Depending on population density, root growth can be retarded strongly and patches of stunted onion plants are the above-ground visible symptoms. Even at low pre-sowing densities, between 25-100 *P. penetrans* per 100 ml of soil, yield is reduced significantly. Pang *et al.* (2009) reported yield reductions of 30% and 73% at pre-sowing densities of 200 and 1600 juveniles per 100 ml of soil, respectively. In field experiments in the Netherlands on a peaty soil the marketable yield of onions was reduced by 15% and 25% at infestation levels of 250 and 500 *P. penetrans* per 100 ml soil, respectively. Complete crop failure was observed at pre-sowing densities of 1000 nematodes per 100 ml of soil or more; onion seedlings died off within a couple of weeks after emergence due to a totally degraded root system.

Fig. 40.1. Necrotic lesions on roots of daffodils caused by infestation of *Pratylenchus penetrans*. Symptoms are similar to those found on *P. penetrans* infested roots of onion. Photograph courtesy of Wageningen University & Research, Field Crops.

The damage caused by *P. penetrans* is enhanced when the crop also suffers from other stress such as a deficit of water and/or nutrients.

Biology and life cycle

Pratylenchus penetrans is a migratory endoparasite. Reproduction is sexual and the nematode completes its life cycle in 30 to 86 days, depending on temperature. The life cycle is shortest at 30°C, but fewer eggs are produced compared to 20°C–24°C. Feeding and migration by *P. penetrans* degrades root cells and root surface symptoms appear as necrotic spots or lesions. Longer periods of feeding may result in cell death (Zunke, 1990), causing a reduction of fine roots and leading to above-ground symptoms of water and nutrient deficiency, such as chlorotic foliage and reduced growth.

P. penetrans is capable of penetrating tubers of host plants such as potato, turning seed potatoes into a potential source of dispersal. The nematode is also spread due to root infection of propagation material such as strawberry, fruit trees, lilies and roses. Winter population decline in the absence of a host plant can vary from less than 10% to about 50%. There are indications that there are strains/pathotypes of *P. penetrans* (France and Brodie, 1996).

Interactions with other nematodes and pathogens

Alternaria porri, Fusarium oxysporum f.sp. *cepae* and *Sclerotium cepivorum* are common soil-borne fungi pathogenic to onion in the Netherlands. Interactions with *P. penetrans* are known for other subspecies of these fungi in other crops but we are not aware of proven interactions in onion. A very harmful and well-known interaction of *P. penetrans* is that with the fungus *Verticillium dahliae* in strawberries and in potato causing potato early dying (see Chapter 50 in this volume).

Recommended integrated nematode management (INM)

There are a number of INM tools that can be used to manage *P. penetrans* in onion. The most important management tool at present is crop rotation with non or poor-host cover crops. Additional measures include black fallow, anaerobic soil disinfestation, flooding/inundation and the use of nematicides, depending on the level of infestation.

Prevention of spread

INM starts with prevention and quarantine to prevent spread to uninfested fields. The use of certified, nematode-free planting material is therefore of the utmost importance to prevent introduction. *Pratylenchus penetrans* can be spread by infested seed potatoes as well as in infested strawberry, fruit trees, lilies and rose transplants. Farm hygiene, including weed control and cleaning machinery, is also important to avoid spread.

Proper analysis of the problem

Also important is knowledge of which nematodes (in addition to *P. penetrans*) are present in a field and at what densities, so appropriate control measures can be taken. Crop inspection and soil sampling can reveal the nematode species and population densities in a field. Accurate estimation of population densities of *P. penetrans* requires extraction of nematodes present both in the mineral and organic fraction containing root fragments in the soil samples. An incubation time of 2 to 4 weeks is needed to extract most of the *P. penetrans* from the organic fraction.

Crop rotation

P. penetrans has a very wide host range, including many cash and cover crops. Reducing populations of *P. penetrans* by a properly chosen crop rotation is therefore complicated. Onions are commonly grown in rotations with barley or wheat (moderate to good hosts), potato (good host) and sugar beet. Sugar beet is a poor to moderate host and one of the few major cash crops that can be included in a crop rotation to control *P. penetrans.*

Marigold, as a catch-crop (discussed later), and resistant black oat (*Avena strigosa*) cultivars are also recommended as good cover crops. Most other major cover crops such as fodder radish, yellow mustard, clovers and rye grasses are very good hosts for *P. penetrans*. It should be noted that black oats is a host of *Fusarium oxysporum* f.sp. *cepae* (www.best4soil.eu/database, accessed 2 February 2021) and may increase fungal disease damage in a succeeding onion crop. Information can be found on the host status and sensitivity of cash crops and cover crops for design of rotation schemes for both plant parasitic nematodes and soil fungi on the 'best4soil' website. This INM tool is available in more than 20 European languages.

Nematicides

The fumigant Monam (a.i. metam sodium), the granular nematicide Vydate (a.i. oxamyl) and Nemguard, a biological-based granular nematicide containing active ingredients of garlic (*Allium sativum*), are registered in the Netherlands for control of *P. penetrans* in onion. Due to government-imposed restrictions and requirements for sealing the soil surface with virtually impermeable plastic or compressed soil films to avoid evaporation, the fumigant metam sodium is no longer an economic alternative treatment for onion growers. A pre-sowing broadcast application of oxamyl can improve yield by 10–25% depending on pre-sowing population density.

Inundation and anaerobic soil disinfestation (ASD)

Inundation and ASD are two different methods of disinfesting the soil by creating anaerobic soil conditions.

ASD is carried out by incorporation of organic material, irrigation and tarping the soil with airtight foil for at least 6 weeks at soil temperatures >16°C. Maximum potential of inundation is reached when the field is flooded for 10–14 weeks in summer. Both methods are very effective for the control of weeds, volunteer host plants, several soil-borne pathogens and plant parasitic nematodes (Lamers, 2014). ASD and inundation will reduce *P. penetrans* populations by more than 99%. These approaches are still too expensive when *P. penetrans* is the only problem, and they are mainly used to suppress species like *Meloidogyne chitwoodi* (see Chapter 48) or *Ditylenchus dipsaci* (see Chapter 41).

Catch-crop

Marigold (*Tagetes patula*) has been known for decades as a very effective catch-crop for *P. penetrans* (Oostenbrink, 1957). A successful grown *Tagetes* will reduce a *P. penetrans* population by more than 95%, a decrease which is much stronger and longer lasting when compared to the natural decline by fallow or when a non-host is grown (c. 75% decrease of the population). Different species of *Tagetes* are used to control *P. penetrans* of which *T. patula* is the most effective species (Fig. 40.2). Under climatic conditions in the Netherlands, marigold has to be grown in the summer for at least 3 months (Evenhuis *et al.,* 2004). As a consequence, the income of a cash crop is lost. The technology was formerly only used for high-value crops like strawberry and roses, but long-term crop rotation experiments have shown that marigold is also an economically profitable measure in arable farming. In a rotation of barley–potato–sugar beet–potato, on fields naturally infested with *P. penetrans*, replacing barley with marigold reduced population densities of *P. penetrans* by more than 99%, and the populations remained extremely low for at least 3 years (Fig. 40.3). This raised the interest of arable farmers, and an increasing number of onion growers are now using *Tagetes* to control *P. penetrans* effectively.

To achieve a maximum and long-lasting control of *P. penetrans,* weed hosts must be controlled.

Fig. 40.2. Field of marigold, *Tagetes patula.* Photograph courtesy of Wageningen University & Research, Field Crops.

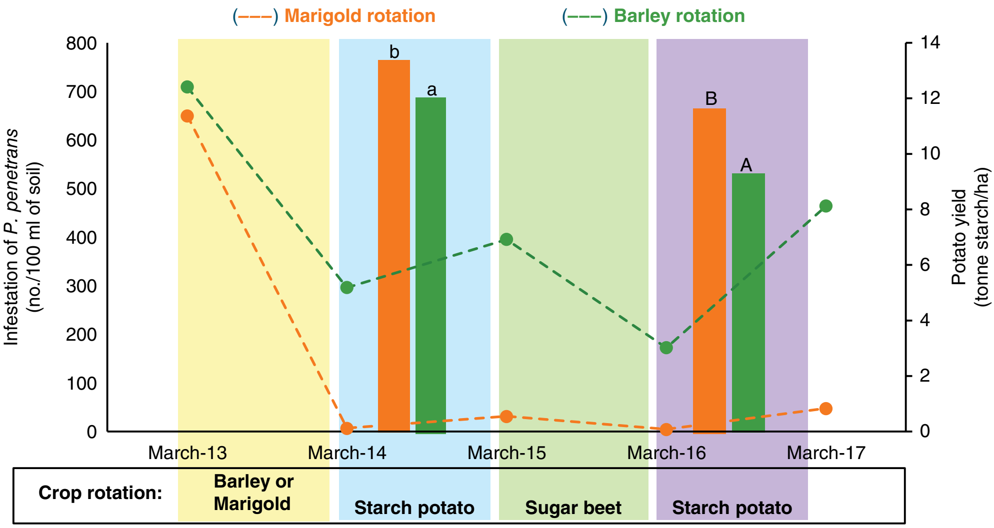

Fig. 40.3. Population dynamics of *Pratylenchus penetrans* in a crop rotation of barley–potato–sugar beet–potato and a rotation in which barley is replaced by marigold (*Tagetes patula*) and yield (bars) of starch potato in these rotations. Figure courtesy of Wageningen University & Research, Field Crops.

Soil compaction

Observations in commercial onion fields and in field experiments (unpublished data) showed that compacting the upper 15 cm of the soil prior to seeding can reduce damage significantly. In two field experiments on peaty soils naturally infested with *P. penetrans*, soil compaction, a granular nematicide (Vydate, a.i. oxamyl, 20 kg/ha) and a combined treatment of soil compaction and oxamyl were compared. Compressing the soil prior to sowing improved seedling emergence, crop growth and yield of onions significantly (Fig. 40.4). Soil compaction, oxamyl and the combined treatment improved yield by 7%, 10% and 13%, respectively, compared to untreated at a pre-sowing population density of 250 *P. penetrans* per 100 ml of soil.

Optimization of nematode management

A more integrated approach is necessary to sustainably improve the quality of arable soils used for onion production. A so-called boost year could be an important part of such an integrated strategy. This is a year in which a farmer does not grow a cash crop but takes the opportunity to implement multiple measures to control plant parasitic nematodes and soil-borne pathogens as well as improve other aspects of soil quality like soil structure or organic matter content. When marigold is grown as a main crop in summer it could be an important part of such a boost year. In spring and at the beginning of summer, before marigold is sown in July, measures like growing potato as a catch-crop for control of potato cyst nematodes, control of volunteers, mechanical weed management, applying organic amendments in the right circumstances, improving the drainage system, and so on, should be undertaken. In autumn, a second green manure crop could be grown. A prerequisite is that the costs of the boost year are recuperated in the further crop rotation. To make marigold use more attractive to growers, new herbicides are necessary. Most weeds are hosts for *P. penetrans* and therefore successful weed control in marigold is of utmost importance.

In order to expand control of *P. penetrans*, properly designed rotations with non-hosts or resistant cultivars of cash crops and cover crops are needed. Crops used in onion rotations, such as maize, need new cultivars that have resistance

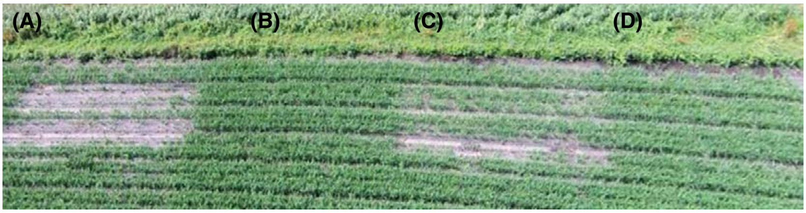

Fig. 40.4. Experimental field, showing the effect of **(A)** untreated, **(C)** soil compaction, **(D)** oxamyl and **(B)** the combined treatment of oxamyl and soil compaction on the emergence of onion seedlings. Photograph courtesy of Wageningen University & Research, Field Crops.

to *P. penetrans*. Even if the resistance is not directly beneficial for maize production it would aid control of *P. penetrans* on other crops in the rotation scheme.

Biofumigation

The use of green manures, which, after chopping and incorporation, release bionematocidal compounds, have not been shown to be effective in reducing densities of *P. penetrans*. Field research has shown that many crops (*Brassica* species) used for biofumigation are very good hosts for *P. penetrans*, causing an increase in the population, and that the amount of toxic compounds (ITCs) produced after incorporation of a biofumigation crop is far from sufficient to control plant parasitic nematodes (Vervoort *et al.*, 2014).

Future research requirements

The mode of action of soil compaction leading to reductions of *P. penetrans* on onion is still not researched in detail. Understanding this mechanism would help to improve the method and to make it applicable for onion and other cash crops.

Furthermore, the damage threshold of *P. penetrans* is much lower on dune sand than on sandy soils. There are indications that this is due to both physical and biological soil properties, in which the amount of organic matter and content/quality may play an important role. Understanding this mechanism can contribute to the development of environmentally friendly management measures and are topics for further investigation.

Mixtures of cover crops should be investigated because multiple aspects of soil quality can be improved and their use reduces the damage caused by nematodes. More information on the effect of these mixtures on population densities of plant parasitic nematodes and on initial damage thresholds is needed.

The bioagent *Bacillus firmus* is registered for control of *P. penetrans* in maize. It is worth exploring the potential of this and other biocontrol agents for management of *P. penetrans* in onion and other crops.

Outlook: anticipating future developments

Changing climate conditions will lead to warmer summers and milder winters, and therefore periods of drought and also locally more extreme precipitation. When summers get warmer, more generations of *P. penetrans* per year will be formed. Population densities will increase and the extent of damage caused by this nematode species on a succeeding crop will change. On the other hand, milder winters could also affect natural nematode decline during winter. Due to locally less precipitation during the growing season, water deficits could occur and increase the level of damage caused by *P. penetrans* because of restricted uptake of water and nutrients in infested and reduced root systems. A warmer climate may also lead to a shift in the incidence of other species like *P. thornei* that favour warmer soil temperatures.

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41 Integrated nematode management of *Ditylenchus dipsaci* in onion: A nematode in a world all on its own

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Introduction

Stem and bulb nematode, *Ditylenchus dipsaci*, is one of the few plant parasitic nematode species infesting above-ground plant parts. This is likely the reason why already in 1857 it was described from seed heads of teasel (*Dipsacus fullonum*), being one of the earlier records of plant parasitic nematodes. It can be transmitted through infested planting material and seed, survive longer periods in the soil, has a broad host range and a relatively short life cycle and high multiplication rate. Further, it is able to cause substantial post-harvest losses in stored onions, making it a difficult nematode to manage.

Economic importance

Until very recently, in the Netherlands stem nematodes were a quarantine pest in onion and lucerne seed, bulb onions and ornamental bulb crops. The quarantine status implied an obligation to try to contain and prevent further spread of stem nematodes. This meant that when stem nematodes were found in the harvested crop, special measures had to be taken to control the nematode in the field. Because of the quarantine status and the consequences that followed from an observed field infestation, farmers in general were hesitant to speak openly about the problem. This made it more difficult to get insight into and manage the problem with stem nematodes. At the end of 2019, the status was changed to regulated non-quarantine pest (RNQP) (EU, 2019). The aim with a RNQP is to prevent economic damage of specific crops. For the cultivation of onions, it is required that at the time of inspection no visual symptoms of stem nematodes should be found, or that infested plants immediately are removed and stem nematodes are not found in a representative sample, or adequate physical or chemical measures were taken and nematodes are not found in a representative sample of the plant material (EU, 2019).

A single bulb onion infested by stem nematodes can be a source of rot to the entire healthy bulb onion lot at storage (Fig. 41.1). Therefore, when stem nematodes are found in an onion lot it is strongly advised to immediately sell it for consumption.

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Host range

Stem nematodes have a very wide host range, including more than 500 plant species and consist of different races that vary in their host range (Botjes and Ritzema Bos, 1905; Sturhan and Brzeski, 1991). Some of the races have a very narrow host range, whereas others are polyphagous. The 'onion race' is known to be polyphagous and can infest onion as well as oats, potato, maize, sugar beet, *Phaseolus* and *Vicia* bean, pea and carrot. Different populations of the 'onion race' have been shown to vary somewhat in host range. Other stem nematode races like rye, daffodil and tulip races are also able to infest onion. Others like the red clover and hyacinth races are not known to infest onion. Besides known hosts of arable crops, many weeds are maintenance hosts to stem nematodes during winter and fallow periods.

Fig. 41.1. A sliced onion bulb infected by stem nematode: typical symptoms of spongy and slimy rottenness. Photograph courtesy of Wageningen University & Research, Field Crops.

Distribution

In the Netherlands, problems with stem nematodes in onions are known from the main onion growing regions in the north and south-west of the Netherlands, but also in the polder area that has been reclaimed from the sea. The south-west of the country originally was an area where *Phaseolus* beans and peas (*Pisum*) were grown to be dried for human consumption. As these crops are very good hosts to stem nematodes, problems were widespread.

Symptoms of damage

Onions that are infested with stem nematodes have twisted and deformed leaves that are brittle and have a bluish colour (Fig. 41.2). The bulbs are often spongy and cracked. In cases of severe infestation, plants die. Especially when the weather is cold and humid, the spread of the infestation is rapid and patches of affected and dead plants quickly increase in size. In such conditions, plant growth is slow and therefore plants are often retarded and unable to survive (Fig. 41.3).

Biology and life cycle

Stem nematodes are known to reproduce sexually. All juvenile and adult stages of the nematode can infect a host plant, although the fourth juvenile stage (J4) is the main infective stage. The life cycle is temperature dependent and takes approximately 20 days to complete at

Fig. 41.2. A plot of onions infected with stem nematodes: typical symptoms of winding and excessive tillering. Photograph courtesy of Wageningen University & Research, Field Crops.

Fig. 41.3. Hot spots of stem nematode infestation foci in onion fields: typical symptoms of retardation and sparse growth pattern. Photograph courtesy of Wageningen University & Research, Field Crops.

15°C. Every female may lay 200–500 eggs. As a result an infestation starting with lower initial population densities can quickly increase to large numbers. The J4 may enter a survival stage ('dauer larva') that is able to withstand dry conditions for a long time. Dauer larvae have been found to be able to become active and infective after more than 20 years. This is the reason why an infestation may be very persistent. It is unknown what conditions are favourable for longer time persistence in the soil.

Generally, all Dutch river and marine clays soils appear to be infested by stem nematodes (Seinhorst, 1956a). In the south-eastern part of the Netherlands, onion bloat caused by stem nematodes is only associated with and spreads easily on sandy and loam soils along the river. In the south-western part it was found that onion bloat is persistent on all heavy clay soils (>30% clay particles), but on light (<30% clay particles) and sandy soils only when onions are grown more frequently, i.e. more than once in 3 or 4 years.

Soil conditions influence the activity of stem nematodes, which are found to be more active (mobile) in clay and loamy soils than in sandy soils (Seinhorst, 1950). Activity of stem nematodes also depends on soil conditions such as soil moisture, temperature and aeration. Moisture equivalent is critical for nematode activity, with moisture equivalent being the percentage of water that a soil can retain in opposition to a centrifugal force 1000 × gravity. Lower soil temperatures (5–10°C) are more favourable for the activity of stem nematodes. A temperature of 20°C does not directly impact the nematodes themselves but activates soil factors that are unfavourable for stem nematodes (Seinhorst, 1950). These factors are suppressed by partial sterilization, which indicates a biological origin. The unfavourable soil condition can also be transferred through addition of a soil extract. At 36°C the activity of stem nematodes stops. Some variation in the optimum temperature has been reported for different races.

Interactions with other nematodes and pathogens

Interactions with other nematodes and pathogens on onion are not known.

Recommended integrated nematode management (INM)

The INM approach that is practiced in the Netherlands is based on prevention, inventory, crop rotation, inundation (flooding creating anaerobic conditions) and, as the last option, the use of chemical nematicides. The majority of growers and advisers who have infestations follow some of the recommendations outlined below.

Prevention, hygiene and field inventory

It is very difficult to control stem nematodes when they are present in a field, so prevention is very important. It starts with the use of certified planting material and seed that is free of stem nematodes. Stem nematodes can be easily transported by seed, planting material, residues like straw and hay and contaminated soil. Thus, a high level of hygiene on the farm, for example cleaning machinery before moving from one field to the other, avoids the spread of nematodes among fields. In both sandy and clay soil, weeds are a source of infection and a maintenance host for stem nematodes and should be managed. Recognition of an infestation in some crops and weeds that do not show any symptoms is difficult. Regular field observations and removing plants with visible symptoms limits further spread of the nematodes. Considering the persistence of dauer larvae, the history of onion bloat incidence in a field may give information about the risk of reoccurrence of stem nematodes even after years of cropping non-hosts. Previous history of failure of onions due to stem nematodes also may help to locate and narrow the sampling unit at the time of sampling to determine the central population density of the infestation foci and to decide on future management based on a decision support system (DSS). Localizing infestation foci using geographic information systems can further be developed to manage the damage of stem nematodes in the future.

Crop rotation

A DSS for management of plant parasitic nematodes is available in the Netherlands. The web-based DSS 'Aaltjesschema' (in Dutch) and 'Best-4Soil' (now available in 22 languages, www.Best4Soil.eu/database, accessed 2 January 2021) help farmers and extension service in selecting the most ideal cropping frequency and order, including the use of green manure crops, to manage population densities below the damage threshold. The websites contain both information on the rate of multiplication of the nematodes on the crop and sensitivity of the crop to damage, as well as additional background information. However, development of stem nematode safe crop rotations is not yet possible due to the occurrence of races and insufficient information on host plant specificity. Knowledge about the host status for stem nematode races can help selecting crops that can safely grow on a certain infestation. Hosts and non-hosts differ in their influence on the degree of stem nematode infestation of the soil (Seinhorst 1957). Seasonal fluctuation of stem nematodes is also affected by soil type. Generally, population densities of stem nematodes decline in the winter in both clay and sandy soils. The rate of decline is much faster in light and sandy soils as compared to that of heavy clay soils (>30% clay particles) (Seinhorst, 1957). In a heavy clay soil in winter, a population declines to densities of <100 stem nematodes/500 g soil, whereas in sandy soils they may decline to <5 stem nematodes/500 g soil. The damage threshold for onions is 0–10 stem nematodes/500 g soil, thus crop rotation is not effective for heavy soils whereas in lighter soils onions can be grown once in 3 to 4 years.

Inundation and anaerobic soil disinfestation (ASD)

In the cultivation of ornamental bulbs and onions, inundation is now widely used as a method to control stem nematodes. The soil infected with stem nematodes must be inundated slowly and remain inundated for a period of 14 weeks, with a soil temperature exceeding 16°C. This means that in the temperate climate in the Netherlands, inundation can only be applied in summer, preferably the latest at the beginning of July. The method is most effective when the water does not leak, indicated by the amount of water that must be added over time. Control below the detection level is possible in lighter soils, but in heavy clay a small proportion of the stem nematodes may remain unaffected. On certain fields that are not suitable for application of inundation, either due to slope, a low groundwater table or lack of access to large amounts of water, ASD may be an alternative. An amount of 40 tonnes/ha of fresh, easily degradable organic material (e.g. grass) is incorporated into the soil to a depth of 40 cm. The soil is irrigated with 15–20 mm water, then covered with virtually impermeable film and left for at least 6 weeks at a soil temperature of at least 16°C. Inundation is more effective than ASD.

Additional measures

As a last option when other control measures fail, non-fumigant nematicides can be used, although the number of allowed nematicides is nowadays limited. The target with chemical nematicides is always to bring down population densities below the damage threshold (fumigants) and/or to postpone the moment of infection (non-fumigants).

Optimization of nematode management

The management of stem nematodes in onion can be optimized by the following:

- Starting with clean plant material requires a method of disinfection that controls the nematodes while leaving the plant material untouched. Ornamental bulbs are heated in a water bath, but the prevalent temperatures that are used seem to be ineffective. For bulb onions this procedure has not been adopted.
- A detailed understanding of the soil properties related to stem nematode distribution might help in the management of stem nematodes.
- The distribution of stem nematodes in the field is highly variable, it is difficult to determine a clear infestation focus and the pattern of spatial distribution is unknown. This information is needed to calculate the detection probability using a standard method of sampling. Developing a standard method of sampling might help to optimize management by early detection of infestations and applying site specific methods of control.

  One of the main objectives of sampling is to estimate initial population densities for advisory DSS in the management of soil dwelling plant parasitic nematodes (Seinhorst, 1988; Been and Schomaker, 2006). So far there is no specific sampling method for stem nematodes. To develop a sampling method, including the bulk sample size needed for estimating nematode density per unit area with a certain degree of precision, understanding the horizontal and vertical spatial distribution of stem nematodes is important. It is known that 80–100% are found in the upper 20 cm of the topsoil. The horizontal distribution of stem nematodes in onions both in clay and sandy soil is mostly roundish and irregular, as active spread by the nematodes is predominant.

  Using a standard method, the detection probability in clay soil for central population densities of 10 and 100 stem nematodes/500 g soil is 23% and 90%, respectively. In sandy soil where the activity of the stem nematodes is lower, the probability of detection is <2% for a central population of 15 stem nematodes/1000 g soil. Considering the low damage threshold and irregular distribution, field monitoring and records of the cropping history are necessary and may lower the sample size that is required to detect the infestation foci. However, even when no stem nematodes are found in the soil, damage might still be observed in the onions (Seinhorst, 1956b). This illustrates the need for the development of reliable methods of sampling and extraction that increase the probability of detecting low levels of infestation.

- DSS for management of plant parasitic nematodes like Aaltjesschema and NemaDecide depend on studies of population dynamics and damage threshold parameters in both sandy and clay soils. The available studies on host status are limited and mostly unsuitable to be utilized for DSS. New studies are required with a full range of nematode densities under controlled conditions to estimate the host status of onion cultivars and other crops that are attacked by the onion race of *D. dipsaci*. This will help to optimize the management of stem nematodes in planned rotations, at least in sandy soils. Results can be applied to field conditions based on estimating initial population densities using an established accurate sampling method. This might further be coupled with geographic information systems to provide scenarios of management options using several crops with known host status and related damage.
- Improvement of the detection method of stem nematodes in soil is needed. At present, stem nematodes are extracted from soil using Oostenbrink and Seinhorst elutriator followed by Baermann tray. When dauer larvae do not become active during the extraction period, they are missed. Further, the very low damage threshold calls for a high level of detection that needs improvement.
- Rapid molecular diagnostic tools to differentiate races of stem nematodes is fundamental to decide on available relevant control measures related to the specific race using a DSS.
- Finally, it is necessary to strengthen the relationship between plant protection service, extension services and farmers, which helps to get accurate insight into infestation of fields with stem nematodes and implement necessary control measures.

Future research requirements

A sampling method needs to be developed based on new sampling data and estimation of the coefficient of aggregation of *Ditylenchus dipsaci*. It is important to upgrade and calibrate techniques used in estimating densities and the activity of stem nematodes. This might help understanding the reasons for an increased activity of stem nematodes in partially sterilized soils, which may be related to the specific niche of stem nematodes in the soil. Further, the chemical nature of the soil extract from soil that inhibits stem nematode activity needs to be elucidated. This requires detailed soil analysis using gas chromatography to identify and quantify the chemicals involved. The information obtained could be the first step to understand why specific infested spots in a field remain static for many years without spreading within and between fields. Moreover, it also helps to understand soil conditions affecting the mortality of stem nematodes.

Breeding for resistance against stem nematodes has been successful in lucerne and in red and white clover, but also in oats and rye. As several stem nematode races can infest onion, breeding for resistance needs to be targeted at a range of possible infective races to be effective as a management tool.

Outlook: anticipating future developments:

In general, problems with stem nematodes are most severe when the spring is cool and moist. Climate change in the Netherlands is predicted to result in increasing temperatures, with less frequent rainfall and a higher incidence of heavy showers. It is difficult to predict how this will affect the incidence of problems with stem nematodes. In practice, it has been found that after a warm and dry spring with a low stem nematode incidence, problems still arose after the first rainfall in summer.

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42 Lowering quality damage in open-field vegetables caused by *Meloidogyne chitwoodi* and *M. fallax* in the Low Countries

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Introduction

The Low Countries (the Netherlands [NL] and Belgium [B]) provide some of the best agricultural soils in the world for open-field vegetable production rendering high yields per hectare. The processing industry for frozen and preserved vegetables has high economic value in both countries. In 2019, 18.2% of the world's frozen vegetables came from Belgium and 4% from the Netherlands (FAOSTAT, 2021). Since the 1990s, pre-harvest quality control of carrots (*Daucus carota*) and black salsify (*Scorzonera hispanica*) showed an increase in tap root damage with severe galling and rough surface rendering the infected vegetables unprocessable. This quality damage was caused by the polyphagous root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax*. *Meloidogyne chitwoodi* was first described on potatoes in the Pacific North-west of the USA in 1980, but re-examination of old specimens and illustrations in the Netherlands and the relatively high genetic distances between Belgian populations suggest a longer presence in the Low Countries. *Meloidogyne fallax* was detected for the first time in 1992 in a field near Baexem (NL) and described as a new species in 1996. Since 1998, *M. chitwoodi* and *M. fallax* have been listed as quarantine organisms in the EU (EC Directive 2000/29/EC).

Distribution

The global distribution of *M. chitwoodi* and *M. fallax* is limited (Fig. 42.1). Their presence in the Low Countries and information on their biology and life cycle are covered in Chapter 48.

Symptoms of damage

Both species can cause severe galling and rough surface rendering of infected vegetables, making them unprocessable (Fig. 42.2). Often these symptoms are caused by the second generation and they increase with longer field periods of the crop. The galls produced on lateral roots or on alternate host crops (onions, peas, spinach, leek, green beans and lettuce) that could be used in

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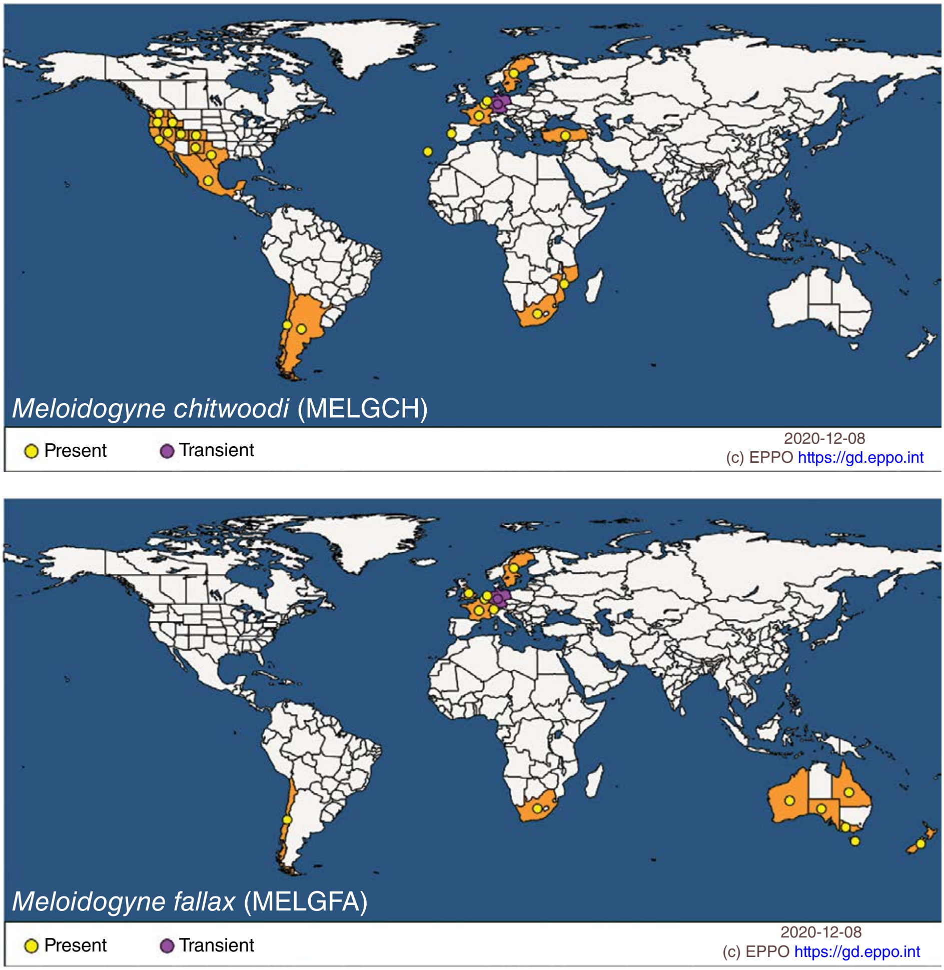

Fig. 42.1. Global distribution of *Meloidogyne chitwoodi* and *Meloidogyne fallax*. Figure courtesy of EPPO Global Database, https://gd.eppo.int (accessed 5 April 2021).

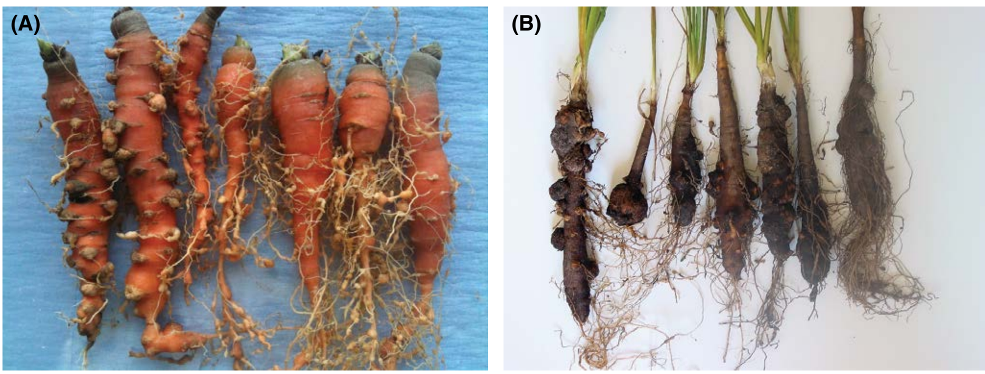

Fig. 42.2. Quality damage on **(A)** carrot and **(B)** black salsify caused by *Meloidogyne chitwoodi*. Photographs courtesy of W. Wesemael.

rotations with tuber vegetables are mostly small and can be overlooked.

Economic impact

A survey in 2015 revealed that 52% of the open-field vegetable farmers in Flanders (B) were confronted with losses due to plant parasitic nematodes. *Meloidogyne chitwoodi*, *Pratylenchus penetrans* and *Heterodera schachtii* were reported as the major problems. At the farmers' level, economic impact due to *M. chitwoodi* and *M. fallax* is reflected in (i) direct yield loss mostly in quality loss; (ii) future loss due to spreading of the nematode to other fields with machinery; (iii) indirect losses when non-susceptible or tolerant, less profitable crops are included in the rotation; and (iv) extra costs related to phytosanitary measures due to the quarantine status.

At low initial *M. chitwoodi* densities (Pi = 3 J2/100 cm^3 soil), 1.5% damage on carrots was reported under field conditions (Wesemael and Moens, 2008a) leading to a financial loss of €500/ha. If pre-harvest quality control of carrots and black salsify show >30% damage then the crops are no longer harvested and financial loss is €2500–5500/ha. When profitable vegetable crops need to be replaced by other crops due to *M. chitwoodi* or *M. fallax*, Belgian farmers often choose maize. Compared to vegetables, maize has about €1400/ha less financial value.

Spreading of *M. chitwoodi* and *M. fallax* puts pressure on the availability of high-quality soils for vegetable production in the vicinity of the processing industry. Allocation of the production to neighbouring countries (France, Germany, Poland) renders a 10% higher variable cost due to increased transport times and costs. Further migration of production and processing to other countries will also have an impact on local employment.

Recommended integrated nematode management

Prevention

With increasing occurrence of unprocessable tap roots, the industry organized preventative soil sampling in collaboration with farmer associations. A contract for sensitive vegetables such as carrots and black salsify will only be given for soils that are free of *M. chitwoodi* and *M. fallax*. About 33% of the sampled fields were rejected. Not all companies follow this strategy but it is highly recommended as it reduced the rejection of produce from 7% to less than 1.5%. Major constraints of preventative soil sampling are the costs for analysis (>€100 per sample) and the potential quarantine status of fields detected with *M. chitwoodi* or *M. fallax*. When *M. chitwoodi* or *M. fallax* are detected in a field in Belgium the infestation needs to be reported to the National Plant Protection Organization (NPPO). In the Netherlands, this reporting is only required when the nematodes are found in propagation material. The diagnostic lab responsible for the analysis also needs to report to its NPPO. In Belgium, the phytosanitary measures require that tubers and roots be cleaned (removal of soil) before they are allowed to leave an infested field. Also, machinery needs to be cleaned before they leave the field. These measures are cumbersome and expensive. After detection, the farmer needs to wait for 3 years before he can ask for an official re-sampling and analysis to declare the field free of *M. chitwoodi* or *M. fallax*. Belgian vegetable farmers mostly work with leaseholds and these can change annually. Landowners might ban farmers who report *M. chitwoodi* or *M. fallax* infestations and as a consequence often infestations are not reported to the NPPO. To overcome reporting by the analysing diagnostic lab, samples are sent to diagnostic services in the Netherlands or France. These labs report to their own NPPO and it takes time before the findings are shared with the Belgian NPPO. Often the exchange of information is limited to once per year and lacks details on exact location.

Harvesting is mostly done by specialized companies who move from field to field and often work 24 hours per day during the harvest period. Soil adhering to machinery is at most brushed away before leaving the field but mostly no actions are taken to avoid further spread. To minimize transportation costs, efforts are made to reduce the soil adhering to the harvested product. At the industrial processing plant this soil is collected and returned to agricultural fields. In theory the soil returns to the field where it comes from, but in reality this is difficult to control. Farmers are willing to take the soil to their fields as it is fertile soil and helps to overcome erosion from their fields. Waste soil is also transported to non-agricultural locations, mostly unknown to the processor. Soil present in washing water and root pieces or peelings are mostly treated before being discarded. In Flanders (B), a processing company is sanitizing the wastewater (anaerobic and aerobic) and this water is used by local farmers to irrigate their fields. Organic waste (roots, tubers, peelings) is used as animal feed or goes to a digester.

Planting material (leek, celeriac, cabbages) is not known to be a source of *M. chitwoodi* or *M. fallax* infestation. Seedlings of celeriac and cabbages are mostly grown in substrates free of nematodes but planting material for leek is often grown in seeding beds in the field. It is important that these seeding beds are checked for the presence of plant parasitic nematodes. Potatoes are often grown in rotation with vegetables and it is of paramount importance that certified seed potatoes are used to avoid introduction of *M. chitwoodi* and *M. fallax*.

Crop rotation

Due to the wide host range of *M. chitwoodi* and *M. fallax* and the high specialization of vegetable farmers, options for crop rotation are limited. At present, it is very difficult, if not impossible, to eradicate *M. chitwoodi* and *M. fallax* with crop rotation once it is present in the field. However, a well-planned rotation scheme can reduce the population to levels that allow susceptible cash crops to be included. Wageningen University and Research field crops (NL) developed a useful tool (www.aaltjesschema.nl/, accessed 12 November 2020) that allows farmers to check the host plant status and damage potential of a wide variety of crops and cover crops for different plant parasitic nematodes. This program is based on the vast amount of data collected over the years and available data from the literature and is updated regularly. The nematode decision tool NemaDecide predicts population development and possible damage depending on the chosen rotation and the initial population density. This allows planning for the longer term.

Chicory is a non-host for *M. chitwoodi* and *M. fallax*. Onions and peas are poor hosts and can be included in a rotation. However, pea might suffer from damage caused by the nematodes. Spinach (*Spinacia oleracea*) and lettuce are poor hosts for *M. chitwoodi* and *M. fallax* and due to the short field period, it can be harvested before a new generation of the nematodes is formed thereby acting as a trap crop. Asparagus is a non-host for *M. chitwoodi* but good host for *M. fallax*. Different cultivars of green beans (*Phaseolus vulgaris*) show reduced or delayed development of *M. chitwoodi* and were a non-host for *M. fallax* and can be used to lower the population (Wesemael and Moens, 2012). It is important to destroy the stubble that remains in the field immediately after harvest to avoid further development of the nematodes. In general, huge population build-ups and damage can be avoided when crops/cultivars with a short field period are chosen that are grown in early spring. Due to market demands this strategy is not always possible.

About 75% of vegetable farmers in Belgium use cover crops in their rotation, basically to improve the soil structure and to control the nitrogen balance. Most farmers are aware that cover crops can have an effect on plant parasitic nematodes but they indicate that they need better support for successful use. Only 14% of farmers use cover crops to manage nematode problems. Cover crops with resistance to *M. chitwoodi* have been developed. For example, resistant cultivars of fodder radish (*Raphanus sativus*) are available and have proved to be successful (Teklu *et al.*, 2014). They are mostly sown in August/September to allow proper crop development and coverage before the temperature drops. The use of cover crops is promoted in the framework of agroecological measures. Farmers get subsidies when using mixtures of cover crops for soil health improvement. It is important that these mixtures are composed of resistant cultivars and non-hosts for *M. chitwoodi*.

Several weeds are host plants for *M. chitwoodi* and *M. fallax* (Kutywayo and Been, 2006), therefore it is important to have proper weed control and volunteer potato removal within the rotation to manage both nematode species.

Optimization of nematode management

Proper management starts with knowledge about the distribution of *M. chitwoodi* and *M. fallax*. The current quarantine status and strict phytosanitary measures are counterproductive for their management. A regulated non-quarantine status would facilitate a better approach to the problem in the Low Countries. A soil passport that combines physicochemical data, cropping history and data on pests and diseases including *M. chitwoodi* and *M. fallax* pressure can be an important step towards proper control. These data can be a guide when both farmers and the processing industry have to make decisions on crop rotations. Moreover, a clear view on problem fields may help to prevent further spread of *M. chitwoodi* and *M. fallax*. Farm machinery or vehicles can spread nematodes through adhering infested soil particles; unfortunately, cleaning of machinery is generally not practiced. If infested fields are localized, field practices on these fields can be grouped and before the machinery is used on *Meloidogyne*-free fields they can be cleaned thoroughly. The same strategy should be used when crops are harvested. It will only be possible to schedule this field practice on infested fields if the distance between them is short. Further development of population growth models such as NemaDecide can reduce sample costs. Implementation of a soil passport also brings responsibility to the landowner who will have to collaborate with the land user to maintain high-quality soils.

As quality damage on tubers and tap roots is caused by the second generation of *M. chitwoodi* and *M. fallax*, the use of crops with a short field period leads to minimum losses. Rotations with crops that have a short field period that can be grown in the spring allow the implementation of a fallow period during the warmer months of the year before a resistant cover crop is sown. During this summer fallow, *M. chitwoodi* and *M. fallax* will require a host plant for survival and their energy reserves will drop, enhancing natural decline. As an alternative for the summer fallow, *Tagetes patula* can be grown as a cover crop. This is a non-host for *M. chitwoodi* and *M. fallax* and actively reduces the population of *Pratylenchus penetrans* which also causes substantial damage to a wide variety of vegetables.

Postponing the sowing date was shown to be successful for carrots and reduced quality damage caused by *M. fallax* (Molendijk and Brommer, 1998). Most likely this is due to a stronger winter decline and hence a lower initial population. For *M. chitwoodi* this might not be successful due to differences in survival strategy (Wesemael *et al.*, 2006) and peaks in the population when soil temperatures increase have been observed (Pinkerton *et al.*, 1991; Wesemael and Moens, 2008b).

The use of decision support tools and computer-controlled monitoring of degree days as well as moisture levels during crop growth will help cover-crop trap cropping by allowing the farmer to destroy the crop before a new generation is formed (see Chapter 60). Cover crops that are fast growing can be used as trap crops in between cash crops. Incorporation of the crop in the soil will also increase the organic matter content and improve the soil quality.

Recently, promising cultivars of potato and sugar beet with resistance against *M. chitwoodi* have been developed and will be available in the near future. These can be included in rotations with vegetables.

In the Netherlands, inundation has been successful to eradicate *M. chitwoodi* in infested marine clay soils. Fields in Belgium are less suitable for this technique. At present, the cost for inundation is also too high for vegetable farmers.

Future research requirements

Breeding for *M. chitwoodi* and/or *M. fallax* resistance in vegetables is limited due to the restricted market and high costs for development. Increasing knowledge on the nematode–crop interactions and related genes, combined with transcriptomic analysis and marker-assisted selection, might facilitate the development. Also, CRISPR-CAS will enable faster development of resistant crops but at present such crops are not accepted in the EU. Breeding of crops with shorter field periods can be another option as this will also reduce the need for irrigation which is more and more required in Belgium. Irrigation with sanitized wastewater will become more important. Economic feasible treatment of waste streams (soil, roots, washing water) needs to be developed to minimize further spread and allow cultivation of root and tuber crops in *M. chitwoodi* or *M. fallax* infested soil.

Further research on *M. chitwoodi*/*M. fallax*–plant combinations, population development and damage potential is needed to improve nematode decision tools that assist farmers in their rotation planning.

Outlook: anticipating future developments

In the future, more generations of *M. chitwoodi* and *M. fallax* will be formed during the growing season and multiplication most likely will continue during mild winters on cover crops. Root-knot nematodes from warmer climates will find their way into our fields and increase the problem. The current mind-set to eradicate *M. chitwoodi* and *M. fallax* seems obsolete. Rather than looking at one pest or pathogen, a holistic approach in which the agroecological system is considered is needed. Looking at shifts in nematode communities, facilitated with next-generation sequencing and meta-DNA barcoding, will allow us to adjust our agricultural practices to minimize losses caused by plant parasitic nematodes. Plant resistance and tolerance in cash crops will play a key role and can be complemented with ecological control measures.

In the future, breeding programmes will produce cover crops with resistance to *M. chitwoodi*, allowing more options for rotations and the additional benefits of cover crops and cover-crop mixtures. In the past, cover crops were destroyed when frost appeared in autumn. Cover crops that are not 100% resistant but delay the development of *M. chitwoodi* were destroyed before new eggs were formed and acted as a trap crop. Global warming reduces the number of nights and days with freezing temperatures and as cover crops are no longer destroyed by frost this might induce an extra generation of *M. chitwoodi*.

Climate change also comes with new opportunities. Crops that were restricted to warmer climates can now be selected for cultivation in temperate areas. Tests with soybean, sorghum, chickpea and sweet potato in Flanders show promising results and might bring new options for *M. chitwoodi* and *M. fallax* management. Knowledge on their host plant status and susceptibility for *M. chitwoodi* and *M. fallax* is needed.

New bioagents or chemical agents are being developed to upregulate or downregulate genes that are involved in the plant–nematode interactions. These products can be applied as foliar sprays and induce systemic acquired resistance. In combination with data on infestations and precision farming, application of these products can be fine-tuned to optimize results.

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43 Face to face: How *Paratylenchus bukowinensis* deals with vegetables

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Introduction

The pin nematode *Paratylenchus bukowinensis* Micoletzky, 1922 belongs to the group of ectoparasitic nematodes. Many, if not most, ectoparasites found in vegetable crops are ignored and are usually not considered to be of economic relevance. However, under favourable conditions, *P. bukowinensis* can cause severe damage on host plants of the families Apiaceae and Brassicaceae, such as celery, carrot and cabbage (Brzeski, 1991; Schmidt *et al*., 2020). Members of the genus *Paratylenchus* are one of the smallest plant parasitic nematodes affecting agricultural crops. Fully grown adult stages of *P. bukowinensis* hardly reach 500 μm in length. Since *P. bukowinensis* is often considered of little relevance, many laboratories do not score this species as a problem when analysing nematode suspensions for plant parasites of economic importance. A further aspect complicating its detection is the fact that males and fourth-stage juveniles, the latter being the most common stage found in soil samples during winter, do not exhibit typical tylenchid stylets. Instead, the stylet is reduced and hardly visible. This explains why those specimens are often missed during routine analysis. Overall we believe that *P. bukowinensis* is one of the most underestimated plant parasitic nematodes in practical agriculture. Thus, it is time to face those limitations and increase the awareness of the importance of *P. bukowinensis* in the nematology community as a severe pathogen.

Economic importance

As for other ectoparasitic nematodes, strong data on economic damage caused by *P. bukowinensis* are scarce. Damage by *P. bukowinensis* is especially pronounced on crops, where the root or tuber represents the harvested produce, such as for celery, carrot or parsley (Ghaderi, 2019). While high nematode numbers early in the season will affect plant establishment, high numbers later in the season will reduce plant growth, yield and, even more importantly, yield quality due to the induction of root necrosis, root or tuber deformation or excessive formation of lateral roots. For celery, the tolerance limit, i.e. when plant growth first starts to be affected, is about 70 nematodes/100 ml soil and the minimum yield at high infestation is about 60% of that of non-infested treatments (Brzeski, 1975).

Economic losses can vary greatly depending on nematode infestation level, host plant species and climatic conditions. Yield reductions in the range of 5–10% usually pass unnoticed by

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the farmer. With increasing infestation levels (above 70 nematodes/100 ml soil) yield losses increase, and under extreme conditions, complete loss of the crop can occur. As mentioned above, economic damage is most severe on tuber or root crops, whereas on leaf crops, such as cabbage or rapeseed, *P. bukowinensis* hardly causes any visible damage and only slightly reduces plant fresh weight (Brzeski, 1971). For example, on cabbage, even though the nematode reduces the size of the produce, the cabbage itself is still of good quality and can be marketed. Therefore, the economic loss is minimal to the grower.

Host range

The host range of *P. bukowinensis* covers species of Apiaceae and Brassicaceae. Good to moderate hosts are carrot, rapeseed, fodder radish, turnip, celery, fennel and parsley, depending on cultivar (Brzeski, 1976, 1991; Schmidt *et al*., 2020). We suspect parsnip is also a host but we found no data for this crop, which again is a problem overlooked. Vetch, belonging to the Fabaceae family, seems to be a poor host. All other plants are non-hosts, such as white clover, red clover, subterranean clover, crimson clover, Egyptian clover, Persian clover, common sainfoin, winter wheat, barley, maize, teff, black oat, naked oat, sugar beet, tomato, lettuce and onion (Schmidt *et al*., 2020; J. Hallmann, personal communication).

Distribution

Paratylenchus bukowinensis was first described based on a single female from a sandy meadow in Romania and named after the Bukowina region in the north of the country. Because of insufficient morphological information the nematode was re-described by Loof and Oostenbrink (1968). It is a temperate species and mainly distributed within Europe. It has been described from Bulgaria, Croatia, Czech Republic, Estonia, Germany, Lithuania, Poland, Romania, Russia and Slovakia (Ghaderi *et al*., 2016). It is also known to occur in the Netherlands and Belgium (L. Molendijk, personal communication). Outside Europe it has been reported from Uzbekistan, Belorussia, Georgia, Iran and the USA. *Paratylenchus bukowinensis* mainly occurs in arable and horticultural soils where it is supported by short rotations. It is rarely found in meadow and pasture soils (Ghaderi *et al.*, 2016). The favoured soil types range from light sandy to heavy clay soils (L. Molendijk, personal communication). A survey of plant parasitic nematodes in organic farming in Germany in the early 2000s detected *Paratylenchus* spp. in about 56% of 246 field sites, with *P. bukowinensis* being the second most abundant species after *P. projectus* (Hallmann *et al*., 2007). Although population data are missing, according to farmers and advisers, *P. bukowinensis* causes damage and is widespread in most of the intensively cropped horticultural regions in Germany and the Netherlands. It can only be assumed that this also applies to other regions in Europe with similar cropping conditions.

Symptoms of damage

Symptoms are best seen on susceptible root crops. For example, parsley and carrot roots and tubers are shorter, deformed or forked. Carrot roots show rusty brown root tips. Celery roots react to nematode infestation by producing lateral roots. Extensive root branching will result in a dense root mass, a so-called root beard. On carrot and celery, root necrosis (Fig. 43.1) is commonly observed and might eventually destroy the whole root system (Brzeski, 1976). An example of this is shown in Fig. 43.2, where the

Fig. 43.1. Root necrosis on carrot caused by *Paratylenchus bukowinensis*. Photograph courtesy of Wageningen University & Research, Field Crops.

right celery plant is heavily retarded in growth. On carrot, initial densities of 200 *P. bukowinensis*/100 ml soil already cause severe stunting of the shoots (Fig. 43.3) and root deformation below ground. Compared to below-ground symptoms, above-ground symptoms are non-specific and best characterized by stunting and delayed maturity. Early signs of nematode infestation are a heterogenous stand (Fig. 43.4) or the inability of a crop to react to fertilizer applications with improved plant growth due to a damaged root system.

Fig. 43.2. Root damage on celery caused by *Paratylenchus bukowinensis* (right) in comparison to a non-infested plant (left). Photograph courtesy of Wageningen University & Research, Field Crops.

Biology and life cycle

Brzeski (1976) noted that females, second- and third-stage juveniles (J2 and J3) of *P. bukowinensis*, the only stages that feed, are found feeding mainly on the epidermal cells and sometimes on cells two layers deeper in the root parenchyma of parsley and cabbage. The fourth-stage juveniles (J4), have a thin, short stylet and a reduced pharynx and do not feed. The root diffusates stimulate the moulting of J4 to adults, although some moulting occurs in the spring in the absence of root exudates. Population increases of 700% in one season on parsley are not uncommon (Brzeski *et al.*, 1976).

The length of the *P. bukowinensis* stylet allows the nematode to feed on cells up to few layers deep into the root parenchyma. Once feeding is finished, the nematode retracts the stylet from the root cell and moves to the next suitable feeding site. As a result of the feeding, the plant cell collapses. While the damage of single collapsed plant cells can be easily compensated by the plant, feeding of several hundred or even thousands by the often high soil populations results in destruction of large amounts of root tissue and thus economic damage.

Temperature

At soil temperatures around 20°C, one generation of *P. bukowinensis* is completed in 3–4

Fig. 43.3. Effect of 0, 200 or 2000 *Paratylenchus bukowinensis* per 100 ml soil on the shoot and root growth of carrot 11 weeks after planting. Photograph courtesy of Julius Kühn Institute.

Fig. 43.4. Celery field infested with *Paratylenchus bukowinensis* showing a heterogenous stand and poor growth of the plants. Photograph courtesy of Wageningen University & Research, Field Crops.

weeks (Brzeski, 1976; Schmidt *et al.*, 2020). This is calculated as a temperature sum of 370–400 degree days assuming 8°C as base temperature for nematode activity. Due to the short life cycle and high reproduction potential of females, *P. bukowinensis* can build up high population densities within a short cropping season. Final population densities in a well-established crop can exceed 20,000 specimen/100 ml soil at harvest time. Reproduction of *P. bukowinensis* studied under greenhouse conditions showed that inoculation of fodder radish and rapeseed with 745 nematodes/100 ml soil lead to a population increase to 18,007 and 38,367 nematodes/100 ml soil, respectively, after 9 weeks (J. Hallmann, personal communication). This translates to a reproduction factor of 180 for fodder radish and 380 for rapeseed, respectively.

Natural decline

According to Brzeski (1991), the natural decline of *P. bukowinensis* reached 75% in the first year and 91% in the second year following the growth of non-host crops such as onion and tomato. However, several individuals were still able to survive even 2 years in the absence of a host plant. This shows that most probably J4 stage juveniles are capable of withstanding cold and dry periods. Population dynamics in the absence of a host plant were also studied under controlled conditions in the greenhouse at room temperature of approximately 20°C (Hallmann, unpublished data). The population density of *P. bukowinensis* decreased from 305 individuals/100 ml soil to 182 individuals/100 ml soil after 14 weeks, which relates to an overall population decline of 40%. This was less than the 60% decline observed for *Meloidogyne hapla* tested in parallel. Overall, this illustrates that population decline rates are species specific and that in the case of *P. bukowinensis*, the presence of the 'survival stage' J4 might have contributed to a better survival of this species and a lower population decline when compared with *M. hapla*. In general, natural decline under field conditions is expected to be highly variable due to the many physical and biological parameters that affect nematode dynamics.

Interactions with other nematodes and pathogens

Unfortunately, little is known about the interaction of *P. bukowinensis* with other plant pathogens. It can only be assumed that wounding of the root by the nematode would foster infestation by secondary pathogens in a similar way as reported for other plant nematode interactions. For example, unpublished reports indicate that *P. bukowinensis* might make carrots more vulnerable for infestation by *Alternaria dauci*, *Rhizoctonia violaceae* and bacterial pathogens, but solid data are missing.

Recommended integrated nematode management (INM)

The problem of INM with this nematode is not the availability of methods of nematode control but the detection and identification of the nematode. Quite interestingly, the specificity of root symptoms on celery and carrot caused by *P. bukowinensis* make them good indicator plants. Thus, farmers can easily perform their own biotest for the presence of *P. bukowinensis* by collecting soil from the field, filling it in pots, buckets or plastic bags, and then growing celery or carrots followed by examination for root symptoms two months later. In many countries routine laboratories provide the service of sampling and diagnostics. Once identified, *P. bukowinensis* can easily be controlled by crop rotation. It is only important to avoid crops belonging to the families Apiaceae and Brassicaceae. Without a food source, *P. bukowinensis* densities will decline by about 40–50% within 3 months. The longer the period without a host plant, the better the decline. However, care needs be taken to also control weeds of those plants in susceptible plant families as they can build a green bridge from one crop to the next. Caution also has to be taken when Brassicaceae green manure crops are grown within a rotation. They often do not show any symptoms of damage, not even at high reproduction rates of *P. bukowinensis*. Fodder radish, for instance, is highly tolerant to this nematode (Schmidt *et al.*, 2020). As is to be expected, farmers look at a healthy fodder crop and assume its use led to a healthy soil, being unaware of the high *P. bukowinensis* densities that have built up. If a highly susceptible crop like those mentioned above is grown afterwards, complete crop failure could occur.

Future research requirements

We have to move away from just looking at one plant–one nematode interrelationship and focus more on the soil ecosystem. For example, in undisturbed ecosystems, such as grasslands and forest regions, well-buffered soils do not allow severe plant damage by plant parasitic nematodes. We need to elucidate the main mechanisms responsible for the buffer capacity of soil and how we can restore those mechanisms by agronomic practices. This would, of course, also require a change in how the crops are grown with a move away from monoculturing.

Remote sensing technology needs to be further developed for early detection of plant damage by *P. bukowinensis*. Future molecular-based diagnostic tools need development that allow faster and more accurate detection and population density estimation that can be coupled with decision support systems that instantly inform the farmer of a problem and give him information on management options.

Outlook: anticipating future developments

Firstly, it is expected that global warming will result in a shorter life cycle of *P. bukowinensis* and thus a higher reproduction rate per year. Secondly, the need to ensure food security on a global scale will enhance production intensity. Thirdly, economic pressure will force farmers to concentrate on fewer crops. The first two events will result in more root biomass per area and thus better food conditions for the nematode. The third event becomes relevant if the farm specialization is based on host plants for *P. bukowinensis*. All three phenomena are expected to increase nematode numbers and thus damage unless other measures develop that react counter wise, like enhanced resilience of soil biodiversity as mentioned above. *Paratylenchus bukowinensis* is not only a herbivorous organism feeding on plant roots, but it also poses a food source for other soil-dwelling organisms and antagonists. This raises the question if and how those natural control forces can be used for nematode management that in the end would lead to a truly sustainable system. One such approach is seen in modern farming practices that rely on minimum tillage. Minimum tillage increases the soil organic matter content of the soil, stimulates soil biodiversity and improves soil health by increasing the antagonistic potential in a soil. Data demonstrating these effects from field studies are not always that convincing. However, should a highly diverse soil ecosystem be established under minimum tillage, plant parasitic nematodes would be at a disadvantage, while at the same time crop tolerance to withstand nematode damage would be improved. In the end, farmers need objective information to be able to make the difference between facts and fantasy. Science should be transparent and make a clear distinction between what is still a hypothesis and what are solid and proven measures that can be taken to economically improve soil resilience reducing the impact of plant parasitic nematodes.

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SECTION VI:

Root and Tuber Crops

44 The need for new approaches for management of potato cyst nematodes: The view from the Rhineland-Palatinate

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Introduction

Potato is an important crop throughout Europe and especially in Germany where potatoes are grown on over 250,000 hectares. Approximately 60% are grown for fresh table use, about 20% for starch and the rest produced for seed potatoes. According to the Federal Statistical Office, Lower Saxony is the largest potato producing county, followed by Bavaria and North Rhine-Westphalia.

Distribution

Potato production worldwide is negatively affected by the presence of the golden and the white potato cyst nematodes *Globodera rostochiensis* and *G. pallida,* in this article abbreviated as potato cyst nematodes (PCN). The two species originated in South America (Niere and Karui, 2018). They were brought to Europe in the 1900s and continuously spread mainly via seed potato.

PCN is present with restricted distribution in all counties in Germany where potato is produced and these infestations are strictly regulated. Both species are present and often in mixed populations. The presence of pathotypes further complicates the use of quarantine measures.

Economic importance

Potato yield and overall production is negatively impacted by both species of PCN from planting to harvest, but there are differences in importance within the country and on crop type. The initial lack of quarantine regulations as well as insufficient research on the epidemiology of PCN led to massive population increases and uncontrolled spread of *G. rostochiensis* in the 1950s. The level of yield loss was dependent on initial soil population densities as well as type of production and environmental factors. However, in some cases *G. rostochiensis* caused complete crop loss (Fig. 44.1). With the introduction of PCN resistant cultivars in the 1970s losses dropped dramatically and the nematode is for the most part unimportant in potato production with a few exceptions. However, the use of resistance to *G. rostochiensis* caused a shift in species importance towards *G. pallida,* which is in the meantime the main potato pest in the major potato growing areas of Germany. *Globodera rostochiensis* is still of some importance in the eastern part of the country and in vegetable growing areas with 'early season potato' (ESP). Losses in the past caused by PCN varied from 10–100% on a field basis when control measures were not practiced.

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Fig. 44.1. Complete crop loss due to *Globodera rostochiensis*. Author's own photograph.

Host range

The host range of PCN is limited to the nightshade family (Solanaceae). Potato is the most important host plant. Tomato, bell pepper and aubergine are also good hosts, but they are not components of potato rotations. More important are thermophile nightshade weeds, like black and bittersweet nightshade (*Solanum nigrum* and *S. dulcamara*, respectively), since they allow PCN multiplication even under non-host rotation crops, e.g. maize or vegetables. Where tomato is grown under near monoculture conditions in greenhouses in untreated field soil, PCN is often detected. In all cases spread on farm machinery and last season's roots is possible.

Pathotypes

The PCN are further separated into pathotypes, with five pathotypes of *G. rostochiensis* (Ro 1-5) and three of *G. pallida* (Pa1-3). In the field, different pathotypes might occur simultaneously. This complicates recommendation of suitable resistant cultivars (Table 44.1).

The tight pathotype classification has been shown to be inappropriate for *G. pallida* as they show a sliding scale of virulence (Nijboer and Parlevliet, 1990).

Distribution

The spread of the nematode became an important factor in potato production and stimulated the plant protection agencies in all countries in the EU to react. Therefore, different levels of quarantine regulations have been put in place in many countries harbouring PCN. Beginning in the 1950s, the German government set laws to regulate crop rotations to prevent the multiplication and spread of *G. rostochiensis*. This will be discussed below.

Table 44.1. Current schemes for identifying and classifying pathotypes or races of potato cyst nematodes. From Brodie, 1993, © CABI

Differential host	Designation of *Globodera rostochiensis* pathotypes							
	Nijboer and Parlevliet (1990)		Ro1			Ro3		Ro5
	Kort *et al*. (1977)	Ro1		Ro4	Ro2		Ro3	Ro5
Differential host	Canto and de Scurrah (1977)	R_1A		R_1B	R_2A		R_3A	–[a]
Solanum tuberosum ssp. *tuberosum*		+		+	+		+	+
S. tuberosum ssp. *andigena* (H_1)		–		–	+		+	+
S. kurtzianum KTT/60.21.19		–		+	–		+	+
S. vernei GLKS.58.1642.4		–		+	–		–	+
S. vernei $(VT^n)^2$62.33.3		–		–	–		–	+
	Designation of *Globodera pallida* pathotypes[a]							
	Kort *et al.* (1977)	Pa1	–[b]	–[b]	–[b]	Pa2	Pa3	–[b]
Differential host	Canto and de Scurrah (1977)	P_1A	P_1B	P_2A	P_3A	P_4A	P_5A	P_6A[c]
Solanum tuberosum ssp. *tuberosum*		+	+	+	+	+	+	+
S. multidissectum (H_2)		–	–	+	+	+	+	+
S. kurtzianum KTT/60.21.19		+	+	–	+	+	+	+
S. vernei GLKS.58.1642.4		+	+	+	–	+	+	+
S. vernei $(VT^n)^2$62.33.3		–	+	–	–	–	+	+
CIP 280090.10d[d]						–	–	+

[a]EPPO (1992) recognizes only Pa1. Trudgill (1985) and Nijboer and Parlevliet (1990) recognized virulence groups but not pathotypes.
[b]No comparable pathotype reported.
[c]New race identified by Franco and Gonzalez (1990).
[d]New differential clone added by Franco and Gonzalez (1990).

The type of potato is the problem in Germany

An economically important problem exists where PCN infestations occur in ESPs that are planted between February and March. ESPs are affected not only in Rhineland-Palatinate (Rheinland-Pfalz), but also in other states in Germany as well as in a number of countries in the EU.

This high-value crop is often produced in intensive crop rotations within distinct areas with soils suitable for early cultivation and irrigation. Frost protection measures (foil and fleece cultivation) are necessary to avoid damage and to accelerate ripening (Fig. 44.2). Because of these requirements, suitable fields are rare and often overused. Early season potato reach maturity within 75–90 days. This allows maximizing profit due to one or two additional crops within the growing season. Mid-season potato mature within 95–110 days and late season around 120–135 days.

Early season potato is especially susceptible to PCN in which management is complicated because of a limited choice of resistant cultivars and market requirements.

Due to the limiting factors affecting ESP cultivation, growers often practice intensive field exchanges. This complicates PCN management since there is constant spread of cysts by soil attached to machinery and to vegetable root crops, e.g. turnip, carrot, radish, parsley and celery.

Symptoms of damage

The symptoms of damage of PCN are unspecific and similar to those caused by other soil-borne diseases. Typically, nests or oval-shaped areas of poorly developing plants are seen in the field. The symptoms are easily confused with nutrient deficiency or differences in soil structure, e.g. compaction (Fig. 44.3). These field symptoms

Fig. 44.2. Foil cultivation of early season potato. Author's own photograph.

Fig. 44.3. Typical *Globodera* nest-type symptoms within potato fields. Author's own photograph.

are often masked by the use of high levels of fertilizer and optimum moisture levels. Irrigation also reduces symptom development. In the end only soil sampling and the examination for the presence of cysts or direct examination of the root system for white to yellow cysts confirm the presence of PCN.

Biology and life cycle

The life cycle has been described in many publications. Even though PCN only completes one generation per year, the enormous reproduction potential of 300–400 eggs/female make them a major pest of the potato crop. Their eggs survive within the dead female body or cyst for up to 10 years in the soil. Juvenile emergence corresponds to the minimum temperature for potato development. This allows them to complete a full life cycle even under ESP, which is in the field for 75–90 days compared to regular season potato of at least 120–140 days. *Globodera rostochiensis* females are at first white in colour, turning golden brown as the cysts mature, whereas *G. pallida* cysts remain white until maturation.

The difference in the temperature requirements of the two species is an important factor. *Globodera rostochiensis* hatches at 20°C with a lower limit of 10°C, whereas the optimum for *G. pallida* is slightly lower and the lower limit is 8°C (Franco, 1979). This means *G. pallida*, which is the dominant species now in Germany, is better able to deal with the cool soil temperatures (Brodie *et al.*, 1993). Nevertheless *G. rostochiensis* is still the predominant species in ESP production. *Globodera pallida* records on ESP fields are comparatively rare. Restricted choice of *Globodera* resistance among the early season cultivars might be a possible reason in that there is little rotation of cultivar resistant types, as well as the temperature compensation by measures of harvest advancement.

Interactions with other nematodes and pathogens

Potato cyst nematodes are often found in the field together with other soil-borne pathogens, e.g. *Rhizoctonia solani* or bacterial diseases (Fig. 44.4). Possible interactions need to be investigated for proper advisory recommendations.

Fig. 44.4. Symptoms of damage caused by interactions between *Globodera* and soil-borne bacterial diseases. Author's own photograph.

Recommended integrated nematode management

Government regulations

In the very beginning government regulations (1992) were restricted to a required examination of the pre-season density of PCN in fields used for seed potato production to prevent further spread. Since then, new regulations (2010) now require monitoring of table potato areas. All states in Germany now have to examine at least 0.5% of their potato production area for PCN infestation. Positive detections are officially documented and reported to the local plant protection agencies and eventually communicated to the EU. When an infestation is detected (cyst with living content) government regulations require the following:

- 6-year ban on potato production;
- 2-year ban followed by the planting of a resistant cultivar;
- 2-year ban after application of a nematicide; and
- other management methods that reduce PCN (government approval necessary).

Rotation

There is no room for grower flexibility in potato production in Germany. A 6-year quarantine in production is a major limitation to the production of ESP and adds to the complexity of planning production over time.

In fields with laboratory confirmed infestations, additional restrictions are in place to limit spread by farm machinery. To make matters worse, there is no guarantee that after the 6-year quarantine the field will be certified PCN free.

Resistant cultivars

Planting resistant cultivars reduces the length of quarantine significantly. The list of cultivars available to the grower, however, is determined by the market and potato breeders. The use of resistant cultivars in infested fields is used extensively by growers for mid to late season and industrial potato production. Their use in ESP is not practical because the available spectrum of cultivars is extremely limited and does not always fit market demands. Also problematic is the determination of the occurring pathotype in a field by the local plant protection service, which takes up to 2 years. This complicates the integration of resistant cultivars in crop rotations within ESP. Resistant cultivars, with resistance to all pathotypes of *G. rostochiensis* (Ro 1-5), could be used to bypass the time-consuming official laboratory pathotype screening process. Unfortunately, very few cultivars are registered and the choice for the farmer is extremely limited.

Nematicides

The use of nematicides for PCN control is basically an approach that ensures acceptable yield.

Presently, Nemathorin 10G is the only nematicide registered in Germany for PCN control. The application of Nemathorin is, however, restricted to late season potato and not registered for use in early or mid-season potato (Anonymous, 2020).

Furthermore, the use of nematicides that do not have nematode population reduction activity are not advocated for PCN management. This is the case in most states in Germany.

Optimization of nematode management

Hotspots

Concentrations of *G. rostochiensis* infection in ESP sites are often found in vegetable production areas. The intensive and very quick crop fluctuations during the growing season (2–3 crops per field), highly specialized producers and the frequent field exchanges cause problems with transparency of the field histories. Field history, especially for sections that include nematode infestation and other diseases (e.g. *Globodera*, *Synchytrium*, *Plasmodiophora*) is absolutely essential for the development of well-adapted strategies. Field history data should include information on crop rotation, choice of cultivar, soil cultivation and machinery disinfestation.

Field passport

A ‘field passport’ would be a good instrument to save important information for company management and aid agricultural extension decision making. Such a field-specific document must be available even for tenant farmers. In times of digital farming, it should be no problem to modify a field plot card index for this purpose.

In addition to quarantine nematodes, the document should also include information on the use of plant protection pesticides (product, quantity, timing and dosages) since the application of certain products is often restricted for several years. Registration of other pests (e.g. *Meloidogyne hapla*, *Ditylenchus dipsaci*, wireworm, clubroot) could be included as additional useful information for the producer.

When the quarantine retention period has expired a new soil examination for PCN would be required before planting a following potato crop. This is necessary because a 6-year period with non-hosts is often not sufficient to eliminate PCN from the soil. Residual populations often remain, due to difficulty in managing volunteer potatoes and nightshade weeds that maintain the population. A large percentage of the ESP growers clean and pack the potatoes in market-suitable packages. There is also a controlled and traceable disposal of the residual soil after cleaning. However, the disposal of wash water involved in the cleaning process is still a problem. The use of appropriate technology (heat, UV exposure, fermentation ponds) is essential so that PCN cysts, eggs and/or juveniles cannot survive and create new hot spots.

Alternative management methods

Between 2010 and 2017, field trials were conducted in order to compare the efficacy of resistant cultivars and Nemathorin 10G with the alternative control techniques biofumigation and sowing of *Solanum sisymbriifolium* that has PCN suppressive activity (Augustin and Wach, 2018).

Regardless of excellent field conditions (temperature, soil moisture, foil cultivation), biofumigation proved to be inconsistent. The biofumigation based on glucosinolate-rich oil radish mulch or pellets gave a maximum reduction of 50%. The efficacy of resistant cultivars was below a 90% reduction because of the negative effects of the presence of volunteer potatoes and weed hosts. The nematicide Nemathorin gave comparable reductions in the fast growing ESP, but since registration is only for late season potato, application in ESP is still not possible.

The highest level of control was obtained with the antagonistic crop *S. sisymbriifolium*, which was equal or better than the nematicide. Control activity of over 90% was reached with *S. sisymbriifolium*. However, the cultivation of this trap crop is highly sophisticated. Because of the high temperature demand during the seedling stage and relatively slow plant development, management efficacy is closely coupled with the proper use of herbicides and adequate irrigation for moisture maintenance.

A successful *Solanum* crop is an environmentally safe method for removing both species of *Globodera* from infested fields regardless of pathotype (Timmermans, 2005).

Growers of high-value sensitive vegetable crops would need to sacrifice nematode infested field sections for one vegetation period for this technology, which would allow them to manage the PCN problem in an environmentally sustainable way.

This integrated nematode management antagonistic trap-crop system is now part of states official regulations (Rhineland-Palatinate) and the first in Germany.

Future research requirements

- There is need to study the post-harvest amount of small-sized potato tubers remaining in the soil in order to effectively reduce the number of volunteer host plants within the crop rotation. This is a challenge for breeders as well as for agronomists with additional effects on other pests and diseases of potato.
- Interactions between *Globodera* and other soil-borne pathogens should be investigated in detail in order to improve strategies.
- A continuous development of new resistant cultivars matching market requirements would promote ESP production within PCN areas.
- The advance of global warming enhances the use of thermophile antagonistic plants for trap and natural control. Since the availability of pesticides has dramatically decreased, there is a need to develop alternative agronomic methods for a successful establishment of weakly competitive crops like *S. sisymbriifolium*, e.g. planting of nursery plants instead of seeding. A faster development is positive for the establishment of the trap crop and for the economy since it allows an additional crop per season.

Outlook: anticipating future developments

- Climate change will in the future shift potato production zones as well as other crops. With changes in consumer attitudes and the new technologies emerging in genetics (as presented in Chapter 64 in this volume), it is not clear which crops will be adaptable to higher temperatures.
- There will be an increase in the crop production intensity in many parts of the world that will lead to increased PCN development over time.
- There is no doubt that strict registration requirements in Germany for new pesticides

will lead to fewer pesticides available for nematode management.

- These changes will force us to work harder to develop field hygiene and alternative techniques to manage PCN.
- Integrated nematode management, as outlined in this book, will require integration of all forms of control methodologies in effective systems and not dependence on one approach whether resistance or pesticide based.

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45 Transporters of trouble: Trichodorids and Tobacco rattle virus in potatoes

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Introduction

Trichodorids may cause both direct damage to the plants as well as indirect damage through transmission of Tobacco rattle virus (TRV). Trichodorids include nematodes of among others the genera *Paratrichodorus* and *Trichodorus*, which are the most common in north-western Europe. The number of species occurring within a given region varies. Ten species are known to occur in the Netherlands and 13 species have been reported for Germany. High densities of the nematodes in spring may cause direct damage to the plants. However, indirect damage may already occur at low nematode densities when only a few nematodes are infected with TRV.

Economic importance

Trichodorids often show a patchy distribution in the field, which makes it difficult to estimate the overall effect of direct damage. When trichodorids are present in high densities (i.e. above 10 specimen/100 ml soil in the case of *Paratrichodorus teres*, above 100 specimen/100 ml in the case of other trichodorid species when based on extraction by Oostenbrink elutriation) (Actieplan Aaltjesbeheersing, 2010), yield loss can be 30–50%. Besides direct damage, even more important is the transmission of TRV by trichodorids. If potato tubers are infested by TRV, the plant tries to hinder further spread of the virus by a hypersensitive response. As a result of this defence response, local lesions, ringspots or line patterns of corky tissue are formed in the tuber. The disease is called 'spraing' or corky ringspot (Fig. 45.1).

Because of this qualitative damage, potatoes are worthless for the processing industry and unattractive as ware potatoes. For seed potatoes, the Dutch inspection service (Nederlandse Algemene Keuringsdienst) allows a maximum of 6% infested tubers (NAK, 2020). Higher levels will result in rejection of the entire containment. The official threshold for ware potatoes is similar, but companies usually set their own threshold at a much lower level. Whereas some companies require ware potatoes to be absolutely free of symptoms, other companies do allow some damage up to 2%. Those requirements may also depend on the amount and quality of the potatoes that are available. With respect to seed potatoes, some countries even require PCR testing for import, with zero tolerance for the occurrence of TRV. This poses the risk that lots visually free from symptoms are rejected because of latent virus infection.

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Fig. 45.1. Symptoms of spraing in potato tubers caused by infection with TRV transmitted by trichodorid nematodes. Photograph courtesy of Wageningen University & Research, Field Crops.

Host range

The host range of trichodorid nematodes is broad, and the host status of many crop species is still unknown. Plants that are known to multiply at least some of the most common trichodorid species are potato, sugar beet, onion, carrot, maize, *Phaseolus* beans, cabbage, wheat, barley, rye and ryegrasses (Best4Soil, 2020), as well as strawberries and a range of tree and fruit tree species. Also, many wild plants that occur as arable weeds are able to multiply trichodorids.

Distribution

In a sampling of 500 arable fields throughout the Netherlands, trichodorids were found in 60–70% of the samples from sandy soils in the eastern part of the country (Keidel *et al.*, 2007). In a large-scale sampling of grasslands in the Netherlands, trichodorids were found in about 40% of the samples from sandy soils (de Boer *et al.*, 2019). When soil conditions are known to be favourable for a nematode species, the area that theoretically is sensitive to damage may be estimated. Based on a description of the texture and organic matter and lime content of the topsoil, and the texture of deeper layers in the soil profile, it was estimated that 18–27% of the soils in the Dutch marine clay area may be sensitive to direct damage by *P. teres* (de Smet and van Soesbergen, 1968). Direct damage is minor in soils with more than 12% clay and silt particles <16 µm, although indirect damage by TRV may still occur.

The four species that are most common in the Netherlands differ somewhat in their preference of soil type. *Paratrichodorus teres* is most common on marine calcareous sandy soils in the west of the Netherlands but is also found in the east. *Trichodorus primitivus* is more commonly found on sandy loam soil and sandy silt loam soil in the west and north of the country and along the rivers. *Trichodorus similis* and *P. pachydermus* often occur in mixed populations on wind-deposited sand layers with a high percentage of sand mainly in the east of the country, although *T. similis* is more widespread and is also found in some other areas. The latter two species are the most common trichodorids reported in general from Germany and in particular as pathogens of potato. Regarding soil type, *T. similis* and *P. pachydermus* are mainly reported from loamy sand to silty loam soils. However, a few records also exist from loamy soils.

Symptoms of damage

Typical for an infestation with trichodorids is the occurrence of some plants that grow well in a patch that is otherwise severely damaged (Fig. 45.2). The symptoms of infestation are stubby roots and a root system with a branched or bushy appearance. Trichodorids feed on the epidermal cells just behind the zone of root elongation, causing swelling and stunting of the root tip (Taylor and Brown, 1997). In cases of severe infestation, the root may stop growing, inciting the formation of new roots. Trichodorids in potato also cause above-ground symptoms, where the sprouts start winding and show elongated brown spots (Fig. 45.3). Sprouts can even die off.

Fig. 45.2. A characteristic field symptom of direct damage by trichodorids is the alternation of severely damaged and seemingly unaffected plants in a patch. Photograph courtesy of Wageningen University & Research, Field Crops.

Fig. 45.3. Trichodorids cause swelling and winding of the sprouts and elongated brown spots. Photograph courtesy of Wageningen University & Research, Field Crops.

This symptom can easily be mistaken for an infection with *Rhizoctonia*. Although the multiplication of *P. teres* on potato is lower than that of the other trichodorids, damage to the plant is more severe and potatoes may have a cracked appearance. Regarding virus symptoms, TRV in susceptible potato may cause yellow blotches on the leaves (stem mottle; Fig. 45.4), but the main cause of yield loss in potato is the formation of arcs and/or spots of corky tissue in the tubers as described above. The symptoms are often confused with the deficiency disease caused by a lack of calcium.

Biology and life cycle

Trichodorids are ectoparasites that primarily occur in sandy soils that are prone to drying in periods of drought. However, damage by trichodorids has also been recorded from loamy soils, at least in some regions. Trichodorids can be found in the entire soil layer where roots occur, but often are found around the area where the topsoil meets the subsoil (Cooper and Harrison, 1973). Different species seem to preferably

Fig. 45.4. Stem mottle is the above-ground symptom of potato infected with TRV. Photograph courtesy of Wageningen University & Research, Field Crops.

inhabit different soil depths where some species may even be found at 60–90 cm depth. Trichodorids appear to be susceptible to drought and the distribution over the soil profile is related to time of the year and soil moisture. The concentration in deeper soil layers seems to be due to low survival in the drier upper layer and not so much to migration to deeper soil layers (Rössner, 1972). This probably is the reason why problems with trichodorids and TRV are lower in years with a dry spring, when the density of trichodorids around the roots of the newly planted crop is low. In autumn, the density of trichodorids in the upper soil layer increases again.

All life stages of the nematode are able to acquire and transmit TRV when feeding on infected plant roots, but the virus is lost from the nematode when moulting. Trichodorids are relatively long-lived species with a lifespan of up to 20 weeks that retain virus also in periods without access to TRV infected plants (Taylor and Brown, 1997).

Interactions with other nematodes and pathogens

Trichodorid nematodes can transmit TRV and pea early-browning virus (PEBV). TRV has a very broad host range that includes many crop species and wild plants including weeds, whereas PEBV has only been found in leguminous crops. Different trichodorid species are known to transmit different serologically distinct types of TRV (so-called serotypes), which vary in the ability to multiply in different plant species. TRV may be transmitted through daughter potatoes, although the rate of transmission decreases over generations. TRV may also be transmitted through infested arable weeds and weed seeds that carry the virus. Trichodorids that feed on the infested weeds may acquire the virus and subsequently transmit it to the crop. No interaction with fungi or bacteria is known.

Recommended integrated nematode management (INM)

The INM approach that is advocated in the Netherlands is based on prevention, soil sampling and monitoring, crop rotation and, as a last resort, supplementary measures including nematicides. As it is very difficult to eradicate trichodorids and TRV when they are present in a field, it should be stressed that prevention is important. Prevention starts with the use of certified planting material that has been inspected for diseases, especially TRV. Further, a high level of hygiene on the farm, cleaning machinery before moving from one field to the other, avoids the spread of nematodes among fields. Hygiene also includes weed management, as many arable weed species may be a source of trichodorids and TRV. Weed management is especially important as a long-term strategy, although in the short term it may not decrease virus transmission. Cooper and Harrison (1973) reported that in one experiment, intensive weed management for one and a half years resulted in a higher level of spraing incidence than no weeding. They hypothesized that the nematodes preferably feed on weeds over potato. In the weeded treatment, infested trichodorids that survived the lay period had no alternative than to feed on potato and as a result transferred the virus, but in the unweeded treatment, they may have fed more on the weeds than on the potato plants.

Secondly, knowledge about the field and the nematode species present is important. The soil type may already give information about the probability of problems with trichodorids, which are very unlikely to occur on heavy soils. Some famers have changed their soil texture by ploughing up heavier soil to the surface or by bringing heavy soils from elsewhere. This measure prevents direct damage but does not prevent damage by TRV. The history of the field, including knowledge about problems that occurred in different crops in the past, and observation of irregularities in crop growth are relevant to note. Soil sampling and assessment of the plant parasitic nematodes that are present are essential to create a sound crop rotation. As different trichodorid species vary in host range, determination to species level is advised. With the use of the websites 'Aaltjesschema' (in Dutch) or 'Best4Soil' (22 languages), the most ideal cropping frequency and order can be determined, including the use of green manure crops. The websites contain both information on the rate of multiplication of the nematodes and sensitivity of the crop to damage, as well as additional background information. Considering the broad host range of trichodorids and TRV, designing a crop rotation may be quite challenging. It is important to take into consideration what trichodorid species is present. For example, fodder radish has been found to be successful in the management of *P. teres* and its associated TRV serotype, as both do not multiply on this green manure crop. Similarly, spring barley can be used to suppress *P. teres*-associated TRV, as it does multiply trichodorids, but not the serotype of TRV that is transmitted by *P. teres*. The level of TRV in the field is brought down when the nematodes multiply and juveniles feed on roots that are free from TRV. However, these measures do not hold for other trichodorid species and their associated TRV serotype. Different potato cultivars vary greatly in their sensitivity to TRV. Growth of a tolerant cultivar yields tubers that are free of symptoms but do contain and multiply the virus.

As supplementary measures, fumigant (metam-sodium that degrades into the biological active compound methylisothiocyanate) and non-fumigant nematicides (e.g. oxamyl. fosthiazate and fluopyram) can be used. Application guidelines can vary a lot and thus regional requirements need to be enforced thoroughly. When nematicides are used to suppress other nematodes, trichodorids may also be suppressed as a side effect. The presence of fresh organic matter seems to hamper the transmission of TRV, maybe through decreased nematode activity. However, addition of compost decreases trichodorid numbers to some degree, but does not influence spraing incidence levels. Anaerobic soil disinfestation has not been studied as a specific measure to manage trichodorids, but in the one experiment where they were measured as a side effect, numbers decreased below the detection level after 13 weeks treatment (Goud *et al.*, 2004). However, the occurrence of TRV was not determined in this study. Inundation for 16 weeks decreased numbers of trichodorids to very low levels, but infection with TRV was still found in some samples (Asjes *et al.*, 1996). Because of its insufficient efficacy, inundation is currently not advised as a control measure for TRV in the Netherlands.

Optimization of nematode management

The observation that direct damage by trichodorids is less severe in dry springs has led to the idea that lowering the soil water table around the time of planting may reduce damage. This would need further investigation but is unlikely to solve indirect damage by transmission of TRV. When the distribution of soil texture in a field is not homogeneous, problems with trichodorids may be localized to patches with lighter soil texture. In that case, if deeper soil layers contain more clay and silt particles, deep ploughing of specific patches may change the soil texture so that it becomes unsuitable for trichodorids. This measure has successfully been performed in the Wieringermeer polder, one of the areas that has been reclaimed from the sea.

Knowledge transfer to farmers, advisers and students is important to implement an INM strategy for trichodorids and TRV. With increasing awareness and knowledge about the TRV problem its management will improve. For the most part transfer of knowledge systems are available and are being used.

Future research requirements

The main focus of research in nematology has been on sedentary endoparasitic nematodes and their relationship to the plant, including resistance breeding. Much less is known about the mode of interaction between ectoparasitic nematodes and plants. It remains intriguing why trichodorids are able to infect many plant species, but not all. Further, it would be interesting to study the cause of differences in host plant range among different trichodorid species. This may aid selecting non-hosts or poor hosts for a better trichodorid and/or TRV management. Furthermore, potato cultivars differ in their susceptibility for spraing disease. However, the underlying mechanisms are far from being understood. Thus, it is still unknown if the lower susceptibility of some potato genotypes is the result of a plant resistance response against the nematode or against the virus or more a tolerance response against the virus. An answer to this question might prepare the way for selection towards enhanced resistance and/or tolerance.

Outlook – anticipating future developments

Effects of climate change in north-western and central Europe will lead to milder winters and warmer summers and an increase in total as well as extreme precipitation. As a result of increasing temperatures, plant evaporation and the chance of a water deficit during the growing season will increase. These scenarios may have different implications for the effects of trichodorids and TRV on potato. On the one hand, a drier spring may limit the occurrence of the nematodes to lower soil layers, decreasing the chance that they will affect the newly planted crop. Dry conditions later in the season will reduce crop yield as roots damaged by the nematode are less effective in taking up soil water and nutrients. Furthermore, milder winters may stretch the length of the growing season and thus the period of nematode activity. Overall, the current scenarios discussed in relation to global warming for temperate regions will most likely increase disease incidence and severity.

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46 Will the *Globodera pallida* epidemic signal the end of the seed potato industry in Scotland?

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Introduction

Of all pests and diseases of potatoes, British potato growers consider potato cyst nematodes (PCN: *Globodera pallida* and *G. rostochiensis*) to cause the most damage (AHDB, 2018). By feeding on the roots, PCN stunt plant growth, adversely affecting yields, tuber size and skin finish. Yield losses depend on the population level of the PCN in the soil, the soil type and the tolerance of the potato cultivar to the nematode damage. Overall, the estimated annual loss to the British potato industry due to PCN is approximately 9% of crop value. Across Britain, *G. pallida* is now the predominant species of PCN.

PCN co-evolved with wild potatoes in the Andes. They were most likely introduced into Europe on several occasions following the European potato famines of the 1840s when expeditions to Central and South America brought back potato breeding material with greater resistance to blight. PCN were first recorded in Scotland in 1913. In 1972, Stone described *Heterodera* (now *Globodera*) *pallida* using populations from two localities, one of which was Duddingston in Scotland. Records referring to *Heterodera rostochiensis* before then would also have included *H. pallida*. Given their sedentary nature and a typical rotation for potato cultivation of 5 or more years, the introduction of PCN into Scotland can be considered relatively recent in terms of the number of host generations.

The extensive economic damage caused by PCN has led to the treatment of both species as quarantine pests (Pickup *et al*., 2018). For Scotland in the 2020s, *G. pallida* must now be considered widely distributed and beyond any stage where eradication is an option. Successful eradication of PCN, while maintaining commercial potato production, has yet to be achieved anywhere. *Globodera pallida* has yet to be found in all potato growing regions of Scotland, so there remains some, albeit increasingly limited, value in containing infestations to already affected areas. Otherwise, for most of the country, regulatory control is targeted at the management of known infestations with the aim of limiting further increase and spread.

Transmission of PCN is associated with the movement of infested seed or soil, predominantly through the trade in seed potatoes. In Scotland, as in the rest of Europe, management of PCN meets the minimum standards set out in the EU PCN Directive 2007/33/EC, requiring testing of soil samples from all land intended for seed potato production. Where PCN are found in these official tests, the land is recorded as infested. Seed potatoes cannot be grown on

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infested land and potatoes other than seed can only be produced under an officially approved control programme. The Directive also prohibits the planting of infested potatoes.

Economic importance

Over 27,500 ha of potatoes were planted on 2600 farms in Scotland in 2016. Of this area, c. 11,000 ha of seed potatoes were planted with the remainder grown for ware (end-use). The estimated value of the potato crop in 2016 was £209 million. Potatoes generally account for around 7% of Scotland's total farm output.

The increase in *G. pallida* has been most marked in the county of Angus, traditionally the part of Scotland most intensively cultivated with potatoes. In 2019, 31% of the Scottish seed potato crop and 38% of the Scottish ware potato crop was grown in Angus. The incidence of *G. pallida* in Angus is estimated as 9% of the area used for potato production, accounting for 68% of the total area of *G. pallida* infested land in Scotland. A recent study (Blok *et al.*, 2020) modelled both the increase in *G. pallida* and its economic impact on potato production.

The area with *G. pallida* testing positive in each year was low from 1995, starting to slowly increase from 2005 and more rapidly since 2010, when the higher sampling rates specified by the 2007 Directive were introduced (Fig. 46.1).

An exponential model shows the area testing positive each year doubling approximately every 7 years. With good agreement between the model output and the data, the model was used to predict the levels of *G. pallida* infestation under the assumption that current management practices continue. By 2040, over 20,000 ha of the land in Angus (of an estimated 50,000 ha otherwise suitable for potato production) is predicted to be infested and therefore, under current regulations, prohibited from use for seed potato production. The production of ware potatoes will also be limited. Using the model suggests losses due to *G. pallida* could rise to as much as £125m per year by 2040. This figure represents the opportunity loss not the actual loss, i.e. the value of potatoes that could have been grown had the land not been infested by *G. pallida.*

The relatively recent development of the *G. pallida* 'epidemic' has yet to have significant economic impact for most farmers. In Scotland, unlike England where the epidemic is more advanced, PCN are considered less of a concern than blight and blackleg. Concern is currently more about the statutory restrictions on seed potato production, rather than impending yield losses in ware crops. The sustainable management of the land is also threatened by the increasing area of potatoes produced on rented land: growers who rent are looking more at profit of their crop and less at the long-term health of the land they have rented.

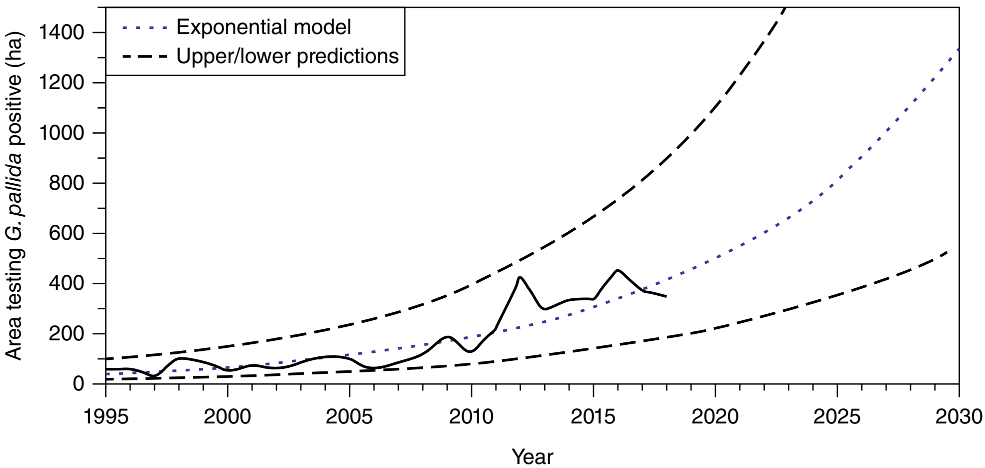

Fig. 46.1. Increase in the area testing positive for *Globodera* pallida (thick line) compared with the model prediction (dotted line) and the upper and lower predictive 95% confidence intervals. Author's own figure.

Host range

The host range for PCN is limited to potatoes as no other host crops can be grown under Scottish field conditions. Outside the crop, populations of groundkeepers (tubers that escape harvest) are potential hosts. The only weed host of PCN commonly found in Scotland, *Solanum dulcamara*, is rarely found in arable land.

Distribution

As of July 2020, the area of land in Scotland officially recorded as infested with PCN is 20,157 ha. Of this total, 14,625 ha (73%) is recorded as infested with *G. rostochiensis* and 6568 ha with *G. pallida* (33%), including 1036 ha (5%) with both species (Fig. 46.2). Until the 1990s, nearly all infestations in Scotland were of *G. rostochiensis*. In 1990, only 179 ha of the currently infested land was infested with *G. pallida*, compared with over 6500 ha in 2020. Over the last 45 years, the incidence of *G. pallida* has effectively doubled every 7 years, or roughly each crop rotation. In contrast, the area infested with *G. rostochiensis* is now relatively constant, having only increased by 2% over the last 7 years. With an estimated 150,000 ha of land used for potato production in Scotland, the area officially recorded as infested with PCN is approximately 13.5%, and the area infested with *G. pallida* is approximately 4.4%.

Symptoms of damage

The nematode cysts lie dormant in the soil until the juveniles are stimulated to hatch by exudates from the roots of a host plant. As the juveniles invade the roots, the plant responds by closing the stomata, retarding plant growth. At low levels of infestation, symptoms are rarely seen in the field. With high infestations, patches of affected plants are more visible as root damage makes them more susceptible to water and nutrient stress. Symptoms include stunted plants, patches of the crop where plants fail to meet across the rows, or delayed flowering and white cysts on the root surface or in the soil (Fig. 46.3). The potential use of spectral reflectance has

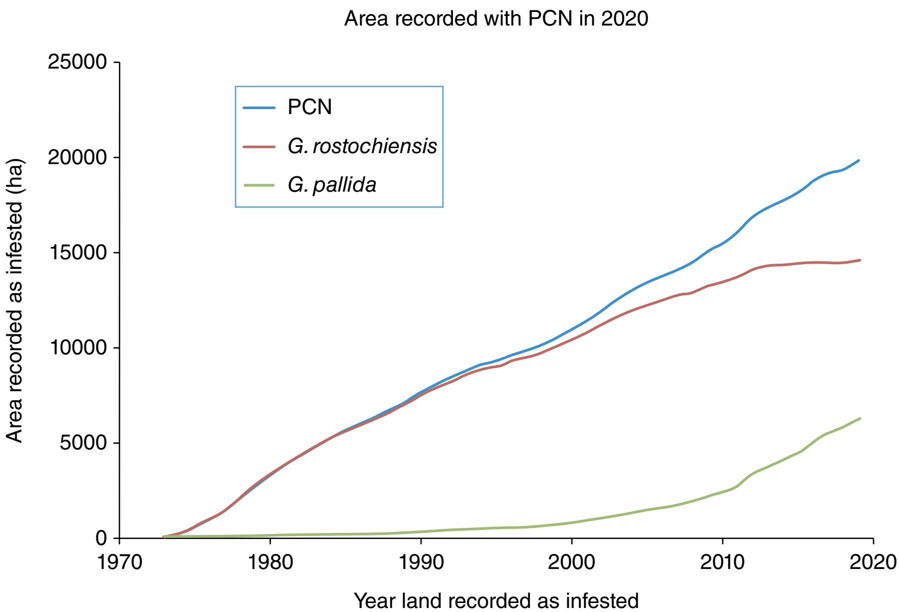

Fig. 46.2. The area of land recorded as infested with potato cyst nematodes, *Globodera rostochiensis* and *Globodera pallida*, as of June 2020. The data are presented by the year in which the land was originally recorded as infested. Author's own figure.

Fig. 46.3. High density of white cysts of *Globodera pallida* on potato roots. Author's own photograph.

been investigated for remote sensing of PCN infestations, but generally any variation requires further investigation, usually involving soil sampling, to confirm PCN as the cause.

Biology and life cycle

The life cycles of the two PCN species are broadly similar (Moens *et al.*, 2018). Where differences occur, they tend to be a matter of degree: the annual decline rates of *G. pallida* (10–30% per annum) tend to be lower than those of *G. rostochiensis* (20–40% per annum) and the hatch of *G. pallida* occurs at lower temperatures and for a more prolonged period than in *G. rostochiensis*. The pathogenicity of both species is generally assumed to be similar in terms of yield loss in the presence of similar population levels under similar conditions, although a third species of PCN, *G. ellingtonae* is of less economic concern due to its much-reduced pathogenicity. The most significant difference in the biology of the two species lies in the extent to which commercially successful varieties are resistant to each species. The first UK cultivar with *H1* resistance to PCN, Maris Piper, bred in 1966, is now the most widely grown cultivar with a wide range of marketing options offered to the grower/producer. In 2019, 55% of Scotland's potato production was of cultivars highly resistant (scores 7–9) to *G. rostochiensis*, whereas only 2.6% had comparable levels of resistance to *G. pallida*. This disparity provides a strong selection pressure favouring the increase of *G. pallida*.

Recommended integrated nematode management (INM)

Decline rates, rotation length and groundkeepers

Long rotations of 5 or more years have been a long-standing part of the INM strategy for the management of PCN in Scotland. For seed production, a minimum rotation of 6 years is a requirement for seed classification, 8 years for the highest grade. Rotations take advantage of the natural decline/annual hatch of c. 20–30% of the population that occurs each year in the absence of a host. For rotations to be effective, fields must be free of groundkeepers: PCN populations will increase on susceptible varieties and groundkeepers of resistant varieties could lead to selection of resistance-breaking populations. Any withdrawal of the herbicide glyphosate is likely to exacerbate the groundkeeper problem.

Nematicides

In Scotland, growers have access to one granular nematicide, Nemathorin (fosthiazate), applied at planting to control juvenile nematodes emerging from cysts to invade the roots of the growing potato crop. By effectively reducing the initial nematode population level, nematicides can prevent root damage and protect crop yields. Any nematodes that successfully invade the roots will still multiply on susceptible hosts. Fumigant nematicides are not used in Scotland. However, with increased pesticide regulation, the withdrawal of all nematicides is highly likely.

Biofumigants

Biofumigation uses natural biocidal compounds (isothiocyanates) released into soils when glucosinolates in plant residues (usually brassicas) are hydrolysed to suppress soil-borne pests. For effective biofumigation, approximately 12 weeks' growth of the crop is required prior to maceration and incorporation into warm soils. In Scotland, this generally means the grower experiencing the economic impact of the loss of a growing season and trials to date have yet to demonstrate sufficiently beneficial reductions in *G. pallida* populations.

Trap crops

Growing a potato crop, which stimulates nematode hatch, and burning the crop down before the juveniles can mature and tubers are formed, typically after only 40 days of plant growth, can reduce PCN populations. This has been used in the Netherlands, but rarely in Scotland, due to production costs and the impact on a short growing season. *Solanum sisymbriifolium* (sticky nightshade or litchi tomato) has great potential to control PCN, stimulating hatch but preventing the nematode from completing its life cycle. Unfortunately, strains of *S. sisymbriifolium* adapted to low temperatures that will establish under Scottish conditions are not yet available.

Resistant varieties

Resistance is the most effective management tool available to control *G. pallida* in Scotland. Resistance is scored on a 1–9 scale, based on susceptibility of the tested potato cultivar relative to a standard susceptible control cultivar (reference score of 2). Scores reflect the ability of a cultivar to limit PCN reproduction to half of that of a cultivar scoring one unit less (Table 46.1). Innovator is the most widely grown *G. pallida* resistant cultivar, scoring 9, reflecting its ability to limit reproduction to less than 1% of that on a susceptible cultivar such as Maris Piper.

Using a scenario of an intolerant cultivar grown on light silt, the PCN calculator (Elliott *et al.*, 2004) can be used to show the rotation period between crops required to prevent an overall population increase following the cultivation of a potato crop over the range of resistance scores. A susceptible cultivar (score 2) would require a 13-year rotation period, compared to 5 years for a cultivar of score 5 and 1 year with scores of 7 or more. If the use of a granular nematicide with an effectiveness of 70% or 50% is factored in, rotations can be reduced further. For susceptible cultivars, nematicides have relatively minimal effect in managing PCN, although they can be effective in reducing the loss of yield. Using nematicides with moderately resistant varieties (scores 4 to 6) has beneficial effects in controlling PCN and reducing rotation periods to less than the rotation periods that are typical for ware potato production in Scotland.

In Scotland in 2019, varieties highly resistant to *G. pallida* (resistance scores of 7 to 9) were cultivated on 5% of the seed potato area but on only 0.4% of the ware potato area (Fig. 46.4). Cultivars susceptible to *G. pallida* (resistance score = 2) were grown on 86% of both seed and ware land. The overall Scottish seed crop has an average resistance score of 2.5 to *G. pallida*, whereas the ware crop has a lower average of 2.2. For comparison, the average score for resistance to *G. rostochiensis* is 5.6 for seed and 6.3 for ware. The most widely grown cultivars with

Table 46.1. Reproduction rate of *G. pallida* on varieties of different resistance scores relative to a susceptible cultivar (score 2). Rotation periods (years) required with and without nematicide treatment to prevent population increase (based on PCN calculator assuming an intolerant cultivar grown on light silt with a decline rate of 20% per annum).

Resistance score	2	3	4	5	6	7	8	9
G. pallida reproduction	100%	50%	25%	12.5%	6.3%	3.1%	1.6%	0.8%
No treatment	13	10	7	5	2	1	1	1
Nematicide – 70% control	11	7	4	2	1	1	1	1
Nematicide – 50% control	12	8	5	4	1	1	1	1

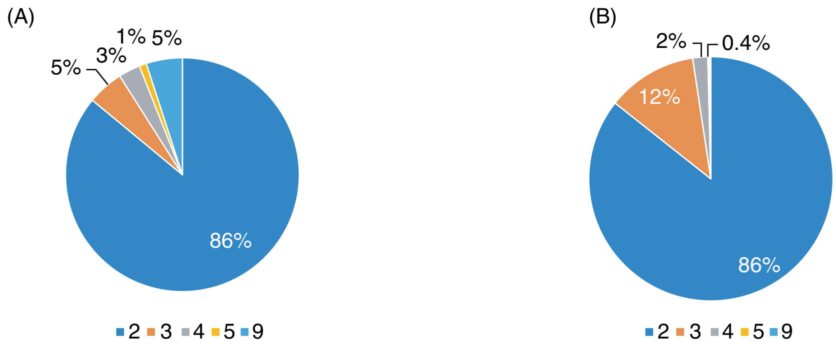

Fig. 46.4. Resistance scores to *G. pallida* of cultivars grown in Scotland for (A) seed and (B) ware in 2019. Author's own figure.

high resistance to *G. pallida* are Innovator and Arsenal, both with an end-use predominantly in the processing sector. Unfortunately, their widespread cultivation is limited by the Scottish environment, which is not suitable for producing the dry-matter content required for processing. Cultivars with lower levels of resistance to *G. pallida*, e.g. Harmony (score 4), Osprey and Vivaldi (both score 3), are more widely grown for a fresh market end-use. Highly resistant cultivars can be grown as seed crops, but this limits their cultivation to land that has passed a pre-planting soil test for PCN. The Scottish potato industry is in urgent need of commercially successful cultivars with high resistance to *G. pallida* suitable for the fresh market to increase the average resistance score of the ware crop to well above 2.2.

Optimization of nematode management

The official EU method for sampling to establish whether a field is free of PCN, outlines a standard sampling rate of 1500 ml ha^{-1} taken using 100 cores. Based on the models of Schomaker and Been (1999) describing typical aggregations of PCN, there is a detection level of 90% of finding one cyst in a population of 3.8 million cysts ha^{-1}. Therefore, it is unrealistic to expect to be able to reliably detect PCN populations at very early stages of infestation. In Scotland, where seed potato classification rules require a minimum rotation of 6 years, such population levels will not generally be reached within 30 years (five rotations) since an original introduction, even if the introduction is of around 100 cysts ha^{-1} (based on the population models developed by Elliot *et al*., 2004). Once populations have reached levels at which detection is feasible, several seed crops will already have been produced from this infested field, potentially spreading cysts into clean land, and the implementation of appropriate biosecurity measures while still of value, will be too late to contain the population to the infested field. Once populations have reached levels at which detection occurs, the land is recorded as infested until another official test has been completed and no PCN have been found. Seed potato production can then resume in this field as the population is now below the detection level, i.e. below 3.8 million cysts ha^{-1}. The continued spread of *G. pallida* in Scotland shows that the statutory measures of pre-crop soil testing of fields prior to seed potato production are not enough by themselves to control this pest. Additional control measures are required to prevent the economic impacts already described.

Widespread cultivation of cultivars with moderate or high levels of resistance to *G. pallida* that are suitable for the table and salad sectors is required to control *G. pallida*. Growers would like 'free' access to resistant cultivars; cultivars with resistance to *G. pallida* have been recently bred and are tightly controlled by the 'seed houses'. Priority should be given to the breeding of *G. pallida* resistant cultivars suitable for Scottish conditions and the development of reliable markers for resistance will allow the more rapid selection of suitable breeding material. The industry also needs to work more closely with end markets such as processors and supermarkets to increase the demand for resistant cultivars, enabling the growers to produce them profitably.

Future research requirements and outlook

If growers had access to a range of commercial cultivars with levels of resistance to *G. pallida* similar to those available for *G. rostochiensis*, then the Scottish potato industry would have sufficient tools to control this 'epidemic'. The increasing incidence of *G. pallida* and the likely withdrawal of the remaining nematicides make breeding for resistance a high priority in Scotland. Unfavourable agronomic qualities and the polygenic nature of the resistance has complicated this process, compared with breeding for resistance to *G. rostochiensis*. It is hoped that improving the efficiency of breeding programmes using molecular markers will overcome these traditional problems.

It is also essential that growers make best use of available knowledge to manage *G. pallida*. Currently too few are aware of how it multiplies, how it is spread and how to control it. This is true for ware growers who currently see little of the economic impact they will soon face, and for landowners who rent their land to potato growers.

Work is also required to develop novel control methods for *G. pallida* suitable to Scottish conditions. Biofumigants have yet to be accepted as sufficiently beneficial results have not been demonstrated here. Trap crops that are available are generally not well adapted to cooler conditions and have yet to show benefits over growing potato cultivars with dual resistance to PCN, producing a crop in its own right. Other potential control options include bacteria, fungi, potential predators and the use of chemicals from potato root diffusates to induce hatching. More work is required to find suitable alternatives to control *G. pallida* under Scottish conditions.

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47 Integrated nematode management of root-knot and root lesion nematodes in Idaho potatoes: Major economic limiting factors

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Introduction

Potato ranks in fourth place next to wheat, rice and maize among the world's food crops. Potato ranks first and third in the list of edible energy and protein production per hectare per day, respectively. Among all pests and pathogens, nematodes are one of the major limiting factors for potato production worldwide. Discovery of root-knot nematode on potato in the US dates back to 1889 when Neal reported *Meloidogyne arenaria* on potato crop in Florida. Considering the economic importance of root-knot nematodes, Idaho potato growers are advised to test their soil for *M. hapla* before planting potatoes. The earliest record of *Pratylenchus* on potato in the US was by Cobb, who found that *P. penetrans* was causing pustules over the surface of potato tubers. In the US, the potato rot nematode, *Ditylenchus destructor*, was first found in Wisconsin in 1953. Earlier, however, it was reported during 1945, that six farms in the vicinity of Aberdeen were infested with stem nematode. More than 68 species of plant parasitic nematodes belonging to 24 genera are associated with potato fields from different parts of the world. Among all, two groups of nematodes are important in potato production in Idaho. These include root-knot nematodes (*Meloidogyne* spp.) and root lesion nematodes (*Pratylenchus* spp.).

Symptoms of damage of root-knot nematodes

In Idaho, root-knot nematodes (*Meloidogyne* spp.) have been recognized as a major nematode pest on potato and are found in abundance especially in sandy soils. Females feeding in the tubers and the development of live young cause enlargement or bumps in the outer layers of the tubers (Fig. 47.1) rendering them useless for either fresh packing or processing. They have a wide host range leading to population increases when other susceptible crops are grown in rotation with potato. Damage by *Meloidogyne hapla* is usually most severe following lucerne hay crops and during years with high spring temperatures. They cause field damage that is localized, usually in circles of various sizes, or spread throughout an entire field with plants becoming chlorotic and stunted. Damaged roots are not able to obtain soil nutrients and symptoms appear as nitrogen or micronutrient deficiencies. Plants

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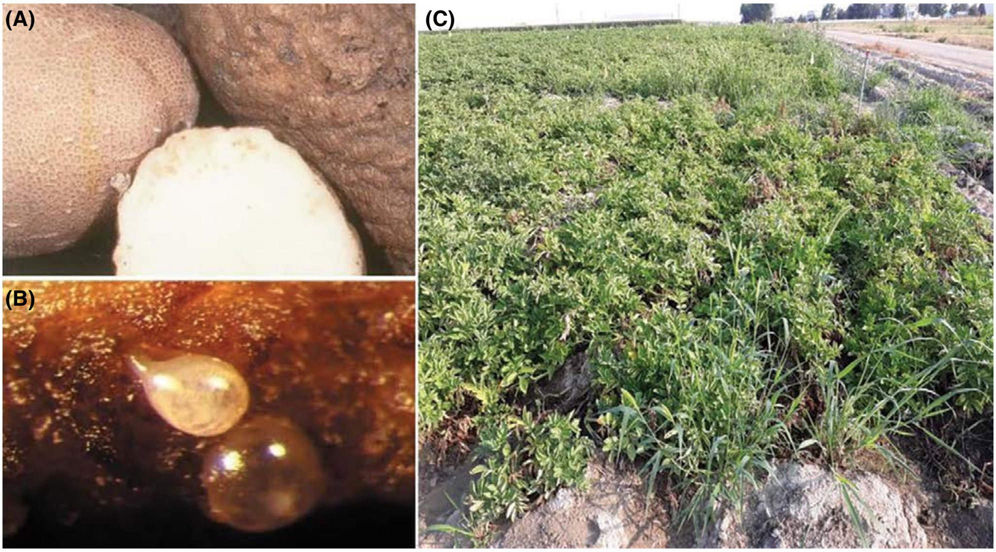

Fig. 47.1. Photo micrograph illustrates symptoms of root-knot nematode infested tubers (**A**), root-knot nematode females dissected from galled root tissue (**B**), and aboveground symptoms of root-knot nematodes in the field (**C**). Photographs courtesy of J.D. Eisenback.

may wilt easily, especially in warm weather, due to root damage even though soil moisture may be adequate.

Distribution of root-knot nematodes

Although there are several species of root-knot nematodes, the two most common on potato in Idaho and eastern Oregon are the Columbia root-knot nematode (*M. chitwoodi*) and northern root-knot nematode (*M. hapla*). *Meloidogyne chitwoodi* was first described on potato in Quincy, Washington, and later in Iron County, Utah. Both species can attack potato and reports of root-knot nematode damage on potato have increased during the past several years.

Host range of root-knot nematodes

Susceptible crops for root-knot nematode include lucerne (*M. hapla*), wheat (*M. chitwoodi*), and other crops that are commonly grown in rotation with potato in Idaho and eastern Oregon and Washington. Three races of *M. chitwoodi* are reported based on their differential reaction and ability to reproduce on different host crops. No host races of *M. hapla* have been reported in Idaho. Pathogenicity studies of *M. chitwoodi* under controlled conditions proved that maize is a better rotation crop than wheat, barley or oats for the susceptible potato crop in the Pacific Northwest. Further studies revealed that *M. chitwoodi* reproduced on 53 of 68 plant species tested under glasshouse conditions. Root-knot nematode populations can increase dramatically when susceptible crops are grown in rotation with potato. Another new species, *M. cruciani* was reported from tomato in the US Virgin Islands, for which potato is also a host.

Recommended integrated root-knot nematode management

Temik offers valuable suppression of root-knot nematode species. If root-knot nematode is a severe economic pest, the use of other nematicides such as metam sodium, Telone II, or MOCAP should also be employed. In University of Idaho studies, *M. chitwoodi* infected plots with Vydate treatment yielded significantly greater tuber weights than MOCAP-treated plots. Maximum and minimum percentages of grade 1 and grade 2 tubers occurred in plots treated with Vydate and MOCAP, respectively. Further, it was found that application of MOCAP either during autumn or spring (2 or 1.5 gallons) along with Vapam (40 gallons) significantly reduced the nematode infested potatoes as compared to the untreated check. The percentage of nematode infestation reduced to 0.2% and 2.7% as a result of autumn or spring application, respectively, with a significant increase in clean and market yield (Hafez and Palanisamy, 2003).

Green manure application along with MOCAP at three nitrogen fertilizer rates revealed that nitrogen application significantly increased tuber yields as compared to treatment without nitrogen. Maximum yield was recorded at nitrogen levels of 760 kg/ha followed by 1521 kg/ha. Among all green manure crops, maximum potato yield was obtained from rapeseed plots, though it was on par with yields obtained from oil radish and buckwheat plots. Application of MOCAP using three application methods indicated that shank-applied and incorporated MOCAP (14 l/ha) plots yielded significantly higher than those treated with the other rates and two methods. Experiments conducted at microplot and field level confirmed that rapeseed 'Humus' and oil radish *Raphanus sativus* reduced *M. chitwoodi* population and increased the potato tuber yield (106–185%) and quality under Idaho conditions (Hafez and Palanisamy, 2001, 2003). Further, it was confirmed that addition of the bacterium, *Bacillus megaterium,* along with rapeseed 'Humus' or oil radish increased the yield and quality of potato tuber and suppressed the population of both *M. chitwoodi* and *M. hapla* under greenhouse and field-microplot conditions (Al-rahiayani *et al*., 1999). The addition of ethoprophos along with rapeseed considerably increased the grade 1 potato yield. Improved efficacy may result from the identification of rotational crops like wheat that is resistant to or a non-host for *M. hapla*. Utilization of such resistant cultivars can reduce nematode survival thereby reducing nematode damage and increasing yield potential.

It was found that of the 800 clones of potato screened for resistance to *M. incognita acrita* under glasshouse conditions, one clone was a non-host, with a root-gall score of zero. The potato cv. BelRus is medium late in maturity and highly resistant to northern root-knot nematode. The occurrence of resistance to races 1 and 2 of *M. chitwoodi* in the F1 hybrids indicates success in the first step of introducing resistance to *M. chitwoodi* into the cultivated potato gene pool. It is anticipated that in the very near future the resistance identified in the present experiments will be transferred to commercial potato cultivars. to be used in the lowland tropics or wherever *Meloidogyne* spp. are of economic importance.

Distribution of root lesion nematodes

Although more than 15 species of root lesion nematodes are reported to attack potatoes, *Pratylenchus neglectus* is the predominate lesion nematode species in Idaho.

Symptoms of damage of root lesion nematodes

Infected portions of the potato roots turn dark brown to reddish in colour and are susceptible to invading secondary pathogens. Lesions on the tubers are usually shallow but sometimes penetrate deeper. In addition to directly causing damage to the potato crop, *P. neglectus* is responsible for potato early dying (PED). Premature death and yield reduction of 30% (6–12 t/ha) due to PED have been documented in Idaho, New York, and Ohio. Potato plants with PED are characterized by stunted growth, chlorotic foliage, deterioration of roots, premature senescence and reduced yields (Fig. 47.2).

Interactions of root lesion nematodes with other pathogens

In Idaho, root lesion nematodes are of concern to potato growers because they reduce yield indirectly by weakening and increasing stress on the plants and by making them more susceptible to fungal and bacterial diseases. There can be a direct relationship between root lesion nematodes and the incidence of *Verticillium* wilt (early die). Two species of root lesion nematode, *P. neglectus*

Fig. 47.2. Root lesion nematode interaction with *Verticilium dahliae* on healthy potato (left) and damaged potato (right). Photograph courtesy of J.D. Eisenback.

and *P. penetrans*, have been shown to increase susceptibility of potato plants to the potato early die complex. *Pratylenchus penetrans* interacts strongly with the fungus pathogen *Verticillium dahliae*, the main cause of potato early die. *Pratylenchus neglectus* is not known to interact directly with *Verticillium*; however, high populations may be associated with other factors that reduce optimal growth, contribute to crop stress, and increase the incidence and severity of potato early die (Hafez *et al.*, 1999).

Reports of a lower incidence of *Verticillium* wilt in potato fields treated with nematicides have provided supportive evidence for the existence of a *Pratylenchus* and *Verticillium* interaction (Fig. 47.3). The infestation of soil with both pathogens resulted in a higher incidence of symptoms expression than when either pathogen is present alone. In a microplot study, it was established that PED can result from synergistic interaction of *Pratylenchus* and *V. dahliae* at population levels commonly found in Ohio soils. It was found that *P. crenatus* did not interact with *V. dahliae* in either year of the tests and *P. scribneri* did not interact with *V. dahliae* in the first year, but it did in the second year when high population levels were used. These results were attributed to high temperature stress during suberization in the second year. Other field studies showed that PED was severe where *P. penetrans* predominated and less severe where *P. crenatus* predominated. Low and high initial populations of *P. penetrans* or *V. dahliae* had no effect on number and fresh weight of tubers, whereas combined infestations of both organisms reduced the specific gravity and yields up to 20%. In another study, up to 50% yield losses of Superior potato resulted from initial populations as low as three *P. penetrans* per 100 cm^3 of soil and one microsclerotium of *V. dahliae* per gram of soil. Differences in the synergistic effect were probably due to the moderate resistance of Russet Burbank to *V. dahliae* as compared to the highly susceptible cultivar Superior.

The most commonly occurring root lesion nematode in Idaho is *P. neglectus*. The nature of *Pratylenchus–Verticillium* interaction in PED is not fully understood. Two theories were proposed in the literature. Firstly, the root wounding by *Pratylenchus* feeding provides entry into the root for *Verticillium*, thus bypassing host defences to disease. Secondly, the interaction between the two pathogens involves modification of host plant physiology. The fact that some species of *Pratylenchus* and not others interact with *Verticillium*, even though all can enter and injure the roots while feeding, weakens the root-wounding theory. In contrast, the second theory seems to have been strengthened, especially as interaction between fungal and nematode pathogens has been observed while the pathogens were physically separated in split root studies

Fig. 47.3. Lesion nematode interaction with *Verticilium dahliae* in the field. Photograph courtesy of J.D. Eisenback.

(Al-rehiayani *et al.*, 1999). Recent histological studies also supported the second theory. Researchers used immunostaining techniques to study the nature of interaction between *V. dahliae* and *P. penetrans* or *P. crenatus* and found that infection of potato by *V. dahliae* primarily occurs through root tips and is not associated with feeding sites or wounds caused by either *P. penetrans* or *P. crenatus*. However, increased vascular colonization by *V. dahliae* of both roots and stems was observed in the presence of *P. penetrans*, compared with treatments with *V. dahliae* alone or with the presence of *P. crenatus*. This suggests that *P. penetrans*, but not *P. crenatus*, may alter the timing of any physiological response in the root tip and that could promote entrance of *V. dahliae* into the vascular system.

Recommended integrated root lesion nematode management

Once these two nematodes are detected after diagnosis, growers practice crop rotation and apply nematicides as a way of suppressing their population build-up. Here are some of the recommended integrated nematode management practices.

Crop rotation and green manure crops

Crop rotation is an effective way for the management of nematodes under field conditions. Potato tuber yields were higher in rotational sequences that began with wheat or barley than in the sequences that began with potato or soybean. Planting a green manure crop before potatoes is an effective way to manage nematodes under field conditions. Green manure crops of oil radish and barley result in decline of *P. neglectus* population densities and tuber yield increases (Al-rehiayani *et al.*, 1999). Green manure crops of oil radish, barley, velvet bean and buckwheat prior to potato resulted in decline of *M. chitwoodi* population densities and tuber yield increase compared to fallow (Hafez and Palanisamy, 2003). Maximum yield followed barley while minimum soil and root population of both nematode species was observed in velvet bean plots.

Nematicides

Nematicides are the first line of defence for root-knot and root lesion nematode management. Temik reduced the numbers of root lesion nematodes in roots with concomitant yield increases in potato and soybean. Efficacy of fosthiazate was compared at 1.0, 1.5 or 2.0 lb/acre banded in-furrow or 7.5EC at 2.0, 4.0 or 6.0 lb/acre broadcast with Temik® at 3.0 lb/acre for control of *P. penetrans* on potato. All treatments provided significant control of nematodes in the roots and the highest rates significantly reduced nematode populations in the soil.

The application of ethoprophos along with green manure significantly increased the tuber yield and grade 1 and 2 tubers as compared to fallow. Invasion of newly reclaimed fields, as well as experimental infection of potato plants by *Pratylenchus coffeae*, were studied at seven locations in Nagasaki Prefecture, Japan. It was concluded that *P. coffeae* may appear in less than 6 years after reclamation, and that rotting of tubers by the nematode may occur within 10 years. Temik is a systemic product that is highly effective in controlling root lesion nematodes that feed inside the root system (endoparasites). Fumigants are not as effective because they do not kill eggs in the soil and do not provide significant residual control. Growers that fumigate should sample for nematodes prior to planting (no earlier than three weeks following spring fumigation) and may use Temik to reduce nematode populations if they remain high. Temik applied at planting remains in the root system and soil profile for 6–8 weeks. When eggs hatch, this long residual results in nematode exposure and subsequent control.

Research at University of Idaho shows that controlling root lesion nematode populations has a positive impact on tuber yield. Twenty pounds of Temik applied at planting significantly reduced root lesion populations and increased tuber yield an average of 38.3 cwt/annum. Years of research and grower experience along the Snake River Plain of southern Idaho have also proven that Temik applications often result in early die suppression with accompanying yield response. These results may be due to several positive effects caused by Temik, including lesion nematode control, impact on the development of *Verticillium* microsclerotia, or plant growth regulatory effects. Early die suppression was documented during the 1997 growing season in the Pacific Northwest trials where foliar insects were not present at levels sufficient to cause economic damage.

Fumigation with metam sodium or Telone® II is usually effective against lesion nematodes. Growers who fumigate should sample for nematodes after fumigation (at least 3 weeks after fumigation) but before planting and may use Temik to reduce nematode populations if they remain high. Temik applied at planting remains in the root system and soil profile for 6 to 8 weeks. When eggs hatch, this long residual results in nematode exposure and subsequent control.

Future research requirements

Additional research is needed into the interaction of the lesion nematode with *Verticillium* wilt and sudden death of potato. There is still some uncertainty as to how important lesion nematodes are with respect to early dying problems in Idaho. Much of the work that showed a relationship with *Verticillium* was done with *P. penetrans* rather than *P. neglectus*. However, reports of a lower incidence of *Verticillium* wilt in potato fields treated with nematicides have provided indirect evidence for the existence of a *Pratylenchus* and *Verticillium* interaction. The infestation of soil with both pathogens resulted in a higher incidence of symptoms expression than when either pathogen is present alone.

There needs to be more effort put into understanding the impact of this unique interaction on potato production.

Outlook: anticipating future developments

- Nematology positions are in jeopardy and a failure to fill them will negatively affect plant health standards.
- There will be a loss of important nematicides soon, alternatives are urgently needed.
- Climate change will impact both the duration of the nematode life cycle and nematode interactions with pathogens.
- There is a direct need to look at means of adapting production systems to climate change in the future. These models should be developed now and not when it is too late.

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48 Integrated management of *Meloidogyne chitwoodi* and *M. fallax* in potato: A complicated agronomical puzzle in the Netherlands and Belgium

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Introduction

Meloidogyne chitwoodi and *M. fallax* have been important nematode problems in arable farming in the Netherlands and Belgium ever since their detection in the 1980s. Their quarantine status and the damage inflicted on product quality in important cash crops such as potato, carrots, black salsify and gladiolus has increased drastically the need for integrated nematode management strategies that prevent yield losses and further spreading.

Economic importance

The Netherlands is a major producer of ware, starch and especially seed potatoes. Belgium is the largest exporter of frozen potatoes in the world (www.fao.org/faostat, accessed 10 November 2020). *Meloidogyne chitwoodi* and *M. fallax* occur in several potato growing areas in the Netherlands and Belgium and can cause severe quality damage rendering them unsuitable for the market. Due to the EU quarantine status of these root-knot nematodes, potato seed tubers have to be free from infection by these two species before being allowed to enter EU traffic. They are a major threat to seed potato production in the Netherlands.

External galling of potato tubers caused by infection of *M. chitwoodi* or *M. fallax* reduces the commercial value of ware potatoes. The extent of this varies depending on the degree of deterioration and what the potato market will accept. Seed potatoes must be free of *M. chitwoodi* and *M. fallax*. In the event of an infection, the phytosanitary certificate is refused and the potato lot can only be sold as ware potatoes when not highly deteriorated or for cattle feed. As such, more than 50% of its economic value is lost. Estimates on economic losses due to root-knot nematodes in ware and seed potatoes are complex and accurate data on the actual financial damage caused by *M. chitwoodi* and *M. fallax* are difficult to obtain. However, economic losses most probably exceed €1 million per year. *Meloidogyne chitwoodi* and *M. fallax* do not cause any economic loss in the production of starch potato because these nematodes only inhibit growth at very high densities and seldom reduce overall yield (galling of tubers is not a quality issue in

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starch potato). However, in Belgium, the phytosanitary measures implied make it impossible to grow potatoes when *M. chitwoodi* or *M. fallax* are detected in a field regardless of the ultimate market targeted.

Economic losses due to the two species is not limited to quality reduction of potato tubers but also to infections on other rotation crops. Other crops in rotations can be seriously infected (carrot, black salsify) and leads to the need for nematode management and intensive soil sampling for detection that generates extra production costs for growers (Wesemael *et al.*, 2011).

Host range

Meloidogyne chitwoodi and *M. fallax* are closely related species and have a broad host range of monocot as well as dicotyl crops, including several major cash crops, cover crops and weeds (Den Nijs *et al.*, 2004; Rich *et al.* 2009). When planning a crop rotation it is also important to be aware of the difference between the host range of *M. chitwoodi* and *M. fallax*. Sugar beet, for example, is a very good host for *M. fallax* but a rather poor host for *M. chitwoodi*. Detailed information can be found in the databases of the EU project Best4Soil (www.best4soil.eu/database, accessed 12 November 2020).

Distribution

Meloidogyne chitwoodi was first detected in the Netherlands in the 1980s. A review of old illustrations and old specimens of *Meloidogyne* suggests that it was probably already present in the 1930s. *Meloidogyne fallax* was detected for the first time in 1992 in a field near Baexem (NL) and described as a new species. In Belgium, *M. chitwoodi* and *M. fallax* were detected for the first time in 1996 but the presence of *M. chitwoodi* in oak forest soil, combined with the relatively high genetic distances between populations, also suggest a longer presence (Waeyenberge and Moens, 2001). In general, *Meloidogyne* spp. occur on a wide range of soil types, but in the Netherlands, *M. chitwoodi* and *M. fallax* are mainly found on sandy, peaty and light marine clay soils. In Belgium, both species are mostly found on sandy soils.

In a survey undertaken in 2006, *M. chitwoodi* and/or *M. fallax* were found in about 20% of the soil samples taken from sandy and peaty soils in the eastern region and sporadically in samples taken from the light marine clay soils in the western region of the Netherlands. A survey in vegetable and potato growing areas in Flanders in 1996–1997 showed that in 1% of the 2877 samples, *M. chitwoodi* and/or *M. fallax* were present (Waeyenberge and Moens, 2001). Since then, more findings have been reported in Belgium and about one out of three fields sampled for carrot or black salsify production are found to be infested in some areas.

Symptoms of damage

Meloidogyne chitwoodi symptoms are very similar to those of *M. fallax*. Both species can infect roots (Fig. 48.1) and tubers (Fig. 48.2). Infected roots show small galls, typically without secondary roots as is the case with *M. hapla*. The spherical bodies of females may protrude from the root surface of small rootlets surrounded posteriorly by a large egg-filled sac which becomes dark brown with age.

Economically, the most significant form of damage due to *M. chitwoodi* and *M. fallax* is

Fig. 48.1. Galling of potato roots caused by *Meloidogyne chitwoodi.* Photograph courtesy of Wageningen University & Research, Field Crops.

Fig. 48.2. Deformation of potato tubers caused by *Meloidogyne chitwoodi*. After peeling, egg masses (brownish spots) become visible. Photograph courtesy of Wageningen University & Research, Field Crops.

quality loss due to tuber infection. Seed potatoes must be free of these quarantine species and can only be grown on fields free of *M. chitwoodi* and *M. fallax*. The development of external symptoms varies with cultivar and infection level. Low pre-plant densities of less than 10 juveniles per 100 ml of soil can cause a total yield loss due to tuber quality defects. In some cases, tubers may be heavily infected without visible symptoms. Only at very high pre-plant densities of >1000 juveniles per 100 ml of soil, growth and total yield is slightly reduced. In general, no yield losses of ware potatoes have been reported in the Netherlands or Belgium caused by *M. chitwoodi* or *M. fallax*.

Biology and life cycle

Meloidogyne chitwoodi and *M. fallax* are sedentary endoparasites. Their life cycle is comparable with most of the *Meloidogyne* spp. Under favourable conditions the life cycle of *M. chitwoodi* and *M. fallax* takes 6–8 weeks. Soil temperature has a major influence on development and reproduction and hence the number of generations per year. Hatching starts at a soil temperature of 5°C and optimum temperature for reproduction is around 20°C (Khan *et al.*, 2014). In the Netherlands and Belgium, *M. chitwoodi* and *M. fallax* can complete two and sometimes three generations per year. In the absence of a host plant the population density of both *M. chitwoodi* and *M. fallax* decreases substantially and a decline of up to 95% has been reported especially during warm summers. During winter *M. chitwoodi* and *M. fallax* survive as juveniles in eggs, which has shown to be an important survival strategy.

Interactions with other nematodes and pathogens

Although root-knot nematodes have been reported to interact with bacterial wilt, *Pseudomonas solanacearum* and *Erwinia* spp. and *Streptomyces scabies*, and fungi such as *Verticillium* spp., *Fusarium* spp. and *Rhizoctonia solani* (Manzanilla-Lopéz *et al.*, 2009) on potato elsewhere, interactions between *M. chitwoodi* or *M. fallax* and other pathogens in potato fields in the Netherlands and Belgium are not known.

Recommended integrated nematode management (INM)

A nematode control strategy, as part of an integrated crop management approach, has been promoted in the Netherlands since the end of the 1990s (Molendijk and Mulder, 1996). This INM strategy was developed to reduce the use and dependency on chemical nematicides and is based on four major pillars: prevention, inventory, crop rotation and supporting measures. In this integrated management strategy nematicides are only applied when necessary as a last resort.

Prevention

It is of great importance to avoid introduction of *Meloidogyne* infestations because damage thresholds are very low (zero tolerance for seed potatoes) and infestations are very hard or nearly impossible to eradicate.

Meloidogyne chitwoodi and *M. fallax* can be spread by infested soil attached to agricultural machinery and to propagation material or by infected planting material such as seed potatoes and flower bulbs (e.g. gladiolus and dahlia). Certified planting material and strict farm hygiene practices are needed to prevent spread of these quarantine organisms.

At the processing plants, tonnes of waste soil are collected and it is recommended that all the waste soil is brought back to the field where it came from but in practice this is almost impossible. The waste soil has to be treated to make sure it is free of this quarantine species. Inundation of waste soil is an effective option.

Inventory

In order to be able to take appropriate control measures, it is imperative to determine nematode species and population densities of a field by soil sampling. In general, soil samples are taken randomly in the upper 25 cm of the soil, collecting approximately 1.5 L soil per hectare. Detection levels increase strongly when samples are taken when the population is expected to be highest either after harvest of a good host and before the middle of November, when populations begin their natural decline. A more sensitive detection method, mainly used by seed potato growers, is a bioassay. Approximately 50 L of soil is taken randomly from 0.33 ha, homogenized and put in a container in which a sensitive potato cultivar is grown. After 3 months, newly formed tubers and soil are inspected for the presence of *M. chitwoodi* and/or *M. fallax*. Field inspection of roots and tap roots of intolerant host crops like carrot or black salsify also may reveal the presence of *M. chitwoodi* and *M. fallax.* It is important to know which *Meloidogyne* species is present because this will determine the options for control with crop rotation.

Crop rotation

Feasible crop rotation strategies to reduce population densities of *M. chitwoodi* and *M. fallax* to levels below the damage threshold of the succeeding crop are limited because of the wide host range of both species. The host suitability and tolerance of cash and cover crops for *M. chitwoodi* and *M. fallax* is shown in Fig 48.3.

Chicory and flax (*Linum usitatissimum)* are non-hosts, peas and barley are poor hosts. Many common bean cultivars are non-hosts for *M. fallax*, but for *M. chitwoodi* this strongly depends on the cultivar. Recently, breeders of sugar beet developed a *M. chitwoodi* resistant sugar beet cultivar.

Growers can also include short season crops like lettuce and spinach in their rotation to reduce population densities of *M. chitwoodi* and *M. fallax*. These are good hosts for *M. chitwoodi* and *M. fallax* and act like trap crops. The nematodes penetrate and start to develop but the crop is harvested and roots are destroyed by soil cultivation before the nematodes can complete their life cycles.

Potato cultivars differ in tolerance for tuber damage symptoms. Therefore, it is recommended that farmers do not grow intolerant cultivars like ‘Hansa’ or ‘Asterix’. At low to moderate infestation levels (<100 juveniles/100 ml soil) it is possible to harvest marketable tubers of more tolerant cultivars such as ‘Donald’ or ‘Première’. Several Dutch breeders are currently developing potato cultivars with resistance against *M. chitwoodi*. These cultivars will be available within a few years and have a very high level of root and tuber resistance (no reproduction) combined with tolerance (no symptoms). *Meloidogyne chitwoodi* resistant fodder radish cultivars (*Raphanus sativus,* Teklu *et al.*, 2014) are recommended as a cover crop after cereals, seed potato or carrots production (www.aaltjesschema.nl and www.Best4soil.eu, accessed 12 November 2020).

In general, the extent of control of *M. chitwoodi* and *M. fallax* by crop rotation is insufficient for seed potato growers because of the zero tolerance needed.

Weed control is of major importance for zero tolerance when a non-host or poor host cash crop is grown for nematode control. Many weeds are good host plants (Rich *et al.*, 2009) and will diminish the effect of the poor/non-host crop on nematode population reduction.

Additional measures

Nematicides

The fumigant Monam (metam sodium) and some non-fumigant granular nematicides (Vydate, Nemathorin, Velum) are registered in the Netherlands and/or Belgium for control of *Meloidogyne* spp. in potato. Due to imposed restrictions

	Root-knot nematodes: *Meloidogyne chitwoodi* Columbia root-knot nematode (1 2 3 4 5)	Root-knot nematodes: *Meloidogyne fallax* False columbia root-knot nematode (1 2 3 4 5)	
Potato	●●●	●●●	Potato
Beet (sugar, fodder)	●	●●●	Beet (sugar, fodder)
Maize (corn)	●●	●	Maize (corn)
Wheat	●●	●●	Wheat
Lucerne (= alfalfa)	●	?	Lucerne (= alfalfa)
Chicory	-	?	Chicory
Carrot	●●	●●●	Carrot
Black salsify	●●●	●●●	Black salsify
Beans	- R	-	Beans
Onion	●	●	Onion
Japanese/Black oat	●●●	?	Japanese/Black oat
Italian ryegrass	●●●	●●●	Italian ryegrass
Vetch	● R	●●●	Vetch
Radish	- R	●● R	Radish

Legend damage	
(white)	Unknown
(green)	None
(yellow)	Little (0–15%)
(orange)	Medium (16–35%)
(purple)	Serious (36–100%)

Legend propagation	
--	Active decline of population
?	Unknown
-	Non host
●	Poor host
●●	Moderate host
●●●	Good host
R	Variety dependent
S	Serotype dependent
i	Some information

Fig. 48.3. Nematode scheme; host suitability and tolerance of cash and cover crops for *Meloidogyne chitwoodi* and *M. fallax*. Figure courtesy of Wageningen University & Research, Field Crops.

and the requirement of sealing the soil surface with virtually impermeable film to avoid evaporation, the use of Monam is no longer an economically feasible alternative for potato growers. Populations of *M. chitwoodi* and/or *M. fallax* can be reduced strongly by fumigation but it does not eliminate populations and therefore fumigation is an insufficient control measure for growers of seed potatoes. The granular nematicides act as nematostatics, paralysing the nematode for short period of time. These nematicides, when broadcast applied, can reduce galling to an acceptable level but have only a limited effect on reproduction. In-furrow application is mostly insufficient to achieve the required control.

Anaerobic soil disinfestation (ASD)

ASD refers to the incorporation of a large amount (>40 t/ha) of easily decomposable organic amendments, with supplemental irrigation and subsequent covering the soil with an airtight foil. The complete depletion of oxygen within 24 hours after tarping (Blok *et al.*, 2000) and the volatile compounds produced during ASD are lethal for several soil-borne fungi and plant parasitic nematodes such as potato cyst nematodes (*Globodera* spp.), root lesion nematodes (*Pratylenchus penetrans*) and *Meloidogyne* spp. (Lamers *et al.*, 2010). *Meloidogyne chitwoodi* populations can be reduced by more than 95%. Due to the high cost of ASD of approximately €4000/ha, ASD is still not an economic alternative method for ware potato growers. In addition, because populations will not be fully eliminated by ASD, this method is not suitable for the zero tolerance needed by seed potato growers.

Inundation (flooding)

Flooding fields for 12 to 14 weeks at soil temperatures above 16°C is very effective for control of several soil fungi, weeds and nematodes. Several field experiments in the Netherlands on marine clay soils showed that *M. chitwoodi* could be eliminated by inundation for 14 weeks during summer. Inundation, originally used by bulb growers to control the stem nematode *Ditylenchus dipsaci*, is now adapted by seed potato growers to sanitize *M. chitwoodi* infested fields. The disinfestation process with inundation can be accelerated by addition of organic material.

Optimization of nematode management

In addition to nematodes, the farmer has to consider all elements of soil quality in his fields. Therefore, the INM tools used for nematode control must not adversely affect soil quality. Optimizing nematode management will only be achieved when an integrated approach including soil quality becomes realistic. In the era of precision farming, big data and computer technology this overall management is on the horizon.

Implementation of a boost year could be a great help in optimizing INM. A boost year means eliminating all cash crops from the rotation and using multiple inputs to improve soil quality, including nematode control. Farmers in the north-east of the Netherlands exchange their barley crop for a clean fallow until June to control volunteer potatoes and other weeds in April/May. In June they sow marigolds (*Tagetes patula*) to control the root lesion nematode *Pratylenchus penetrans.* Long-term field experiments showed that the increase of potato and sugar beet yield made this boost year very profitable (Chapter 40).

Most major cash crops are moderate to good hosts for *M. chitwoodi* and *M. fallax*. Breeding for resistance in major cash crops like maize, cereals and legumes would enhance the opportunities to control *M. chitwoodi* and *M. fallax* by crop rotation in the potato crop.

Future research requirements

To improve nematode support systems used by growers when setting up their crop rotation, quantitative research on reproduction (population development) and tolerance (relationship between initial population and yield) of major cash crops and cultivars is needed. Methods for disinfestation of propagation material and economically applicable sanitation techniques for waste products (soil and water) in the processing industry and tare soil of potato storage need to be developed to minimize the spread of *M. chitwoodi* and *M. fallax*. As the damage threshold of many crops for *M. chitwoodi* and *M. fallax* is very low, research to improve detection techniques by increasing the detection limit, will prevent unexpected damage and economic losses.

Outlook: anticipating future developments

Climate change will lead to warmer summers and milder winters. This may lead to the introduction and spread of *Meloidogyne* spp. from warmer climates in southern Europe to the more temperate regions in the north. The presence of root-knot nematodes in greenhouses is also a focal point for spread. Increasing temperatures will also have an effect on population dynamics. Most likely, more generations will be formed during the cropping season. If increased soil temperatures during winter lengthen the activity of nematode antagonists of *M. chitwoodi* and *M. fallax* this might lead to a stronger winter decline. Very mild winters could cause an increase in root-knot on surviving weeds and volunteers of host crops.

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49 Economic importance of the potato tuber nematode *Ditylenchus destructor* in Russia

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Introduction

Ditylenchus destructor Thorne, 1945, the potato tuber nematode (PTN), is ranked second only to the potato cyst nematode, *Globodera rostochiensis* in importance in Russia. *Ditylenchus destructor* is distributed throughout the former Soviet Union, now the Russian Federation, but has had no significant economic impact in the past. This historically low impact was due to the fact that around 80–90% of potato tuber yield had been produced on small private gardens or fields of approximately 600 square metres. Potato tubers in these smallholder fields, when diseased with PTN rot, are sorted out and discarded by hand. This reduced the overall spread of PTN within and outside the region.

Economic significance

Many farmers today have potato fields with an area of 1000–5000 ha. The common problems these large holder farmers face are associated with quality control of potato seeds, lack of proper crop rotation, and a lack of access to suitable management technologies.

Consequently, the distribution and level of damage caused by the PTN has increased each year since 2010. At present, it is estimated that around 40,000 ha of industrial potato cultivation are highly infested with *D. destructor*. Harvested potato tubers usually contain up to 5% infected tubers. PTN infections can be elevated by excessive irrigation resulting in high moisture levels, thereby increasing the percentage of infected tubers to 10% or more (Shesteperov *et al.*, 2018). In 2015-2019, fields in the Central and Volga regions of Russia lost around 30% of yield due to PTN infected tubers. In rare cases, the number of infected tubers can reach 80% in which case potato harvesting may be discontinued.

Host range

The PTN, like other stem nematodes in the genus *Ditylenchus*, has a wide host range of between 70 to 150 plant species. Hosts include many major crops plants, flowers, shrubs, wild grasses and weed species. It is difficult to develop an effective crop rotation for control of PTN, because in addition to potato, this nematode can reproduce on important crops such as garlic, onion, oat, maize, sunflower, red beet,

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sugar beet, carrot, lupin, pea, lucerne and clovers. *Ditylenchus destructor* can infect roots and stems of tomatoes, aubergines, pepper, cucumber, pumpkin, melon, strawberry and many other vegetables.

Distribution

During 2010–2020, *D. destructor* was reported in the territory of 25 regions of the Russian Federation where potato production is present (Fig. 49.1). However, the real distribution is probably more extensive and requires further investigations.

Biology and life cycle

Ditylenchus destructor is a migratory facultative endoparasite of roots and underground parts of plants such as potato tubers. They enter potato tubers through the lenticels, begin to multiply rapidly and invade the whole tuber. They can continue to live and develop within harvested tubers. Without a host plant, *D. destructor* may multiply by feeding on roots of alternative weed hosts or on mycelia of soil fungi and wild mushrooms (Goodey *et al.*, 1965). The PTN multiplies best in soil with high levels of soil moisture. At soil moisture levels of 40%, about 10% of tubers are infected by *D. destructor*; with a soil

Fig. 49.1. Regions of the Russian Federation with known infection of *Ditylenchus destructor*. Author's own figure.

moisture of 60%, up to 60% are infected; and at a soil moisture of 80%, up to 90% (Dekker, 1972). In soils with constantly changing moisture levels during the potato growing season, tuber rot caused by *D. destructor* increases to a greater level versus that seen in controlled irrigation with stable levels of soil moisture. During wintertime, *D. destructor* survive as adults, juveniles or eggs in infected unharvested potato tubers as well as roots of agriculture crops or weeds.

Symptoms of damage

Potato tuber nematode infects all tissues of potato plants below the soil level. Potato tubers contain a large amount of readily available nutrients that result in rapid PTN reproduction and accumulation of large population densities that lead to destruction of the tuber tissue (Fig. 49.2). Early infections can be detected by peeling the tuber, which can reveal small, off-white spots in the otherwise healthy flesh. These later enlarge, darken, become woolly in texture and may be slightly hollow at the centre. On heavily affected tubers, there are typically slightly sunken areas with cracked and wrinkled skin that is detached in places from the underlying flesh. The flesh has a dry and mealy appearance, varying in colour from greyish to dark brown or black (CABI Invasive Species Compendium, 2020). However, the symptoms of 'dry tuber rot' caused by PTN are very similar to symptoms caused by some parasitic fungi (e.g. *Fusarium*, *Verticillium*, *Phoma*) making identification of *D. destructor* infections in farmers' fields difficult.

Interactions with other nematodes and pathogens

There is no proof of consistent synergetic effects of *D. destructor* with other phytopathogenic organisms associated with potato tuber rot. Around 20–25% of potato tuber rot is caused by invasion of *D. destructor* alone. Other forms of potato tuber rot are caused by mixed invasion with microorganisms. *D. destructor* usually enters the potato tuber through the young healthy tuber skin or through opened lenticels after which they begin to multiply rapidly within the invaded tissue. This form of infection with the PTN alone develops only if the tuber's skin is not broken (see Fig. 49.2).

If the skin of the potato tuber is cracked, other soil-borne microorganisms invade the tuber tissue. There are a number of soil facultative fungi such as *Fusarium*, *Verticillium* or *Alternaria* that are associated with this type of infection. These fungi colonize the tuber tissue remaining after nematode feeding. In other cases, the tubers

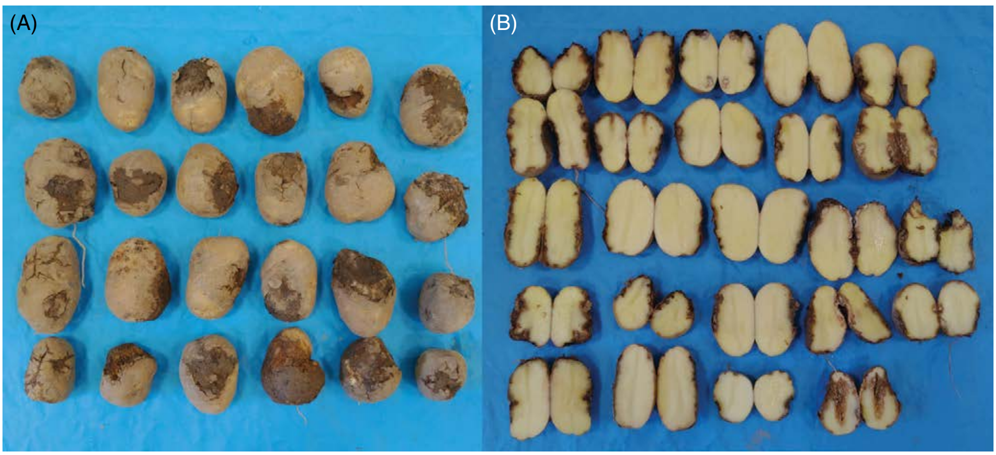

Fig. 49.2. Symptoms of dry tuber rot damage caused by the potato tuber nematode, *Ditylenchus destructor*: (**A**) surface cracking of potato skin and (**B**) damage to the potato due to nematode penetration of the pulp. Author's own photographs.

with *D. destructor* are affected by soil bacteria and fungi (Fig. 49.3). In this case, wet or soft tuber rot develops.

Recommended integrated nematode management (INM)

Some recommended strategies of integrated management of the PTN are published in scientific and applied literature in the Russian Federation. However, choosing the right strategy for control of PTN on smallholder private fields lies totally on the farmer. The two strategies of management outlined below are designed to reduce the spread and damage caused by the PTN.

Smallholder farmers

The first strategy is acceptable to most smallholder farmers that produce potatoes for sale or so-called 'market potato'. These farmers are advised to use one or more of the following methods for control of PTN: crop rotation, green manures and chemical nematicides depending on the PTN infestation level. This strategy allows production of good potato yield with only 1–10% infected *D. destructor* tubers. Government regulation does not prohibit the sale of such potatoes for local market purposes. This strategy is acceptable and economically suitable to most of these farmers. It should be noted that Super-elite seed tubers (commercially certified PTN and disease free) are not required for planting in these

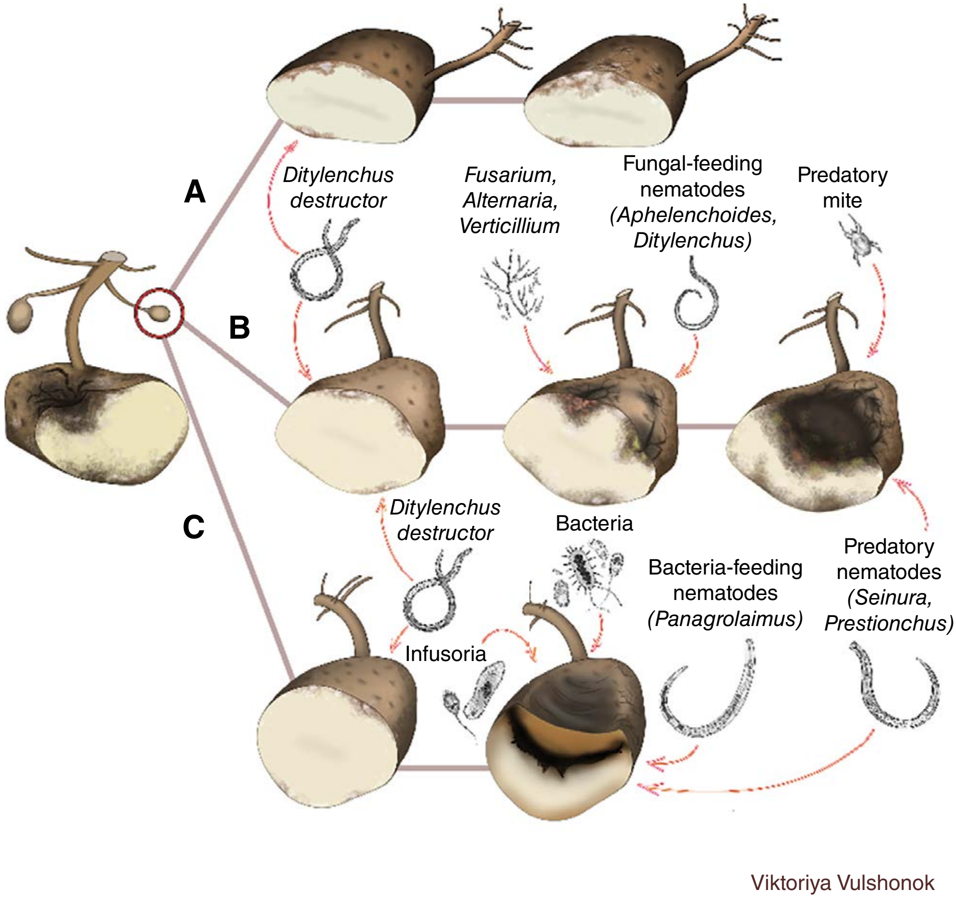

Fig. 49.3. Disease cycle of the interaction between *Ditylenchus destructor* and other microorganisms resulting in wet or soft tuber rot. Figure courtesy of V. Vulshonok, Moscow, Russia.

smallholder situations. Therefore, contaminated seed tubers can circulate in the farmers' planting system.

Commercial large holder farmers

The above strategies are not appropriate for large holder farmers that grow potato for seed or potato for processed commodities such as chips, French fries or other types of potato-related products. Government regulations and industry standards are very strict about the quality of tubers for industrial processing. For example, the GOST 33996-2016 in the Russian Federation regulates potato seed production in all territories of the state. Super-elite reproduction seed tubers cannot contain any tubers infected by *D. destructor*.

Optimization of nematode management

Any INM strategy, therefore, should include two milestones: preventive action and control action, as listed below.

Preventive action

Detection

In 1956, Paramonov stated that 'It should be noted that the main problem with the PTN, as well as with many other plant parasitic nematodes, is that most plant protection specialists have little or no knowledge about nematodes' (Paramonov and Bryushkova, 1956). Therefore, first and foremost all farmers or agriculture personnel should be trained in optimization of nematode management. It is not possible to control this pathogen without an awareness of the symptomology, identification, biology and life cycle of this nematode or what is called '*Praemonitus, praemunitus*'. Identification is very difficult (see Symptoms of damage section) for an untrained person. Highly qualified nematologists are therefore needed to carry out proper identification. One of the primary reasons for the wide distribution of *D. destructor* in the Russian Federation is considered to be false negative results in the diagnostics of potato seed tubers and the further use of the resulting material for planting. Adding to the problem is the fact that the morphology of fungal-feeding species of *Ditylenchus* and that of *D. destructor* is similar. This again makes proper identification of the PTN by untrained personnel very difficult.

Quarantine regulation

The prevention of the introduction and spread of PTN on agricultural land across all territories is needed and this requires training of both extension experts and farmers.

Farm hygiene

PTN can survive in soil particles on machines and farm implements and in storage facilities, and can then be transported to the immediate farm or further to other potato growing areas. Therefore, it is important to clean all surfaces of residues of plant tissue and soil particles.

Weed control

Potato tuber nematodes can reproduce on many weeds in fields during growing of potato plants, after harvesting, and during growing other crops. Control of weeds is an important part of the strategy for reduction of PTN infestation levels in the field. *Ditylenchus destructor* can infect and reproduce on many weeds: *Cirsium arvense* (Canadian thistle), *Cirsium setosum* (field thistle), *Galeopsidis* spp. (hemp nettle), *Sonchus oleraceus* (common sowthistle), *Bidens pilosa* (Spanish needle), *Gnaphalium spicatum* (shiny cudweed), *Oxalis corniculata* (creeping woodsorrel), *Amaranthus deflexus* (smooth pigweed), *Eupatorium pauciflorum* (purple Joe-Pye weed), *Mentha arvensis* (corn mint), *Potentilla anserine* (silverweed), *Solanum nigrum* (hounds berry), *Urtica dioica* and *Urtica urens* (stinging nettle), *Plantago major* (common plantain), *Elytrigia repens* (couchgrass), *Artemisia vulgaris* (sagebrush) and *Rumex acetosella* (sheep sorrel) (CABI Invasive Species Compendium, 2020).

Soil and plant inspection

Ditylenchus destructor can survive for long periods of time in field soil without plant roots by feeding on mycelium of soil fungi. Therefore, it is important to check soil samples from the field to identify plant parasitic nematode diversity before potato planting. Phytosanitary inspection of plants should be carried out during the potato growing season, especially before harvesting.

Control action

Crop rotation

Use of short 2- to 3-year crop rotations is not recommended for the production of high quality potatoes. In this situation, many plant pathogens such as fungi, insects and plant parasitic nematodes can quickly increase in number and jeopardize the quality of future yields. A 4- to 5-year rotation with planting of resistant or non-host crops is recommended. Growing green manures have a positive control effect and reduce the abundance of PTN.

When choosing a crop rotation, it should be borne in mind that *D. destructor* can colonize roots of some potato cultivars and other crops in the field and actively develop and multiply in plant tissue without developing visual above ground symptoms. A striking example is maize, which often had been used in rotations in the Russian Federation before potato planting. *Ditylenchus destructor* can reproduce in maize roots without an effect on the developing aerial parts of a plant. In this case, all plant debris in fields should be destroyed before potato seeds are planted. Using clean fallow is also effective in destroying such plant debris.

Seed stock

The use of certified potato seeds without PTN infection should be used for planting. It is the basis for obtaining healthy tubers and good yield. Official regulations for reduction of *D. destructor* spread in the Russian Federation recommends using high-quality potato seed tubers that are PTN-free. The seeds from Super-elite reproduction (Mini-Tubers, Original Seeds, Super-Super Elite, Super Elite and Elite) are recommended. However, reproductive seeds of the first and second field reproduction process can contain 0.5% infected tubers (GOST 33996-2016, 2016). Each lot of potato seeds should have the official certificate of conformity which contains information about cultivar, place of growing seeds, reproduction number, presence or absence of various pathogens (fungi, viruses, bacteria, nematodes and insects). It is not allowed by law to plant potato seeds without this certificate.

Resistance

Potato cultivars with resistance to PTN are recommended for planting on fields with high nematode infestation levels. Unfortunately, there is not a single potato cultivar known with a 100% resistance to PTN available on the Russian market. Some potato cultivars are very susceptible to *D. destructor*: Bafana, Colombo, Désirée, Eurobola, Gala, Grata, Innovator, Lady Claire, Santana, Challenger, Sylvana, Ivory Russet. However, there also are cultivars that are less susceptible: Achilles, Adretta, Darwina, Festien, Fresco, Hansa, Hela, Laura, Orfei, Santé, Memphis, Panther, Red Scarlett (Mwaura *et al.*, 2015; Ryabtseva, 2018). Research carried out on wild potato species for resistance in the former USSR has demonstrated that resistance to PTN is present in a number of these species: *Solanum chacoenense*, *S. yungesense*, *S. infundibuliforme*, *S. simplicifolium*, *S. catarthrum*, *S. bucasovii*, *S. sucrense*, *S. acaule*, *S. semidimessium*, *S. stoloniferum*, *S. pinnatisectum* and *S. jamesii*. Five forms of tetraploid species of *Solanum andigenum* also have been reported to have a high level of resistance to PTN: *S. andigenum* f. *quieoense*, *S. andigenum* f. *herrera*, *S. andigenum* f. *stenotonum*, *S. andigenum* f. *cuarentona* and *S. andigenum* f. *ocellatum* (Olefir, 1969).

Nematicides

All farmers dream about the 'magic pill' which can solve all problems caused by pathogenic fungi and insects, as well as plant parasitic nematode in the field. The Russian chemical nematicide market contains only one nematicide Vydate® 5G (oxamyl). Application with doses of 30–40 kg/ha in-furrow at planting (cv. Innovator) reduced infection of potato tubers by *D. destructor* up to 93–98% versus untreated variants, but this nematicide paralyses nematodes rather than killing them and thereby is only effective in preventing early root seedling damage.

Biological nematicides

These are not available at this time. Research on the application of nematophagous fungi (predatory and ovicidal) which showed good effects on root-knot or cyst nematodes did not produce positive and significant control results against *D. destructor* in Russia.

Future research requirements

New technologies could improve the present situation with regards to PTN. I believe the technologies listed below in the context of INM will have an impact in the future.

Breeding

Unfortunately, potato breeding for resistance to *D. destructor* is not interesting for the overall European market at this time, because the PTN is not present and/or damaging in Western Europe. There are many potato cultivars with resistance to *G. rostochiensis* and *G. pallida* potato cyst nematodes, but there are no potato cultivars with resistance to *D. destructor*. In contrast to mono genus resistance against potato cyst nematode, resistance against PTN is physiological and identified by many genes. Perhaps, new breakthroughs concerning the genetics of resistance to potato cyst nematodes will make it possible to involve PTN genes in the breeding process. For example, the use of CRISPR-Cas9 technologies or gene modification as outlined in other chapters in the volume will produce good resistance to the nematode. At the present time there are no breeding programmes in the EU or Russian Federation aimed at developing resistance in potato against the potato tuber rot nematodes.

Seed quality

In vitro technologies for potato seed (tuber) production leads to the production of good-quality seed material that is free of viral, bacterial, fungal and nematode diseases. Those technologies are widely implemented in practice by all large European potato companies and are actively implemented by Russian potato seed companies.

Another means of obtaining good-quality seed material is using true potato seeds or botanical seeds that are collected from the berries of the potato plant. It is a technology that is not widely distributed at this time, but it could increase in the future.

Seed-sorting technologies are the next important part of good seed potato production. Advanced machinery such as Miedema™ Smart Grader use scanning by colour and infrared cameras for separating tubers according to size, shape and quality. Some systems provide reliable identification of surface defects on tubers caused by *Rhizoctonia*, potato scab and surface cracks, etc. Developing of spectral or microwave sensors could allow recognition of latent infection in tubers caused by *Phytophthora*, plant viruses or the PTN.

New technologies in plant protection

There is a need to develop new chemical compounds with systemic nematicidal activity coupled with low toxicity for humans and animals as outlined in Chapter 62 in this volume. New non-fumigant nematicides (fluensulfone, fluopyram and fluazaindolizine) are being considered for control of some plant parasitic nematodes, although their effectiveness against *D. destructor* is still at the testing stage.

Future development of bionematicides and/or biocontrol are developments needed for nematode management. However, most of the present bionematicides have little effect on the control of *D. destructor* due to their isolated and prolonged life cycle inside plant tissue where the nematodes are not easily exposed to fungal spores and bacteria. Research is needed on biological control of the PTN that takes into consideration knowledge concerning nematode biology and the uniqueness of the PTN host–parasite interrelationships in field potato production.

New and very promising methods of plant nematode management include the use of RNA interference (RNAi) that has been demonstrated in plant parasitic nematodes. RNAi should help to identify genes and, hence, protein targets for nematode control strategies. Applied research on RNAi in plant protection follows two main directions: construction of transgenic plants and cultivating of transgenic bacteria or viruses (gene therapy).

Transgenic plants would be more effective for PTN control in agriculture but commercial planting and growing of transgenic crops in the Russian Federation are banned and only research projects can be funded.

Outlook: anticipating future developments

Climate change will influence all nematode–crop interactions including factors such as soil, temperature, alternate drying and/or wetting of soils, temperatures in storage facilities, planting dates and the length of the growing season. Climate change will therefore impact PTN as a problem and INM strategies needed for control.

Finally, volatile world markets that influence farm profits as well as the cost of inputs will impact the ability of farmers to manage nematodes in Europe and the Russian Federation.

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50 *Pratylenchus penetrans* and the potato early dying disease

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Introduction

Pratylenchus penetrans is a cosmopolitan species reported from 69 countries representing every continent except Antarctica. One of many species referred to as root lesion nematodes, *P. penetrans* has a wide host range including potato and is found throughout the potato growing region of the northern USA. Most potato fields are infested with the fungus *Verticillium dahliae* as well as root lesion nematodes, and a disease interaction between the two has been demonstrated for multiple soil types, potato cultivars and production regions (Rowe and Powelson, 2002). The fungus infects roots and grows internally through the vascular tissue of stems and leaves, causing wilting in the absence of moisture stress. Just like *P. penetrans*, the 'dose makes the poison' for the *Verticillium* wilt disease and management is based on the density of the fungus in soil at planting. The significance of the interaction between *P. penetrans* and *V. dahliae* is that it is synergistic rather than additive. That is, a much greater level of disease occurs when both pathogens are present than would be indicated by the sum of their individual effects. The nematode appears to impede plant defence responses to the fungus, exacerbating the onset and severity of fungal symptoms. Symptoms that manifest synergistically include loss of yield and tuber solids, impaired photosynthesis and symptoms related to premature vine death, including leaf life span, wilting and yellowing. Disease due to *Verticillium* and nematodes is referred to as potato early dying (PED) to distinguish it from the *Verticillium* wilt disease caused by the fungus alone. Managing *P. penetrans* is important for mitigating the yield losses it causes as well as losses due to PED.

Economic importance

Reduced potato yield by *P. penetrans* alone or *Verticillium* alone has been demonstrated, but most potato fields in the north-central USA are infested with both pathogens. Population densities of fewer than ten *Verticillium* propagules per gram of soil and one *P. penetrans* per cm^3 of soil reduced tuber yield 20–36% in Wisconsin (MacGuidwin and Rouse, 1990). Comparable yield loss was reported for *P. penetrans* alone, but at higher population densities (Bernard and Laughlin, 1976).

Studies in Wisconsin also demonstrated yield loss due to *P. penetrans* for soybean and

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maize, two crops commonly grown in rotation with potato. Models estimated 0.03% and 0.01% loss in yield per nematode in 100 cm^3 soil (and root fragments therein) at planting for soybean and maize, respectively. Based on the models and results of 3024 soil samples, 5% of Wisconsin's soybean fields lose at least 16.5% yield and 20% of Wisconsin's soybean fields lose at least 4.5% yield to this pest. Neither crop is a host for the *Verticillium* fungus.

Distribution

Pratylenchus penetrans has been reported from 39 US states, including Wisconsin. Populations from 34 Wisconsin counties were confirmed to be *P. penetrans* using molecular diagnostics. Surveys of Wisconsin fields planted with grain crops showed positivity rates for root lesion greater than 90%, with *P. penetrans*, *P. neglectus*, and *P. crenatus* as the most common species. Positivity rates for potato are also greater than 90% with *P. penetrans* as the predominant species.

Symptoms of damage

Feeding by *P. penetrans* causes root, stolon and tuber tissues to discolor and form visible lesions (Fig. 50.1). The impact of feeding extends to cells beyond those touched by nematodes, so lesions are initially discrete but increase and coalesce over time as nematodes feed, congregate and reproduce. Nematode harm to the plant goes beyond diminished uptake of water and nutrients by disrupting the allocation of resources to tubers. Shoot growth is rarely affected, and potato fields infested with *P. penetrans* may appear healthy until undersize tubers are harvested. In contrast, the PED disease displays as foliar symptoms that begin before natural senescence (Fig. 50.2).

Biology and life cycle

Root lesion nematodes have remarkable plasticity in their life cycle and are highly adapted to agricultural soils, which makes them virtually impossible to eliminate. They live on the root surface or burrow completely into roots or tubers where they live as internal parasites, never losing the capacity to change position. They traverse soil pores in water films but can suspend growth and development when soil or the root they occupy dries. Movement in the field is restricted by their small size, but *P. penetrans* can disperse passively in displaced soil or as sheltered parasites in transported roots or tubers. Genetic similarity in populations of *P. penetrans* in Wisconsin, other US states, Europe, South America and Asia suggest humans serve as dispersal agents (Saikai and MacGuidwin, 2020). Potato may be particularly important for the spread of this important pest, as *P. penetrans* survives in stored potato seed (Holgado *et al*., 2009).

Fig. 50.1. Lesions made by *Pratylenchus penetrans* in the absence of other pathogens. Author's own photograph.

Recommended integrated nematode management (INM)

Both *P. penetrans* and *V. dahliae* are soil-borne pathogens; that is, the life stage that initiates infection survives in the soil, infection takes place below ground and the severity of their impact depends on their population densities at the time crops are planted. They are highly adapted to survive in the absence of a host and should be considered as a fixed characteristic of fields where they have been detected. Management

Fig. 50.2. Potato early dying disease caused by *Pratylenchus penetrans* and *Verticillium dahliae*. Author's own photograph.

recommendations are aimed at keeping initial population densities low, which can be challenging in fields infested with both pathogens because of differences in their host range, life history and vulnerabilities. The fungus is a critical factor in the PED disease (Rowe and Powelson, 2002) and should be considered in nematode management plans for potato.

Soil sampling for *P. penetrans* and *V. dahliae* is recommended in the autumn with soil samples divided for nematode and fungal assays. They have an aggregated distribution at the field scale and no correlation in their spatial distribution has been demonstrated, so the accuracy of estimates for *P. penetrans* and *V. dahliae* may differ. It is common for sample collection to be biased toward the fungus since *V. dahliae* causes aboveground symptoms and *P. penetrans* does not. Assay results should be used to guide nematode management decisions.

A 3- to 4-year rotation is recommended for potato to break the cycle of multiple diseases. Rotation crops grown in Wisconsin such as maize, soybean and vegetables support reproduction by *P. penetrans* equal to or greater than potato (Morgen *et al.*, 2002). Nematodes must feed on plants to complete their life cycle, so planting two short-season crops such as pea or snap bean with a bare fallow mid-season can reduce reproduction. Damping the increase of *P. penetrans* decreases the need for aggressive measures such as fumigation in the potato year and increases the profitability of the targeted crop, as *P. penetrans* decreases yield of maize (MacGuidwin and Bender, 2016), soybean (Saikai and MacGuidwin, unpublished results), and carrot (Teklu *et al.*, 2016). *Verticillium dahliae* has a more restrictive host range so new propagules of the fungus are not added during the rotation. Potato breeding programmes are currently aimed at developing resistance to *Verticillium* wilt and cultivars differ in susceptibility to fungal infection (Simko and Haynes, 2017). There is no resistance to *P. penetrans* for potato.

Cover cropping and soil amendments help to build soil organic matter, which is the cornerstone of soil health, but *P. penetrans* has a wide host range so only select crops are recommended for nematode infested fields. Forage pearl millet, *Pennisetum glaucum*, is an excellent choice for *P. penetrans* management. Recommendations for other root lesion species and a good choice for *P. penetrans* include sorghum and saia oat (*Avena strigosa*). They support low reproduction by *P. penetrans* and decrease survival of nematodes and *Verticillium* when incorporated as a green manure. Green manures of biofumigant crops in the cabbage family are biocidal but support a high level of reproduction by *P. penetrans*. There is a risk of increased nematode population densities if something goes wrong with the execution or timing of incorporation.

Nematicides or seed treatments are recommended as a needs-based option to support rather than replace cultural tactics. Products are designed to prohibit infection for 30-45 days after planting, thereby delaying reproduction and an increase in nematode population densities. Growers are encouraged to limit pesticide use to *P. penetrans* or PED ‘hotspots’ as informed by pathogen test results and historical yield data. The same advice applies to rotation crops, some of which have more product options for nematode management than potato.

Most potato fields in the US, including 80% of the fields in Wisconsin, are fumigated with biocidal organosulfur or organochlorine chemicals. In the northern USA, fumigation occurs in the autumn preceding the potato crop. Nematodes and fungal propagules that receive a sufficient dose of the product die immediately. Many of those that receive a sub-lethal dose enter winter in a weakened state and lose viability by the next spring. Fumigants reduce population densities of PED, and a fumigated control is used as the benchmark for evaluating new chemical products. Data also show significant non-target impact which contributed to the decision by some countries to ban soil fumigation. Fumigation is an expensive input, but it addresses multiple pests so the return on investment is high. Given the popularity of the tactic, recommendations are aimed at reducing the frequency of fumigation or the rate of fumigant applied.

Growers are encouraged to learn more about nematodes by attending commodity-sponsored educational events and reading information distributed in print and online. The caveat to the recommendation is to be discerning about the pest target of the advice. Information that does not specify the nematode genus can mislead growers into using inappropriate management practices. For example, some rotation crops that suppress the Columbia root-knot nematode, *Meloidogyne chitwoodi*, support a high rate of reproduction by *P. penetrans*. Networking at events helps growers address common challenges such as incorporating nematode management practices into every year of the rotation and developing confidence in using nematode population densities to inform management decisions.

Optimization of nematode management

Soil testing is the foundation for nematode management, so providing results that accurately reflect the status of a field is a top priority. Labs should be encouraged to use recovery methods that account for both soil-dwelling and root-sheltered nematodes. Some rely on root testing only, which is adequate for making a one-time decision but expressing nematodes in soil-based units (grams or cubic centimetres) is better for monitoring population densities over time. Sample collection is often delegated to crop consultants so support for developing sampling plans and records needs to include this group. Map-based records that incorporate observations of disease, pathogen data and production details are the gold standard for integrated management that spans the rotation. Data-driven management plans are less likely to rely on ‘clean-up’ tools like soil fumigation and to be more sustainable in the long term.

Most people are uninformed about nematodes so there is a lot of confusion about their impact and features that distinguish different genera and species. Clear unified messaging is needed to educate professionals and the public about root lesion nematodes. A common misconception is that nematodes are a type of insect, which suggests they come and go. A unified message combining brand recognition and recommended action is possible, as demonstrated for the soybean cyst nematode which is recognized in the US by its acronym (SCN) and the logo 'Take the test. Beat the pest'. Messaging that characterizes *P. penetrans* as soil residents and captures their importance to vegetable, fruit and grain crops would be helpful to growers.

Future research requirements

Science-based evaluations of management options are critical. Efforts are ongoing, but the scope and rate of experiments is insufficient to meet demand. A shortage of applied nematologists in the US and other countries constrains progress in nematode management for all commodities, but especially for specialty crops like potato. Evaluating potato germplasm for *P. penetrans* and other root lesion species is an important activity yet to be addressed. Commercial products for nematode management throughout the rotation such as seed treatments and nematode-suppressive cover crop cultivars are entering the market at an unprecedented pace, creating demand and opportunity for more applied peer-reviewed research.

Soil fumigation has become more regulated in the US and banned in some countries, so the long-term outlook for this practice is not promising. Research on management tools specific to *V. dahliae*, such as host resistance, are underway and likely to replace fumigation for fungal control. Research aimed specifically at *P. penetrans* is needed, particularly regarding diagnostics and host resistance. Nematode tests that inform management use relatively large soil samples and rely on human experts for quantifying nematodes to the genus level. New tools to automate the process, such as molecular and computer-recognition assays are available, but significant research is needed to develop laboratory practices that ensure accurate species-specific results relevant at the field scale. False-negative results due to the small size of molecular samples is an issue for nematode diagnostics so solutions require nematology expertise. Potato breeding and cis-genic programmes have a likewise need to involve nematologists and extend beyond cyst and root-knot nematodes to include *P. penetrans*. Host resistance will be increasingly important in the future but will never reach its full potential if *P. penetrans* and other root lesion nematodes are excluded. Molecular tools for sampling and molecular breeding technology are covered in Chapters 57 and 58 of this volume.

Precision application of farm inputs reduces the cost of potato production and is likely to continue its upward trajectory. Electrical conductivity field maps are being trialled for variable rate fumigation. Research showed the spatial distribution of *P. penetrans* in fumigated fields was uniform during the potato year and increasingly aggregated in the rotation years (Morgan *et al.*, 2002), indicating a need for research to identify edaphic variables that could serve as proxies for nematode sampling. Remote sensing, used now to monitor the potato crop, should be expanded to bare soil with the goal of correlating edaphic data with nematode distribution (see Chapter 59 in this volume). Coordinated collection of geo-referenced nematode and landscape data and data sharing will become increasingly important for modelling nematode population dynamics and targeted management.

The role of the soil microbiome in potato and soil health is an exciting and vibrant research area that has the attention of the potato industry and nematologists. More research is needed to understand the direct and indirect influence of rhizosphere organisms on *P. penetrans* behaviours such as infection, root egress and mating. The impact is reciprocal as shown in controlled studies where *P. penetrans* influenced rhizodeposition of potato which, in turn, affected germination of *V. dahliae* microsclerotia (Bowers *et al.*, 1996). The soil health research arena extends to the entire nematode community and learning more about the ecological relationships of *P. penetrans* and *V. dahliae* in the field holds promise for identifying interactions that can be exploited to manage the individual and joint effects of these important potato pathogens.

Outlook: anticipating future developments

Pratylenchus spp., including *P. penetrans* are highly adapted soil-borne pathogens likely to increase in importance in the coming years. Trends over the last decades indicate population densities of *P. penetrans* have increased in potato production systems and *V. dahliae* have stabilized. Experts attribute this discrepancy to changes in fumigation that target the fungus and increased use of potato cultivars tolerant of *V. dahliae*. Breeding programmes are aimed exclusively at developing resistance to *Verticillium* wilt to address the PED disease. Unless the fungus is eradicated, the PED disease is not likely to disappear in nematode infested fields due to their synergism at very low population densities and capacity for long-term survival, even under a changing climate.

Pratylenchus penetrans is among the most damaging species of plant parasitic nematodes. Its wide host range, capacity for anhydrobiotic survival and flexibility in completing its life cycle in soil or inside roots, suggests that it will be less impacted by a changing climate than more niche-restricted pests such as cyst nematodes. The economic important of *P. penetrans* in rotation crops, such as soybean, is likely to increase as damage caused by the SCN is likely to be mitigated due to intense research efforts already in place today. The likely demise of soil fumigation, now a mainstay for *P. penetrans* management, underscores the importance of improving current technology and discovering novel tools to keep population densities of *P. penetrans* in check.

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51 Modifying a productive sweet potato farming system in Australia to improve soil health and reduce losses from root-knot nematode

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Introduction

Sweet potato (*Ipomoea batatas*) is the world's sixth most important food crop after rice, wheat, potatoes, maize and cassava. More than 105 million metric tonnes are produced globally each year, with more than 90% coming from developing countries. Australia is a relatively small contributor, with total production of around 100,000 tonnes. However, its sweet potato industry has grown remarkably in recent years, with sales increasing by about 20% per annum. Most of the crop is grown in subtropical regions along the east coast, with Bundaberg (southern Queensland) and Cudgen (northern New South Wales) the main centres of production.

Economic importance

Australian growers produce some of the highest sweet potato yields in the world (commonly 60–90 t/ha) but often suffer losses from root-knot nematodes (*Meloidogyne javanica*, *M. incognita* and *M. arenaria*). Commonly, 5–20% of the marketable product is discarded due to nematode damage but yield losses in some fields may be as high as 75%.

Distribution and host range

Root-knot nematode is a ubiquitous problem on sweet potato in Australia for several reasons.

- The subtropical climate is ideal for nematode multiplication. Nematodes develop and reproduce throughout the year because mean maximum temperatures range from 21–30°C.
- Crops are sometimes grown for 7–9 months, so there is time for the nematode to complete as many as six life cycles between planting and harvest.
- The three *Meloidogyne* species are widely distributed. Many weeds, and widely grown crops such as sugarcane, pineapple, tomato, capsicum, cucurbits and ginger, are hosts.
- The major soil types (sands and well-structured clay soils of volcanic origin) are ideally suited to root-knot nematode.
- Widely grown sweet potato cultivars such as Orleans and Beauregard are highly susceptible.

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Symptoms of damage

Collectively, these 'warm-climate' species cause heavy losses, largely because they damage storage roots, the product that is being marketed. Second-stage juveniles invade roots and grow to maturity within the roots, causing longitudinal cracking, uneven protuberances on the root surface and necrotic lesions within root tissue (Fig. 51.1).

Recommended integrated nematode management

When a susceptible sweet potato crop is harvested, root-knot nematode populations are usually relatively high (>1500 nematodes/100 g soil). Numbers must be markedly reduced before the next crop is planted and this is currently done in two ways. First, volunteer plants that grow from small roots left behind at harvest are eliminated, as they carry-over the nematode to the next crop. Most growers use tillage and herbicides, but another alternative is to erect an electric fence and use domesticated pigs to consume the volunteers. Second, a forage sorghum rotation crop is grown for at least 6 months, as it produces a large amount of biomass and many cultivars are resistant to the three common *Meloidogyne* species (Stirling *et al*., 1996).

Although the above practices are effective, root-knot nematode populations are rarely reduced below the damage threshold, which may be less than 0.5 nematodes/100 g soil for crops where the marketable product is roots or tubers (Hay and Stirling, 2014). Pre-plant counts of 10–40 nematodes/100 g soil are relatively common in sweet potato fields, and so growers either apply a nematicide or plant a nematode resistant cultivar such as Bellevue. Four nematicides (1,3 dichloropropene + chloropicrin, metham sodium, fluensulphone and oxamyl) are currently registered for use and growers planting susceptible varieties would choose one of them.

Farming systems and their impact on soil health

Although the farming system used to produce sweet potatoes in Australia is productive, the soil is repeatedly tilled to kill volunteer sweet potatoes, incorporate cover crop residues and prepare beds for planting. It is disturbed again when the swollen roots are harvested and is also subjected to random wheel traffic during the harvest operation. Collectively, these practices have disastrous effects on the health of the soil. The level of degradation varies with soil type, previous cropping practices and the length of time the land has been cultivated (often more than 100 years), but soils with compacted layers, low moisture-holding capacities, poor rainfall infiltration rates and low organic carbon levels are relatively common. From a biological perspective, frequent tillage and the regular use of pesticides and fumigants have modified the soil biology to such an extent that the mechanisms which normally regulate nematode populations are no longer likely to be operating effectively.

Over the last 30 years, Australian farmers have made a major effort to farm more sustainably and improve the health of their soils. Conservation agriculture (i.e. no-till planting

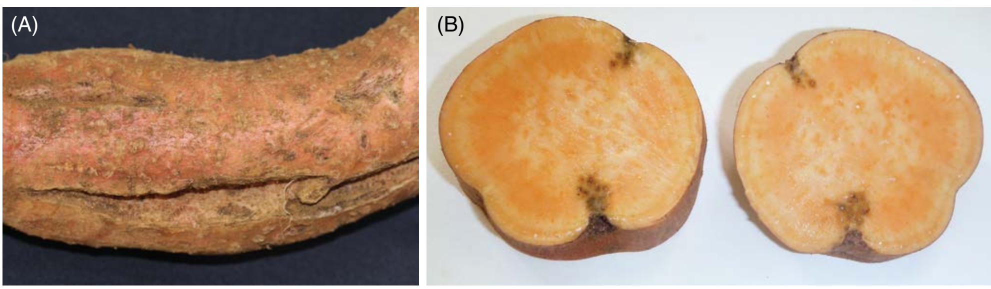

Fig. 51.1. Symptoms caused by root-knot nematode on sweet potato. **(A)** Longitudinal cracking and pimples on the surface of a storage root. **(B)** Internal lesions, each containing several nematode females and egg masses. Author's own photographs.

systems, rotation of crops and surface retention of crop residues) is now standard practice in the grains industry, while crop rotation, minimum tillage, residue retention and controlled traffic are key components of sugarcane farming systems. As decades of research have shown that the above practices help maintain a range of vital ecosystem services (Lehman *et al*., 2015; Stirling, 2018; Pratley and Kirkegaard, 2020), it was clear that the current sweet potato farming system should be redesigned. Thus, research was undertaken to develop more sustainable methods of growing the crop.

Field trials with more sustainable sweet potato farming systems

After considering options that would improve soil health, enhance sustainability and possibly provide acceptable levels of nematode control, a collaborating grower agreed to try an approach that Stirling (2014) termed 'integrated soil biology management'. The basis of the concept is that a range of nematode management practices are integrated into a farming system to not only reduce pest nematode populations, but also improve the physical, chemical and biological health of the soil. The farming system tested on-farm is summarized below.

- Immediately after harvest, the soil was tilled and herbicides were applied to kill volunteer sweet potatoes and weeds.
- Once most of the volunteers had been eliminated, beds 35 cm high and 1.5 m apart were formed.
- A forage sorghum cover crop was grown on the beds during summer and autumn, and it was followed by oats as it produces much more biomass in the cooler winter months. Both cover crops were slashed and then terminated with herbicides, with the residues being retained on the soil surface as mulch.
- Ten months after bed formation, sweet potatoes were established using a strip-till process. A tine was used to form a channel in the middle of the undisturbed beds and stem cuttings were planted in the channel.

As organic amendments are known to increase soil carbon levels, enhance biological activity and provide worthwhile levels of nematode control, two trials were undertaken using waste materials that are readily available to sweet potato growers. In the first trial, sawdust and chicken litter were broadcast on the soil surface and incorporated into the soil during the bed formation process. In the second trial, a V-shaped furrow about 14 cm deep and 9 cm wide was prepared in the centre of the bed and various amendments (compost, sawdust and a mixture of sawdust and chicken litter) were placed in the furrow. Sweet potato was then planted in the furrow so that the swollen roots were surrounded by an amendment as they developed.

The results: minimum till sweet potato production is feasible

When strip tillage was used to plant sweet potato cuttings into beds covered with a thick layer of residue from the cover crops, the mulched residues did not cause any problems (Fig. 51.2A,B). The crop established well and 7 weeks after planting there was no obvious difference between plants growing in the strip-tilled beds and those in beds formed immediately prior to planting (Fig. 51.2C). The yield in the mulched, undisturbed beds was 93 t/ha, demonstrating that minimum till sweet potato production was possible.

Organic amendments for nematode control

The first trial discussed above was done in a field where the previous sweet potato crop had suffered severe damage from root-knot nematode. Thus, the amendments were applied at very high rates (72.2 and 43.9 t dry weight/ha for chicken litter and sawdust, respectively). Initial results suggested that the combination of minimal tillage, cover cropping and organic amendments had been effective, as the pre-plant nematode count was about 1 root-knot nematode/100 g soil. However, the situation changed markedly when sweet potato was planted, as about 70% of the storage roots were unmarketable due to nematode damage when the crop was harvested 31 weeks later. Various soil health parameters had improved (i.e. increased soil carbon levels, greater microbial activity and higher numbers

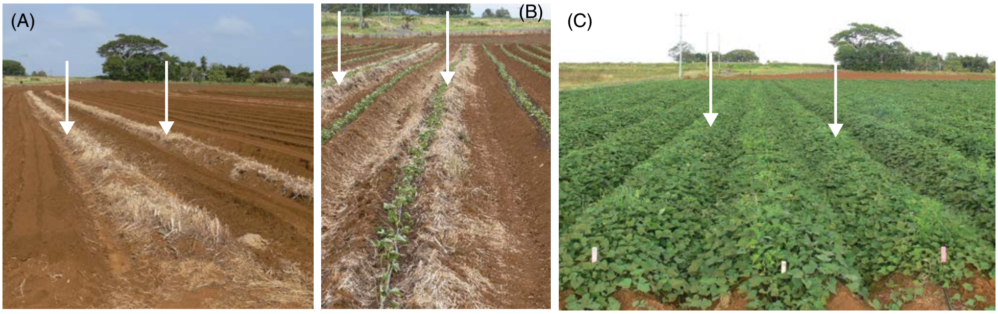

Fig. 51.2. **(A)** All beds were prepared immediately prior to planting sweet potato, except for the two arrowed beds, which were formed 10 months previously and left undisturbed, with cover crop residues retained on the soil surface as mulch. **(B)** Four days later, after the arrowed beds had been strip-tilled with a tine and sweet potatoes planted. **(C)** The crop 7 weeks after planting. Author's own photographs.

of free-living nematodes), but root-knot nematode caused heavy losses.

The trial in which sweet potatoes were planted into furrows filled with three different amendments produced more promising results. When roots were collected from the centre of the bed about 7 weeks after planting, the number of galls produced by root-knot nematode was much lower where organic amendments had been applied than in non-amended plots. A mixture of sawdust and chicken litter had a major effect, reducing the number of galls by more than 90%, while compost and sawdust reduced galling by 71% and 56%, respectively. The results obtained at harvest were also encouraging, as the marketable yield in both the sawdust and sawdust/chicken litter treatments was 93 t/ha. When compared to the non-amended control, both treatments increased marketable yield by 29% and reduced final populations of root-knot nematode by 43% and 39%, respectively (Stirling *et al.*, 2020).

Follow-on work in the greenhouse confirmed that sawdust-based amendments generally increase swollen root production and reduce root-knot nematode populations and the severity of nematode damage. Allowing the amendment to decompose for 6 months and adding an organic nitrogen source usually improved the level of control (Stirling, 2020). This work also showed that the amended soils were biologically suppressive to root-knot nematode, and that a wide range of natural enemies were contributing, including predatory nematodes, mesostigmatid mites and nematophagous fungi.

Optimization of nematode management

The Australian sweet potato industry has grown significantly in the last 20 years but must now make decisions about its long-term future. It has two choices:

- Continue with a farming system that is degrading its soils and rely on nematicides to reduce losses from nematodes.
- Move to a more sustainable farming system that will improve soil health and gradually build a soil biological community capable of suppressing nematode pests.

The danger with taking the first option is that increasingly costly inputs will be required to grow sweet potatoes in soils that are eroded by tropical storms, degraded by tillage and compacted by farm machinery. Also, growers are likely to have to cope with the loss of a key nematicide at some time in the future, as experience over the last 40 years indicates that nematicides often lose efficacy due to enhanced biodegradation or are removed from the market for health or environmental reasons. Nevertheless, many growers will maintain current practices because nematicides such as fluensulphone are effective. Also, there is no incentive to improve soil health in situations where land is leased, as it is usually only used for one or two crops.

The sustainable pathway is most likely to be taken by growers who own their land and want their farm business to remain viable in the long term. Given the results of the study discussed earlier, the first step in that pathway is to integrate basic practices such as early bed formation, cover cropping, minimum tillage and organic amendments into the farming system. The next step is to consider how the new system could be improved. There are many improvements that could be made but perhaps the most important is to incorporate traffic control. Soil is compacted by random traffic and mismatched machinery wheel spacings, and this reduces aeration and rainfall infiltration and impedes root growth. A move to controlled traffic farming would reduce soil erosion, increase soil water retention, improve nutrient use efficiency and enhance soil biological activity. It would also provide economic benefits, as less energy is required to move farm equipment over compacted traffic lanes.

Although many improvements are possible, it is impossible to be prescriptive about future best-practice farming systems. Many potentially useful tactics are available, and it is up to growers to adapt them to local conditions. For example, in regions where sugarcane is the dominant crop, one of the best options may be to grow sugarcane using the sustainable farming system described by Stirling (2018) but utilize sweet potato as the rotation crop rather than soybean or peanut.

With regard to reducing losses from nematodes, forage sorghum will always be an important component of integrated nematode management programmes. However, there is a need to find nematode resistant crops that grow well in winter, as most potentially useful cover crops are susceptible to root-knot nematode. Minimizing the presence of weeds and the carry-over of volunteer sweet potatoes will always be important, but the key question is whether this is best done using tillage, herbicides or livestock; by mulching cover crop residues; or modifying harvesting equipment so that small swollen roots are not left in the field.

Initial work has shown that compost and sawdust-based organic amendments are useful, and that it may be better to place them in a furrow well before planting. However, these amendments may not be effective enough to fully control root-knot nematode when susceptible cultivars are grown for more than 5 months. In such situations, growers could establish a mid-season monitoring programme and harvest the crop before severe nematode damage occurs. Another option would be to apply a nematicide through the trickle irrigation system. However, this could only be done if an effective chemical or biological product was available that is not detrimental to the many natural enemies of nematodes.

Some growers will be reluctant to apply organic amendments because they are relatively expensive, but if they are used in an appropriate manner, costs are likely to decline with time. For example, if a controlled traffic farming system was established; precision agriculture techniques were used to ensure that amendments were always placed in the same position; cover crops were grown for 6–9 months; and carbon losses were reduced by minimizing tillage; then organic carbon levels, soil biological activity and biodiversity should gradually increase over time. Ultimately, this may mean that organic inputs from amendments could be reduced.

Given that sweet potato cultivars with resistance to root-knot nematode are available, growers will always have the option of using them. However, most of those cultivars have been tested for resistance to only one species (*M. incognita*), so the key issue from an Australian perspective is to determine whether they are also resistant to *M. javanica* and *M. arenaria*. It is also important that growers do not become reliant on resistant cultivars, as their overuse may result in the development of resistance-breaking pathotypes.

One question that always creates debate amongst those developing nematode management programmes is whether bionematicides will play a role in future programmes. Many products are available in the marketplace but none are widely used, largely because results are inconsistent. Encouraging natural enemies that are already present at a location is a much better strategy, as naturally occurring biocontrol agents will already be adapted to the local environment. Also, numerous antagonists with different modes of action are more likely to be effective than one or two introduced antagonists.

Regardless of the control tactics used by growers, nematode monitoring will always be an important component of future nematode management programmes. Australia is fortunate in having a commercial DNA-based analytical service (PreDicta®) that quantifies nematode populations in soil and provides species identifications. Growers would use this service to identify the root-knot nematode species that occur in a particular field and assess nematode populations prior to planting, in an established crop, or after harvest.

Future research requirements

It is a challenging task to modify a productive and well-established farming system but the first tentative steps have now been taken. Controlled traffic, early bed formation, effective weed and volunteer control, minimum tillage, cover cropping, retention of cover crop residues as mulch, and organic amendments will be key components of any new system as they are known to improve soil health and enhance nematode-suppressive services. It is now up to land managers to assess these practices in on-farm trials, learn from the results and then integrate some or all of these practices into their farming system. Research on potentially useful nematicides is also required, but it should focus on chemical and biological products that are safe to apply to sweet potato and have little impact on the fungi, microarthropods, predatory nematodes and other organisms that regulate nematode populations.

Outlook: anticipating future developments

Although root-knot nematode is the only serious nematode pest of sweet potato in Australia, reniform nematode (*Rotylenchulus reniformis*) is waiting in the wings. Considered to be more damaging than root-knot in some areas of the US, it is relatively common in tropical Queensland but has been detected in the subtropics, where most sweet potatoes are produced. Consequently, the sweet potato industry must set up a biosecurity programme to limit its spread, and growers need to establish on-farm biosecurity measures to protect their business.

The guava root-knot nematode (*M. enterolobii*) is also a serious biosecurity threat. It occurs in China, South America, the US, Europe and Africa, and has the capacity to reproduce on all sweet potato cultivars, including those that are resistant to *M. incognita*. Australia has an effective quarantine system and hopefully it will prevent the importation of this potentially devastating pest.

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52 Importance and integrated nematode management of the yam nematode (*Scutellonema bradys*) in yam cropping systems of West Africa

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Introduction

Yam, *Dioscorea* spp., one of the oldest food crops known to humans, is the fourth most important root and tuber crop globally (Fig. 52.1). It is a tropical plant that provides food and income for the people in the regions where it is grown. Some *Dioscorea* species are cultivated for their hormones used in pharmaceuticals. Yams are normally vegetatively propagated from whole, small tubers (seed tubers/yams) or cut portions of tubers (setts). West Africa accounts for 90% of worldwide production of yams, where the crop represents key sociocultural and traditional symbols.

Major nematode pests reported on yams include *Scutellonema bradys*, *Meloidogyne* spp., *Pratylenchus* spp. and *Rotylenchulus reniformis*. This chapter addresses *S. bradys*, causing dry rot disease of yams in field and storage. When *S. bradys* infected seed tubers are planted, plant survival is reduced, and the speed of the disease cycle is amplified leading to reduced yield.

A similar effect on plant establishment is caused by *P. coffeae* although the nematode occurs less often in the region. *Meloidogyne* spp., on the other hand, causes characteristic tuber galling and proliferation of roots from the tuber surface referred to as crazy roots that affects marketability of tubers.

Economic importance

Tubers with dry rot have been observed in almost all yam barns surveyed in Ghana and Nigeria. The nematode causes a marked reduction in the quality, seed tuber viability, marketable value and edible portions of tubers. The proportion of tubers with *S. bradys* symptoms in markets in West Africa averages between 2–47% depending on the country (Coyne *et al*., 2006). Depending on the level of infection, *S. bradys* can reduce harvestable yield between 0–50% (Wood *et al*., 1980), closely correlated with 0–40% pre-planting damage observed in seed barns. Storage losses are high (40–80%) from a few weeks to 4 months after storage and can be total when secondary infection occurs. Increased moisture loss and conversion of the complex starchy tissue contributes to the storage weight loss of infected tubers. Infected seed perpetuates the disease cycle, affects sprouting and may

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Fig. 52.1. Relative size of yam to potato tuber. Author's own photograph.

cause die back, resulting in reduced plant stands and fewer tubers. Nematodes are implicated in the increasing scarcity of healthy seed material and may even have contributed to the loss of some highly susceptible crop germplasm. Moreover, infected tubers have to be peeled deeper when prepared for consumption with 10–30% of the edible portion lost.

Host range

The major host of *S. bradys* is yam. However, with the right conditions it has the potential for becoming a pest of concern to a few other crops given ease of germplasm movement and the trends in worldwide climate change. On potatoes, *S. bradys* causes severe tuber cracking, deformation and reduced tuber size (Coyne and Claudius-Cole, 2009). Traditionally, intercropped vegetables in the subsistence farming system including cowpea, fluted pumpkin, okra and aubergine sustained similar *S. bradys* populations as yam in addition to several weeds.

Distribution

Scutellonema bradys is widespread in the yam belt of West Africa (Fig. 52.2) as with other yam growing regions. It occurs in all yam growing countries in the region from Senegal to Nigeria. The proportion of *S. bradys*-damaged tubers to the most popular yam species, *Dioscorea rotundata* (white guinea yam) collected from markets ranged from 1.5% in Cote d'Ivoire, to 14.8% in Burkina Faso and 48% in Nigeria (occupying the greater acreage of the yam belt) (Coyne *et al*., 2006). *Scutellonema bradys* is encountered in 100% of fields during the rainy season and yam tubers in storage during the dry season in Benin, Ghana and Nigeria.

Symptoms of damage

Dry rot, flaking and cracks are the effect of feeding and movement of *S. bradys* in yams. At first, the lesions appear as small yellow spots just beneath the outer skin, which then gradually turn brown to black (dry rot) with progression of the disease (Adesiyan *et al*., 1975) (Fig. 52.3). The outer skin remains intact at the early stages of infection, disguising the damage. The damage can progress to about 2 cm or more deep into the tuber. As the necrosis advances, tubers lose moisture and the skin may shrink and become flaky, which is the characteristic symptom of infection of *S. bradys*. Infection is sometimes associated with cracks; however, this is not a characteristic symptom as other stresses can cause tuber cracking. *Scutellonema bradys* can serve to facilitate secondary infection by fungi and bacteria, resulting in wet rot.

Biology and life cycle

Scutellonema bradys is a migratory endoparasite of roots and tubers. The life cycle from newly hatched second-stage juveniles to adult in yam

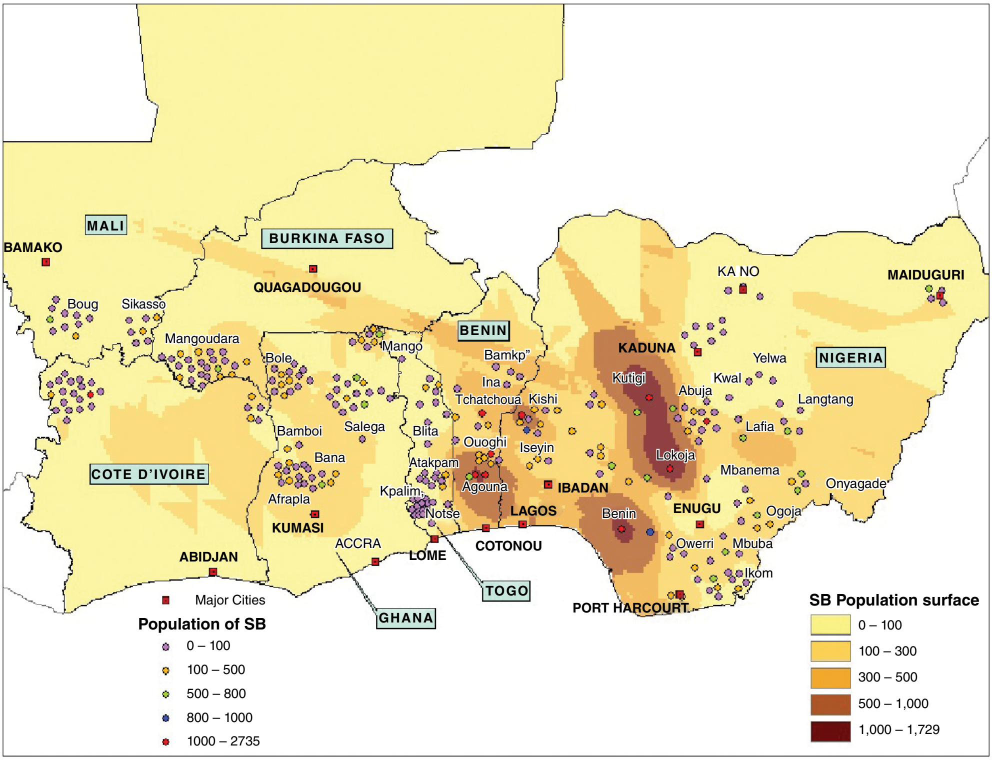

Fig. 52.2. Distribution and population of *Scutellonema bradys* (g^{-1} peel) in West Africa. Figure courtesy of D. Coyne.

takes between 21 and 26 days at 26 ±2°C. Being a tropical nematode that is endoparasitic in tubers, reproduction is continuous until tubers disintegrate. The nematode is spread both in and between fields, but also over long distances via infected seed yams. Yams are in the field for 8–10 months from planting either in November/December (the dry season) or March/April (onset of rains). This long period of exposure ensures continuous build-up of the nematode. *Scutellonema bradys* migrates out of the infected tuber or from soil into the developing roots then into the tuber at initiation. As the tuber matures, the roots begin to disintegrate, releasing nematodes which may enter into the tuber, migrate into roots of alternate hosts (weeds and intercropped plants) or remain in the rhizosphere. The continued cycle arises when harvest is conducted in the late growing season (July/August) and the same land is prepared for the next planting or cultivated with a susceptible legume or vegetable before the next yam crop. *Scutellonema bradys* thus survives periods of absence of its main host. Infected tubers harvested after planting healthy (non-symptomatic) yams serve as evidence that the nematode survives long periods without its yam host. Although the mechanism is not clear, there is a possibility that *S. bradys* may be similar to *S. cavenessi*, which can survive up to 30 months in anhydrobiotic conditions.

Interactions with other nematodes and pathogens

Secondary invasion of fungi and bacteria post-*S. bradys* infection causing wet rot can lead to total tuber loss in yam tubers during storage. The nematode acts as a wounding agent and possibly predisposes tubers to secondary invasion. Reports implicate *Lasiodiplodia theobromae*, *Fusarium* spp. and *Erwinia* spp. in rots initiated by *S. bradys*. However, *Aspergillus* spp., *Penicillium* spp. and

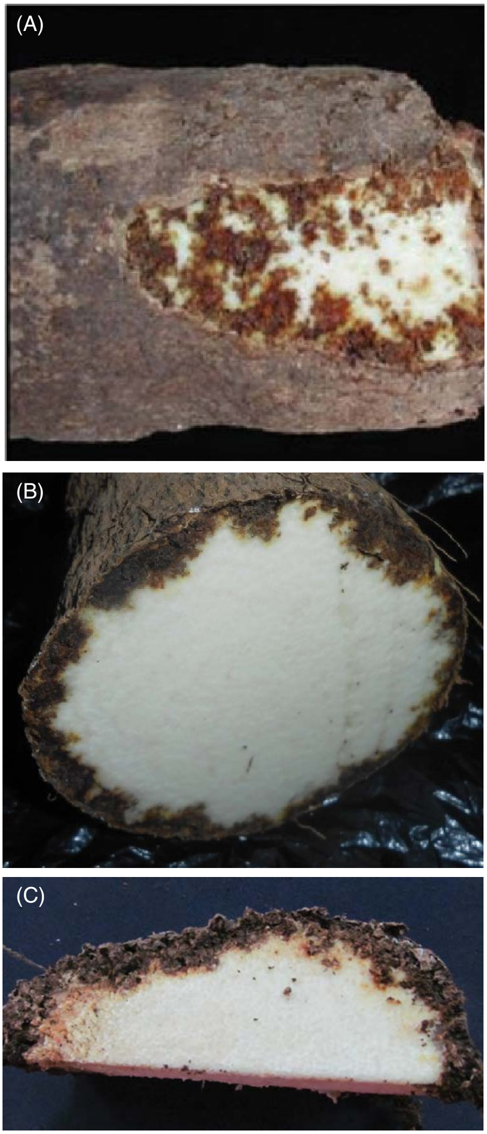

Fig. 52.3 **(A)** Brown lesions induced by *Scutellonema bradys* below the skin of a yam tuber. Photograph courtesy of D. Coyne. **(B, C)** Dry rot of yam tissue with flaking skin caused by *Scutellonema bradys*. Author's own photograph.

Rhizopus spp. are also associated with rotting tubers with initial dry rot symptoms indicating that they may become 'attracted' to the tubers as a result of the products of decomposition from nematode activity. Both the yam and lesion nematodes are sometimes found co-infecting yam tubers with dry rot symptoms. Symptoms of dry rot and galling are observed together in tubers from simultaneous infection with *S. bradys* and *Meloidogyne* spp.

Recommended integrated nematode management (INM)

The majority of the farmers in the region are subsistence farmers who have a tendency to consider costs of management as the major criterion in making the choice for a management option. Their situation is not improved due to the knowledge gap about nematode diseases and the need for their management. Therefore, farmers in the region have relied mainly on traditional methods with a few adopting 'modern' management options as a result of extension efforts.

Resistance to *S. bradys*, a low-cost option, has remained elusive in spite of the wide search among the main food yams with the exception of *D. dumetorum* (bitter yam), a less preferred species. This leaves cultural methods and use of nematicides as the main approaches to *S. bradys* management in the region. Since the main method of spread of the nematode is via tubers, one of the most effective methods for managing the dry rot disease is the use of nematode-free planting material.

Seed tuber selection is most practiced among growers in which tubers showing symptoms of dry rot (cracking and flaking) are avoided as planting material. Mild to moderate symptoms may not be externally observable; however, the presence of dry rot in tubers can be detected by light peeling of the tuber skin to expose the tuber tissue for inspection. Symptoms, even when mild, are easily observed when cut setts are used because the lesions become exposed and the infected sett/tuber can be separated from the lot for planting.

Pre-treatment of setts for seed yam production with a pesticide cocktail (insecticide/nematicide + fungicide) as a dip for 3–5 minutes has proved effective for the production of disease-free planting material with a consequent increase in yield (Claudius-Cole *et al.*, 2017). Recommended nematicides were ebufos and carbosulfan, insecticides were diazinon and chlorpyrifos while mancozeb was the recommended fungicide. Farmers willingly practice this method in combination with seed selection as opposed to the traditional treatment of dusting cut yam surfaces with wood ash. This is in spite of challenges due to the inconsistent availability of recommended active ingredients which differ across the region, and between years.

Production of disease-free planting material through high throughput techniques such as tissue culture, vine cuttings, aeroponics and semi-autotrophic hydroponics (SAH) are relatively new methods of generating healthy tubers for yams. The SAH is a propagation system that uses vines from disease-free botanical seed. The vines are planted in sterilized potting media and supplied with nutrients to obtain large quantities of mini-tubers. Tubers produced from these technologies are targeted for germplasm conservation, seed yam production and germplasm exchange between countries in the region for seed production rather than ware yams for the market. Selected farmers have been trained in these techniques in the region with the aim of encouraging dedicated seed yam producers who will provide disease-free seed to yam farmers.

Hot water treatment of yam tubers at 50–55°C for 25–40 minutes has been practiced by research institutions and enterprises that provide nematode-free planting materials for farmers. The method is used for both seed and ware yam to maintain tuber health. Treatment during/after storage appears to produce better effects than treatment at harvest due to increased rotting of tubers. The cost of the heating equipment, and the difficulties of maintaining constant temperatures, are the main constraints that prevent its use among subsistence farmers in West Africa. Furthermore, some cultivars appear sensitive to hot water treatment and may not sprout after treatment (Coyne *et al.*, 2010).

Yam is usually the first crop in rotation after a fallow, which makes recommendations for crop rotation tricky, especially with increasingly shortening fallows and challenges with access to land in the region. Options of crops/plants to use in rotation are few due to the risk of increasing populations of *Meloidogyne* spp. *Scutellonema bradys* management can be accomplished in this system with cover crops like *Centrosema pubescens*, *Pueraria phaseoloides*, *Mucuna pruriens* (*utilis*), *Stylosanthes guianensis* and *Tagetes erecta* (Claudius-Cole *et al.*, 2016) as intercrops or rotation crops. Susceptible crops like sesame, okra, cowpea and tomato usually planted as intercrops should be avoided. Similarly, weed hosts like *Commelina*, *Eupatorium*, *Synedrella* and *Chromolaena* should be removed.

Field application of nematicides for nematode management in yams is not widely practiced by growers in the region. Availability and quality of pesticides combined with highly variable understanding of pesticide use by farmers pose an on-going challenge in West Africa. Registration of new chemical actives for pest management in the recent decade has generally left nematicides behind in the region. Using Nigeria as an example, carbofuran has been the only registered available nematicide for years until recently. Although carbofuran is highly regulated/banned in most countries, it finds its way into many West African countries even though it is highly toxic and appears no longer effective. The recently registered low-dose fluopyram is effective; however, the available packaging size is very expensive for non-commercial farmers who require smaller quantities.

Several plant extracts have been identified for nematode management for field applications and as seed treatments but are yet to be commercialized. Growers sporadically use aqueous extracts of what is available in their location, usually neem, as recommended by extensionists. However, few packaged products with clear recommendations are available on the markets in the region.

Optimization of nematode management

The general approach for *S. brady* management is: (i) interrupting the nematode spread with seed tubers and (ii) breaking the nematode life-cycle in soil. The use of healthy plant material and host plant resistance are the best options for tackling the first approach. While resistance development is in progress, healthy seed material generated from high throughput methods, including *in vitro* tissue culture, vine cuttings aeroponics and semi-autotropic hydroponics, can be developed as a package for uptake by the private sector. The development of biological control agents to treat soil and seed has huge potential in the region with the diversity of such organisms available in the tropical soils. Strains of Actinobacteria, endophytic fungi, mycorrhizal fungi and *Trichoderma* spp. have been applied effectively for the management of *S. bradys* in studies (Tchabi *et al.*, 2016). Upgrading their delivery method and improved persistence using nano techniques or combinations with organic amendments will foster development of products for large-scale use. Although West Africa is catching up with nematicide use, especially for yams, efforts to tackle barriers in stewardship, safety and suitable packaging that hinder willingness to register or develop nematicides in the region need to be doubled.

Future research requirements

One of the most effective and practical methods of producing nematode-free yams is the preplant seed dip method. This method, though effective, is challenged with the inability to recommend a safe nematicide in the formulation required to dip, and the availability of such a nematicide in the region. Even when the nematicides are available, the quantity packaged is often too large and too expensive for most subsistence farmers to obtain. Nematicide development has advanced worldwide, and recently less toxic and low-dose formulations have become available. However, these nematicides have not found their way to the markets in West Africa. Therefore, there is a huge research gap of information on the performance of recently developed nematicides on the yam–*Scutellonema* relationship. Identified resistance in *D. dumetorum* and other wild *Dioscorea* species should be explored in breeding programmes to develop resistance in edible yams using conventional and advanced procedures. Indigenous biological and botanical agents for nematode management have been identified in the region, with some targeted specifically at the yam nematode. Rather than stop at identification, further research to understand mechanisms, persistence in soil and effective formulations should be the next focus. Yams are exported from the region to other parts of the world. Therefore, for phytosanitary purposes, studies on irradiation may be considered for eliminating the yam nematode in tubers to be exported. This method has the potential to eliminate issues of the presence of the pest on tubers as well as possible challenges of pesticide residues. Not strictly research, but of major concern in West Africa, is the knowledge gap of farmers about nematode pests and the need for their management. For research outputs to effectively reach and be utilized by the target growers, extension bodies need to be strengthened to provide growers with information on the importance of nematodes as a yield-limiting stress, how to recognize their symptoms and what management practices are available.

Outlook: anticipating future developments

In many African countries, where food security and poverty remain a concern, crop losses associated with nematode pests could result to a threat to the source of livelihood of many resource-poor farmers. This bears consequences on the economy and foreign exchange earnings of the developing nations. Dry spells already appear during the season and if trends continue, they will get longer and more frequent with increased possibilities of sporadic flooding. Yam is a long season crop (8–10 months) and the growing period translates to production of larger tubers meaning that tuber sizes may reduce as planting date extends. This will cause an increase in the cost of production of smaller tubers, affecting both income security for the grower and food security for consumers. In addition, re-prioritization or complete change of favoured cultivars by farmers in the region have been observed due to what growers claim to be ‘bad soil’ or susceptibility to disease, thus leading to loss of germplasm. Loss of germplasm and diversity in the region can impact on the resilience of the farming communities in terms of food and income security. Coyne *et al.* (2012) identified various strains of *S. bradys* with different levels of pathogenicity on yams. Environmental pressure is known to have a selecting effect on aggressive species. The implication of the forgoing is that yam productivity may continue to dwindle due to shortened cycles combined with pathogen aggression, not only of *S. bradys* but also with root-knot nematodes. A typical scenario is the resistance-breaking *M. enterolobii* on yams and other crops in the region. These conditions may also influence the development of new/emerging pests as has been observed in cassava with high populations of *Gracilacus* spp. causing marked reduction in feeder roots and tuber yields in Nigeria.

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53 The resilient cassava: Undermined by root-knot nematodes

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Introduction

Cassava is, with good reason, viewed as a hardy, resilient crop – one that perseveres in the face of adversity and provides a yield even under the most extreme conditions. It is also traditionally regarded as being particularly resistant to pests and diseases. Historically, it has therefore been viewed primarily as a subsistence crop, although it is extensively produced as a cash crop, as an urban food staple, as well as for industrial uses (Nweke *et al.*, 2002).

Growing cassava successfully relies upon climate, soil type, vegetation, topography, degree of mechanization, availability of labour and the traditional cropping system. The plant prefers well-drained soil and modest rainfall, but it can survive where soils are wet. Cassava roots do not tolerate freezing temperatures and it grows best in full sun. The best and commonly used planting materials are stem cuttings of 20–25 cm long with five or six nodes (Adekunle *et al.*, 2005). These are planted about 1 m apart, by inserting two-thirds of the cuttings vertically, at an angle of about 45° or by placing the entire cutting horizontally in the soil at a depth of ~10 cm. The crop develops a woody, spindly aerial architecture with much leaf cover above ground and storage roots below ground (Fig. 53.1). In some cultures the leaves are important as a leafy green vegetable, while the storage roots are the main focus for production.

With respect to nematodes, cassava is often viewed as having no nematode problem. Indeed, even seasoned nematologists are taken aback by the notion that cassava is affected by nematodes. While folklore respects cassava for its resilience, the reality is that no crop is immune to nematode infection, including cassava. The myth that cassava is immune is probably not without reason, however. Cassava roots are naturally knobbly and distorted, which casually disguises the damage caused by root-knot or other nematodes. Cassava has traditionally also been cultivated under marginal conditions, with stems planted and left to survive. Those genotypes that survive, therefore, have also overcome, and possibly adapted to, local conditions and prevailing pests, aiding the selection by farmers of those genotypes most resistant and tolerant to nematodes.

This chapter dispels the myth, placing cassava alongside other crops as a susceptible host to nematode pests. It provides an overview of the damage caused by root-knot nematodes, the main pest, as well as how seriously they can undermine cassava production and impact the quality of cassava storage roots.

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Fig. 53.1. Harvesting a cassava crop growing in Kenya, East Africa. Author's own photograph.

Cassava is a tropical semi-perennial crop that takes at least 6 months to harvest from planting but more often is harvested from 9 months onward, depending on genotype, climate and soil conditions, etc. Storage roots can be harvested piece-meal by removing roots on a regular basis, while leaving the plant to continue growing, effectively *ad infinitum*. It is also harvested en masse, especially in commercial plantations, by uprooting the whole plant and removing the whole storage root yield per plant. Once harvested, storage roots deteriorate quickly and need to be prepared, processed or eaten within a few days. In tropical smallholder systems, cassava is principally grown on small plots, between 0.5–2.5 hectares for home consumption or local sale for household income. However, cassava is also an important source of starch and ethanol, for which it is produced on an industrial scale in large-scale commercial plantations across the tropics. In Africa, cassava is the most important of all root and tuber crops as a source of food for both humans and livestock. Nigeria is the world's largest producer of cassava with a production of over 47 million tonnes (MT) per year, followed by Thailand (~30 MT), Indonesia (~24 MT) and Brazil (~21 MT). Under intensive commercial situations with optimal cropping conditions, cassava yields can reach 80 tonnes per hectare, compared to the global average yield of just 12.8 tonnes (FAO, 2013).

Although revered as a hardy crop that produces even under the most challenging conditions, it also responds well to a favourable climate, good agronomy and more commercial production conditions. In terms of pests and diseases, it is prone to a number and can be heavily affected, such as by the African cassava mosaic virus disease, increasingly the cassava brown streak virus disease and cassava mealy bug. Less well known is its susceptibility to root-knot nematodes (*Meloidogyne* spp.). Early infection of roots by root-knot nematodes will lead to their deterioration and death, which, over the course of the cassava maturation ultimately leaves little indication of root-knot nematode infection by the time of harvest, if all roots have died back (Coyne and Affokpon, 2018). To the casual observer, the naturally distorted roots of cassava also likely disguise the knots and galls caused by nematode infection.

Economic importance

In general, nematode problems on cassava tend to be overlooked and ignored although there is substantial documentation of nematodes attacking cassava, dating back some time. Reports on the level of nematode damage to cassava and their impact vary, with some indications of almost total destruction of fields (Théberge, 1985). With growing evidence of the damage they inflict, there is an increasing interest in root-knot nematode infection of cassava towards addressing the issue. This reflects the need to reduce in-field losses on the one hand, but also to improving post-harvest quality and the all-important need to extend the storability of storage roots. Recently, the nutrient quality has been shown to be substantially reduced following nematode attack, such as in biofortified cultivars. Biofortified cassava cultivars with high levels of pro-vitamin A carotenoid content have been developed by conventional plant breeding methods and released for use in Nigeria and the Democratic Republic of the Congo (HarvestPlus, 2012). These biofortified cultivars are aimed at addressing vitamin A deficiency, an important public health problem in sub-Saharan Africa.

As with most crops, attributing direct yield losses to a single element under field conditions is difficult to determine, more so under tropical conditions and especially in smallholder systems. Losses from individual components may therefore be overlooked or may even be confused with and/or disguised by the presence of other constraints. Consequently, the variable conditions under which cassava is grown needs to be considered.

Numerous controlled pot experiments, however, have demonstrated the potential damage that root-knot nematodes are capable of inflicting. Damaged root systems, stunting of plants, reduced aerial growth and lower storage root yield have all been observed. In the field, correlation with nematode densities is less clear. The correlation of nematode densities at harvest with yields has been one method for relating damage to the pathogen. But it appears that with high levels of infection early in the season, root damage can be high, with roots deteriorating and dying, leaving few feeder roots (and nematodes) for assessment at harvest, masking the damage caused by nematodes, giving a false correlation. Comparing yields between sterilized or nematicide treated plots, with untreated control plots where there are relatively high nematode densities have worked though. By creating significant differences in nematode densities early in the season a reasonable comparison can be made. In Nigeria, yield increases as high as 200% were recorded following solarization in farmers' fields (Abidemi, 2014), while yield differences of 64% following nematicide treatment provided convincing demonstrations on the correlation between nematode presence and yield (Akinsanya *et al*., 2020).

One reason behind the general view of cassava being unaffected by nematodes may stem from the traditional use of landraces and long-term local cultivars. These preferred cultivars are selected over many years and generations of farmers, for suitability and ability to survive and produce under local conditions, including exposure to nematode infection. Following the development of cassava breeding programmes to breed for improved genotypes, numerous improved cultivars have been and are currently being developed and released. Improved cultivars developed in one geographical area and distributed to another may not necessarily be adapted to the local conditions, including nematode populations. A good example was the development of elite lines in Nigeria, which were then distributed across Africa for suitability. In Mozambique, lines that performed well in Nigeria were found to be highly affected by root-knot nematodes. They performed poorly, were heavily damaged and were not selected further (see Coyne and Affokpon, 2018). Root-knot nematodes can consistently undermine cassava production and as a group pose a critical threat to tropical crop production as a whole (Coyne *et al.*, 2018).

Host range

Meloidogyne incognita and *M. javanica* are among the most polyphagous plant parasitic nematodes known. They can be found infecting an extremely wide range of crops and non-crops across the tropics and sub-tropics. Consequently, apart from posing a formidable threat to the production of numerous economically important crops, their broad host range creates a major obstacle to their management.

Distribution

The root-knot nematodes most commonly recorded from cassava are *Meloidogyne incognita* and *M. javanica*. These two key species have a pan-tropical distribution, as well as also being among the most commonly occurring root-knot nematodes under tropical conditions. They are both found wherever cassava is grown. Other species, such as *M. arenaria, M. enterolobii* and *M. hapla* have been reported but much less. The diversity of root-knot nematodes infecting cassava has not been extensively assessed. However, many reports refer simply to *Meloidogyne* spp. without identification of the species, so there remains the possibility that other species of root-knot nematode occur on cassava.

Symptoms of damage

The most typical and characteristic symptom is the galled, distorted or knotted feeder roots following root-knot nematode infection (Fig. 53.2). Other symptoms can include wilting and stunted growth but which are not always obvious or present. It is therefore necessary to uproot plants to determine whether root-knot nematodes are infecting the plant. Because of the natural contorted architecture of cassava roots, root-knot nematode damage is not always immediately obvious without close inspection. However, due to the extended period in the ground it is very much suspected that infected roots deteriorate after some time, die and decompose, such that at harvest there may be few roots to provide any evidence of infection. On storage roots, symptoms are sometimes visible with galls 'bubbling' on the surface or forming encrustations, which present a rough surface (Fig. 53.3). Sub-cortex necrosis on storage roots is rare.

This is quite likely genotype dependent but is not very common. These symptoms can also be accompanied by sub-surface brown discoloring or necrotic patches, which can be seen if a thin slice is removed using a knife. In addition to subterranean symptoms, non-distinctive above-ground symptoms, such as stunting, reduced girth width and reduced foliage may occur and can cause wilting. Reduced sprouting and establishment of stems can occur under

Fig. 53.2. Cassava roots heavily galled following root-knot nematode infection. Author's own photograph.

Fig. 53.3. Cassava storage roots encrusted with galls due to root-knot nematode infection. Author's own photograph.

heavy infestations. To detect this and visibly observe the differences, however, it may be necessary to have infected versus non-infected plots growing side-by-side. Ultimately, yield, marketability and quality of storage roots is reduced, with losses generally increasing with the level of nematode infestation. Near total losses due to root-knot nematode infection have been observed (Théberge, 1985). Makumbi-Kidza *et al.* (2000) demonstrated that storage root formation in cassava is initiated when plants are 1–2 months old. At this time, young cassava plants are most prone to root-knot nematode damage, and they determined from their study that yield loss is caused by root-knot nematode reduction of storage root number rather than a reduction in individual storage root weight. When infected with root-knot nematodes, improved, biofortified cultivars also had reduced nutrient quality (Akinsanya *et al.*, 2020).

Biology and life cycle

The life cycle of root-knot nematodes on cassava reflect very much a similar pattern as in other crops. *Meloidogyne incognita* and *M. javanica* are common species with life cycles that differ little between each other and between crops. Typical duration is 30–40 days depending on climatic and edaphic conditions. In the thicker, woody roots of cassava, the egg masses produced by the females, are more likely to be embedded within the tissue, while on the finer, more tender feeder roots, egg masses will be exposed, and found extruding from the roots.

Interactions with other nematodes and pathogens

As demonstrated for other crops, it is anticipated that cassava is similarly more prone to pests and diseases when infected with root-knot nematodes. Of particular note, however, is the increased incidence and level of rots associated with nematode infection. High levels of rots have obvious implications to the quality and marketability of storage roots but rots also affect the in-ground longevity and as well as post-harvest storability of the storage roots. Furthermore, as enhanced levels of storage root rot damage can indeed be an indirect consequence of root-knot nematode infection, the management of these nematodes can, by default, reduce rot incidence. Infection of cassava with root-knot nematodes therefore has broader implications to storage and post-harvest quality, than direct root yields.

Recommended integrated nematode management (INM)

Although *M. incognita* and *M. javanica* are highly polyphagous, crop rotations between different types or botanies of crops will help to suppress nematode densities, as the populations tend to be influenced by the prevailing crop. The continuous cultivation of cassava will influence *M. incognita* and *M. javanica* populations to become increasingly adapted to cassava. Mixed cropping with antagonistic plants, such as *Tagetes* spp. (marigold), or rotations with different crops will help suppress the build-up of populations that are aggressive to cassava. The reality, however, is that managing root-knot nematodes is challenging and complex, given their recalcitrant nature and polyphagous feeding ability. The use of nematicides is not a practical consideration for smallholder farmers given their limited investments to the crop in the first instance. For more commercial systems, using costly nematicides would be an economic consideration, depending on the level of infestation. However, the limited understanding of the damage caused to cassava production by nematodes will restrict the use of nematicides or any nematode management practice for that matter. The use of resistant material is therefore advised wherever possible as a primary foundation to underpin the suppression of nematode damage. This, however, requires that root-knot nematode pests are treated as a serious threat and become a focus for breeding programmes. It also requires knowledge on the prevailing nematode species.

Optimization of nematode management

Optimal management of root-knot nematodes currently depends on the identification and deployment of cultivars with resistance against the key species. This is an effective and attractive management strategy for cassava. Ideally, of course, such resistance needs to be integrated together with additional resistance traits, as well as consumer preferences. In addition, crop rotation practices, using crops or fallows that are suppressive against root-knot nematodes will be important in limiting the build-up of aggressive populations. However, this all relies on the improved awareness of nematodes as pests.

Future research requirements

The optimization and development of appropriate and adoptable management mechanisms is dependent on the involvement of nematology as a discipline in research programmes. Consequently, the development of resistant cultivars also requires that nematology is included as a component within breeding programmes. In this respect, the integration of nematode resistance, especially multiple species resistance, should be a key future objective as the intensification of cassava production using high-yielding cultivars advances. The association of root-knot nematodes with post-harvest quality and storage of cassava raises important concerns, however, which require particular attention. This is especially noteworthy, given that the improvement of cassava storability is a highly sought trait to breed for. This is in addition to the current efforts to improve the nutritional quality of cassava, which is undermined when infected by root-knot nematodes.

Future research should also consider the roles of other nematode genera that are presently found in association with cassava, but which are either considered unimportant or have unknown implications to cassava production. Such broader integration of nematology in plant health research and breeding ultimately relies on improved awareness of nematodes as economically damaging pests.

Outlook: anticipating future developments

The link between nematode infection and storage root quality and post-harvest storability provides an important aspect that may create more focus on nematodes as a constraint to cassava production. This is not simply as yield-suppressing pests, but as a factor affecting storage root quality, which is a focus for breeding programmes. However, if sustainable nematode management is not ensured on time, high levels of nematode damage can be envisaged on cassava, as the crop becomes more intensively cultivated. Losses due to root-knot nematodes are inevitably likely to increase before their importance as a threat is properly realised.

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54 The stem nematode *Ditylenchus dipsaci* in sugar beet: A species of extremes

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Introduction

The stem nematode *Ditylenchus dipsaci* is a migratory endoparasitic nematode of worldwide importance. In 1857, Julius Kühn was the first to discover *D. dipsaci* infesting the heads of Fuller's teasel, *Dipsacus fullonum*, in Bonn, Germany (Sturhan *et al.*, 2008). *Ditylenchus dipsaci* was long considered as a species with up to 30 different host races with specific host crop spectra (Kühnhold, 2011). However, more recent phylogenetic studies showed that isolates from agricultural plant species, including sugar beet, should be considered as *D. dipsaci* sensu stricto (Subbotin *et al.*, 2005). *Ditylenchus dipsaci* is regulated as a quarantine species in many countries and classified as a regulated non-quarantine pest in the European Union, to avoid further spread of this nematode by infested seeds or planting material. Nevertheless, research on this species is still limited although it is ranked fifth in the top ten list of plant parasitic nematodes (Jones *et al.*, 2013). This leaves plenty of room for new research on developing measures for the integrated management of this nematode.

Economic importance

Economic damage due to *D. dipsaci* mostly occurs in temperate climate zones. It causes significant losses on onion, garlic and ornamental bulbs, because infected crops are unmarketable (Jones *et al.*, 2013). The stem nematode has been reported to cause yield losses as well as an increase of secondary products undesirable for industrial sugar production in sugar beet (Castillo *et al.*, 2007). Yield losses can range between 10% and 60% in Germany and Switzerland, but under optimum conditions for the nematode (cool and moist), 90–100% is possible. However, next to the yield loss, if more than 10% beets show crown rot symptoms the complete harvest will be rejected by the sugar factory (Storelli *et al.*, 2020). Germany and Switzerland consider *D. dipsaci* to be of high economic importance in their main sugar beet production areas. Other countries who reported infections and damage to sugar beet by *D. dipsaci* never reported these high levels of economic damage (Castillo *et al.*, 2007).

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Host range

Ditylenchus dipsaci has an extremely wide host range with around 500 host plants belonging to over 40 plant families. With this spectrum, *D. dipsaci* belongs the group of plant parasitic nematodes with the widest host range worldwide. In addition, the nematode infects blossoms, seeds, stems, leaves as well as tubers, stolons and rhizomes and surpasses the range of damage by the polyphagous species *Aphelenchoides ritzemabosi* and *A. fragariae* (Sturhan *et al.*, 2008).

Distribution

Ditylenchus dipsaci is cosmopolitan with a worldwide distribution, but the main problems are present in temperate regions with significant importance in field and horticultural crops. Several countries, including Spain, Canada, France, England, Hungary, Iran, Morocco, Netherlands, Romania, Serbia and Ukraine have reported infestations and damage on sugar beet by *D. dipsaci* (Castillo *et al.*, 2007). However, only Germany and Switzerland repeatedly report severe damage occurring in their main sugar beet production areas (Kühnhold, 2011; Storelli *et al.*, 2020).

Symptoms of damage

Ditylenchus dipsaci symptoms on sugar beet differ depending on the level of infestation, environmental conditions and the developmental stage of the plant. Early spring symptoms can be swellings and distortions on seedlings (Fig. 54.1). Later in the season, small white callus pustules can appear on the surface of the beet crown (Fig. 54.2). These pustules contain thousands of nematodes per gram tissue and are a clear indicator for high levels of infestation in a given field. Later in the season, crown rot develops without visible symptoms such as yellowing or wilting of leaves (Fig. 54.3). In some cases, *D. dipsaci* infestation can be confused with crown and root rot caused by *Rhizoctonia solani*, which appears on the outside on the upper region of the beet body and at the base of the leaf petioles (Hillnhütter *et al.*, 2011). At harvest time, crown rot symptoms increase until complete decay of the sugar beet plant occurs (Figs 54.4 and 54.5).

Fig. 54.1. Early season symptoms of *Ditylenchus dipsaci* damage in sugar beet seedlings, often appearing after cool and moist conditions in spring. Photograph courtesy of Julius Kühn Institute.

Fig. 54.2. Sugar beet showing callus pustules due to heavy *Ditylenchus dipsaci* infestation during mid-season. Photograph courtesy of Julius Kühn Institute.

Biology and life cycle

Mainly fourth-stage juveniles (J4), the survival stage of *Ditylenchus dipsaci*, penetrate above-ground plant parts early in the growing season and feed upon parenchymatous tissue leading to the breakdown of the middle lamellae of cell walls. By withdrawing cell contents through its stylet, the surrounding cells divide and enlarge, which results in malformation of the affected plant tissue (Jones *et al.*, 2013). Reproduction of *D. dipsaci* is by amphimixis and multiplication rates are very high, as a female of *D. dipsaci*

Fig. 54.3. Crown rot developing inside a sugar beet due to heavy *Ditylenchus dipsaci* infestation during mid-season. Photograph courtesy of Julius Kühn Institute.

Fig. 54.4. Crown rot damage due to *Ditylenchus dipsaci* at the time of harvest. Photograph courtesy of Julius Kühn Institute.

Fig. 54.5. Rotten beets due to *Ditylenchus dipsaci* at the time of harvest. Photograph courtesy of Julius Kühn Institute.

produces up to 500 eggs when the temperature is in the optimum range of 15–20°C. Second-stage juveniles hatch within 2 days and further develop into females within 4–5 days, which can live for more than 10 weeks (Jones *et al.*, 2013). *Ditylenchus dipsaci* is a classic example of a species surviving severe desiccation in a dormant state. At the end of the sugar beet growing season, development stops at the J4 stage. These J4s either leave the rotten beets and move to the soil or coil and clump together in a desiccated state as 'nematode wool' and stay in dried plant debris, where they overwinter (Jones *et al.*, 2013). J4s of *D. dipsaci* have been reported to survive for more than 20 years in this anabiotic state (Sturhan *et al.*, 2008; Jones *et al.*, 2013). This extreme capacity to survive desiccation and freezing temperatures also helps to withstand nematicides and facilitates dispersal by plant debris and wind. *Ditylenchus dipsaci* has a very low damage threshold level with only 1–2 juveniles per 250 ml of soil. Egg production is linearly related to temperature, with 0.158 eggs per day degree under optimal conditions (Jones *et al.*, 2013). This rapid population growth results in severe crop damage, even when the initial population density is low.

Interactions with other nematodes and pathogens

Plant parasitic nematodes can interact with other plant pathogens in many ways. Mostly, this interaction results in synergism that influences the level of damage to the plant. Growers are confronted with the interaction of *D. dipsaci* and *R. solani*, both causing crown rot symptoms. *R. solani* benefits from *D. dipsaci* and not vice versa, as the nematode provides penetration points for the fungus (Hillnhütter *et al.*, 2011). As *D. dipsaci* is an obligate biotrophic and cannot multiply on rotten plant tissue, high levels of infection by *R. solani* negatively affect nematode reproduction. Field trials demonstrated that sugar beet cultivars tolerant to the sugar beet cyst nematode *Heterodera schachtii* allowed high reproduction rates of *D. dipsaci* with severe crown rot symptoms. When both *H. schachtii* and *D. dipsaci* are present in a field, they do not interfere with each other as they have different distribution patterns (patchy versus broad) and optimum temperature ranges with higher temperatures leading to greater damage by *H. schachtii*. Conversely, *R. solani* tolerant cultivars also showed tolerance to crown rot induced by *D. dipsaci*, but did not mitigate reproduction (Hillnhütter *et al.*, 2011).

Recommended integrated nematode management

The first recommendation after detection of *D. dipsaci* severely damaged sugar beets in a field should be: 'Stop growing sugar beets'. In many cases this is not possible because sugar beet production areas have to be close to the sugar factory and quotas of beets to be delivered are pre-set. However, in this case other recommendations to mitigate yield and quality losses due to *D. dipsaci* can be made as listed below.

- Delayed planting helps to avoid cool and moist conditions in the spring, which will result in reduced damage by *D. dipsaci*, but also affects overall root and sugar yield due to a shortened cropping season.
- Early harvest is recommended when fields have a known history of *D. dipsaci* and crown rot. These beets have to be processed immediately after harvest as the nematode still causes severe levels of root rot when beets are in field storage before processing. However, harvesting early in the season, again, reduces overall root and sugar yield.
- Use of highly tolerant cultivars, which do not develop severe crown rot symptoms even under high *D. dipsaci* pressure. This measure, together with delayed planting gives the best protection against severe losses due to the nematode fungus beet rot complex. It can, however, lead to the lowest yields when conditions for the nematode are unfavourable due to the fact that the cultivars only yield well when nematode damage occurs.
- Crop rotation is difficult to apply due to the extreme wide host range and extreme capability of *D. dipsaci* to survive in soil and on infested plant material for many years. However, several crops such as barley and triticale or non-host intercrops such as ryegrass can be chosen that are poor or non-hosts, suitable to reduce population densities in soil. In addition, good weed control is critical, as weeds are very good hosts for *D. dipsaci* and can help to maintain population densities above the threshold level (Sturhan *et al.*, 2008).

Optimization of nematode management

Farmers often neglect the fact that *D. dipsaci* has an extremely wide host range and can multiply on many crops without causing symptoms of damage or notable yield loss. In addition, a range of common names is used for the same species. Therefore, it is often not realized by the growers that this single species attacks several field and vegetable crops within their crop rotations. To determine the risk of damage before a field is chosen for the production of sugar beet, soil sampling in the autumn before the next sugar beet crop is advised. However, farmers do not use this tool, but rather follow historic or personal preferences to choose their fields, which consequently results in severe losses and rejection of their harvested beets at the sugar factory. Intensive soil sampling should therefore be used, as it is the only measure to detect high-risk fields with population densities above the threshold of 1–2 nematodes per 250 ml of soil. Data from soil sampling should be used to develop a software-based nematode advisory system in support of crop advisers and extension personnel.

Application of nematicides is no longer an option because of the past phase-out of the chemicals in the EU. Furthermore, recent studies demonstrated that *D. dipsaci* showed a low sensitivity to the nematicide fluopyram (Storelli *et al.*, 2020). Although fluopyram applied at planting reduced initial penetration rates under field conditions, it failed to suppress the reproduction of the nematode until harvest. Consequently, development of crown rot symptoms was not reduced (Storelli *et al.*, 2020). This leaves few options for control besides the use of cultivars highly tolerant to secondary infection by *R. solani* and thus to the development of crown rot symptoms.

Future research requirements

There is a need for molecular analysis of soil samples for the nematode because of the low threshold level and difficulty in detecting such population densities (see Chapter 57 in this volume).

Few studies on the molecular basis of the interactions between *Ditylenchus* spp. and host plants have been published (Jones *et al.*, 2013). In particular, little is known about *D. dipsaci* penetration of seedlings and how it interacts with cultivars carrying tolerance to *R. solani*. A better understanding of the factors affecting the successful penetration of the host and the development inside the beet root is required to develop new and effective control options. Genome and transcriptome sequencing will help to better define targets for breeding for resistance or for other means of control. In order to effectively manage *D. dipsaci* in the future, the focus should be on developing a breeding programme for resistant sugar beet cultivars. To date, the classical breeding approaches have not yielded resistant, but only highly tolerant cultivars (Kühnhold *et al.*, 2006; Kühnhold, 2011). However, breeding programmes might focus on different objectives that target reduced attraction to the seedling, penetration and reproduction rates in addition to suppressing crown rot symptoms.

Outlook: anticipating future developments

Farmers and sugar factories will face many problems in the future. The declining market prices for sugar forces companies to concentrate their production on fields close to the factories to minimize costs for transport. These fields, however, are already infested with *D. dipsaci*, which leaves no choice, but to intensify the search for new control measures.

Increasing temperatures due to global warming might benefit sugar beet production as warm and dry conditions in spring or hot and dry conditions in summer will negatively affect the penetration and development of *D. dipsaci* and thereby will reduce crown rot symptoms. However, future breeding programmes must address these conditions in developing new cultivars adapted to the growing region and producing high root and sugar yields. As *D. dipsaci* is a species of the extremes, it might adapt to these conditions and spread to other regions of the world where sugar beet production will be intensified in the future. Strict enforcement of quarantine regulations is therefore necessary to keep *D. dipsaci* out of these regions to avoid future severe economic losses.

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55 The beet cyst nematode (*Heterodera schachtii*): An ancient threat to sugar beet crops in Central Europe has become an invisible actor

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Introduction

The beet cyst nematode (BCN) was one of the first discovered plant parasitic nematodes. *Heterodera schachtii* was observed in 1859 in Halle in Central Germany by the botanist Herman Schacht and described later by Adolf Schmidt in 1871, who named this cyst nematode species after its original discoverer. At about the same time, Julius Kühn provided proof that BCN reduced plant growth and suppressed yield of sugar beet, symptoms that were previously called 'beet weariness'. Partly due to the lack of knowledge about the effect of sugar beet monocultures on the population build-up of BCN, this nematode had a devastating impact on sugar production in 1876 that led to the shutdown of 24 sugar factories in Germany (Hallmann *et al.*, 2009).

Economic importance

In Europe where awareness of BCN problems has a long history, estimates of the percentage of infested sugar beet growing area is largely based on personal experience of experts and rarely on systematic surveys that are mostly conducted on national scopes. Combining both information sources, about 10–20% of the European sugar beet growing area representing more than 164,000 ha (Eurostat, 2020) on average, can be assumed as BCN infested. In the core production areas of Europe (France, Germany, Netherlands and Belgium) with intensive cultivation of sugar beet, local BCN infestations have a long history and might locally exceed 50% of the regional sugar beet areas. Over the period 2010–2020, sugar beet production experienced a significant change and high-yielding tolerant cultivars became available to growers. Thus older approximations on economic impact of BCN are probably not up to date, though in 1999 economic damage of BCN in Europe was estimated to be up to €90 million annually (Müller, 1999).

Traditionally used standard sugar beet cultivars were susceptible and sensitive to BCN. To control population build-up of BCN, resistant sugar beet cultivars were available but unattractive for growers due to their low yield potential. Both cultivar types were gradually replaced by a high-yielding sugar beet cultivar with tolerance to BCN that was introduced in 2004. Cultivation of

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tolerant cultivars gradually increased primarily in regions with well-known BCN infestation levels, reaching over 80% in some regions. Between 2004 and 2020, sugar yield per hectare in Germany approximately increased by 25% and is currently at an average of 13.4 t/ha (VDZ/VWZ, 2020).

Host range and distribution

Heterodera schachtii is globally distributed and occurs mainly in temperate but also in Mediterranean and sub-tropical climates. Its presence is confirmed in 87 countries across all continents (CABI, 2020). While BCN is associated with 218 plant species, it is mainly reported to reproduce on brassica crops (*Brassica napus, B. rapa, B. oleracea, Raphanus sativus, Sinapis alba*), chenopodium crops (*Spinacia oleracea*) and economically it is most important on beets (*Beta vulgaris*), where it causes significant damage to sugar beet (Turner and Subbotin, 2013; Hemayati *et al.*, 2017).

Symptoms of damage

Damage results from water and nutrition deficiency in plants, as a response to disturbed root growth that is caused by nematodes penetrating the roots. As a result, affected sensitive cultivars show wilting and stunting of plants. In heavy BCN infestations, older leaves show yellowing as a consequence of manganese deficiency. As BCN penetrate roots, plants try to compensate for the damage caused by the development of lateral roots. In sensitive genotypes, this appears as root bearding (Fig. 55.1). Damage is most severe for seedlings up to 2 months after sowing. The presence of BCN at later growing stages is largely tolerated by sugar beet. Thus the extent of damage is mainly determined by the initial population density (*Pi*) of BCN and the sowing conditions in spring, specifically sowing time, water availability and temperature. Sugar beet can tolerate a BCN population pressure below a certain damage threshold. A succession of field trials (2007–2011, unpublished data) conducted at the Julius Kühn Institute field station in Elsdorf (Germany) showed the relation between *Pi* densities and relative damage for a susceptible, a resistant and a tolerant sugar beet cultivar (Table 55.1).

Fig. 55.1. Heavy beet cyst nematode damage and root symptoms (bearding) of a susceptible sugar beet cultivar sampled 90–100 days after sowing. Photograph courtesy of D. Daub, Julius Kühn Institute.

At a *Pi* class over 1500 eggs and juveniles/100 ml tolerant and resistant cultivars still showed 16% yield damage. Irrespective of *Pi* densities, sugar yield achieved with tolerant cultivars was always one to two tonnes per hectare above the susceptible cultivar. Hauer (2016) found a similar yield reduction in tolerant cultivars.

Biology and life cycle

Population dynamics of BCN is mainly affected by crop rotation. Under Central European conditions two to three generations of BCN can develop per year. *Pi* density increases with concentration of susceptible hosts in a crop rotation. Critical for control is a minimum 2 years non-host break in the rotation. For Central European conditions it can be assumed that control of BCN in 4-year crop rotations usually is not necessary. In addition, alternative hosts like cabbage, spinach, Swiss chard, beetroot or oilseed rape, that are cultivated in the same rotation with sugar beet, have to be considered too.

Like many other plant parasitic nematodes, BCN populations are distributed in coherent foci of varying dimensions. Foci of older BCN populations in the field extend ellipse-like in the direction of the soil preparation (Fig. 55.2). Different soil types alter the abundance of BCN; for example, higher numbers of BCN may occur in sandy

Table 55.1. Relative damage[a] (%) of different beet cultivars at different *Pi* density classes of *Heterodera schachtii*.

	Pi class in eggs and juveniles/100 ml soil		
Cultivar type	500–1000	1001–1500	>1500
Susceptible	16.2	19.7	28.3
Resistant	7	13.9	15.7
Tolerant	2.7	4.5	15.9

[a]Per cent of yield detected at *Pi* class <500 eggs and juveniles/100 ml soil

Fig. 55.2. Two *Heterodera schachtii* foci in the direction of soil preparation in a sugar beet field. The *Pi* levels inside foci exceeded 2000 eggs and juveniles per 100 ml soil. Photograph courtesy of D. Daub, Julius Kühn Institute.

patches (Hbirkou *et al.*, 2011). Higher numbers of BCN cysts also may occur at the edges of fields where beets are piled up at harvest before they are loaded onto trucks.

The root depth of sugar beet determines the vertical distribution of BCN. Cysts can be extracted from soil sampled as deep as 1.2 m. The vertical distribution of BCN is variable, and they may be concentrated either in the top or subsoil, or equally distributed over both soil layers. Although it has been known for a long time that BCN occurs in deeper soil layers, this was not considered relevant for damage caused to sugar beet.

Interactions with other nematodes and pathogens

It is possible that tolerance against BCN disappears if other diseases (e.g. viruses, leaf spot, soil pathogens) simultaneously damage sugar beet. On the other hand, penetration of sugar beet roots by BCN juveniles and reproduction may give access to other pathogens or suppress infections by other pathogens (Heijbroek *et al.*, 2002; Hol *et al.*, 2013).

Recommended integrated nematode management

Before tolerant sugar beet cultivars were available, cultivars with $HS1^{pro1}$ resistance against BCN were used specifically in cases where other control measure against BCN were restricted or growers favoured a dual use strategy of production and control. The resistance mechanism in this genotype is based on a hypersensitive reaction in tissues, which implies a high grade of intolerance towards penetration by BCN juveniles. Therefore, the management value of this resistance type is possibly limited to the reduction of BCN populations over crop rotation (Roberts, 1992). Although efforts have been made, the yield potential of resistant cultivars have not met grower's expectations and therefore they are no longer available for cultivation. Instead, the cultivation of nematode tolerant cultivars increased in sugar beet growing regions where BCN infestation traditionally was expected.

Presumably, the majority of sugar beet genotypes that were used for breeding tolerant cultivars derive from partially resistant *B. maritima* accessions (Blok *et al.*, 2018). Growers use tolerant cultivars primarily due to their significant yield benefit, thus partial resistance traits in tolerant cultivars are not used on purpose. Partial resistance in tolerant cultivars allows some reproduction of BCN below a specific equilibrium density. Therefore, reproduction of BCN in these tolerant cultivars at very low *Pi* densities (<500 eggs and juveniles/100 ml soil) usually results in higher reproduction rates, which decreases with increasing *Pi* densities. Tolerant cultivars show differences in partial resistance. Testing partial resistance requires a definition of quantitative resistance. Such a definition strictly would require a calibration to a standard *Pi* density and a standard susceptible reference to determine relative susceptibility.

Sufficient weed control in sugar beet rotations is crucial as some weeds are suitable hosts for BCN. Information on host range among weeds is not readily available and is often disjointed in relation to specific weed species. A recent survey of 27 plant species only confirmed *Thlaspi arvensis* and *Stellaria media* as being hosts with evident reproduction potential for BCN, but only if higher plant densities occur (Meinecke and Westphal, 2014).

If oilseed rape is cultivated in the same rotation with sugar beet, a specific point of concern is the appearance of volunteer oilseed rape post-harvest. Depending on weather conditions they can germinate in very high densities of several hundred plants per square metre. This takes place during summer months at soil temperatures above 20°C. The concurrent encounter of BCN, a very good host at high densities, optimum temperatures for the development of BCN and a period at cereal harvest where growers do not pay full attention to these fields, induce a high risk for an uncontrolled reproduction of BCN. The development of volunteers needs to be interrupted at a certain stage to prevent completion of the BCN lifecycle. A control method achieving this was developed based upon use of a degree-day model that provides a trap crop effect which enables the integration of oilseed rape and sugar beet in the same rotation (Daub, 2020).

In Central Europe, a standard method to control BCN is the cultivation of resistant white mustard (*Sinapis alba*) and oilseed radish (*Raphanus sativus*) as a catch crop prior to the cultivation of sugar beet. Breeding for resistance started over 30 years ago, and ongoing selection has achieved a very high grade of resistance in these cultivars today. In the field, catch crops achieve reduction rates of BCN population density of between 20% and 60%, depending on sowing time, plant density and crop performance. This, in turn, depends on water and nutrient availability. Due to the natural decrease of soil temperatures in autumn, sowing catch crops at the beginning of September does not show a sufficient effect on BCN reduction. Since new subsidy programmes to promote environmentally friendly cultivation have been established by many European countries, there has been a significant increase in growing mixed intercrops. The use of fertilizer is not allowed in these programmes. So far, this regulation does not meet requirements for the establishment of an effective catch crop against BCN. Growers have to check if seed mixtures contain registered cultivars with approved resistance as this is not regulated for seed mixtures.

Optimization of nematode management

The significant yield benefit of sugar beet cultivars with tolerance against BCN in infested and non-infested fields has been accompanied by disregard of traditional integrated control management strategies for BCN. Despite being tolerant, some cultivars can experience damage by BCN. There are three reasons for this: (i) tolerant cultivars primarily mask damage effects, like stunting, wilting, discoloration or root symptoms, due to their physiological adaptability; (ii) partial resistance in current cultivars with tolerance implies reliability towards sustainability of yield; and (iii) following an integrated approach to control BCN is more complex and time demanding than just using a tolerant cultivar as an all-inclusive solution. This trend ultimately results in a dramatic loss of information that prevents an overview of the real BCN situation in growing areas. Cultivation advice has to deal with this erosion of information as cultivation conditions in future possibly will foster the risk of BCN damage in sugar beet that will not appear visually.

Future research requirements

A major goal for future research on BCN and other plant parasitic nematodes will be to make direct and indirect damage impact visible on a plant and a crop scale in the field to provide data for multifactorial models. Future solutions have to cope with a more complex interaction between BCN and other biotic factors and the invisibility of damage in the field, due to the fact that breeding has focused on the selection of tolerance to many different pests and diseases. This specifically will be of some importance if the BCN population should adapt to the gene for partial resistance due to continuous field exposure, as was recently experienced with *Globodera pallida* in potato crops in north-west Germany. Due to the complexity of integrated nematode management and the fact that a huge data set on damage impact relation in the BCN–sugar beet pathosystem is already available, it would be very supportive for growers to develop an extension to established decision support systems like NemaDecide (see also Chapter 60 this volume), which is available for the potato cyst nematode.

Outlook: anticipating future developments

An average temperature increase and the occurrence of temporary droughts in spring are predicted in future climate scenarios for Central Europe (Ruosteenoja *et al.*, 2018). This might enhance the potential risk of increasing the number of generation cycles in a season and also increase the damage impact of BCN to sugar beet. In turn, this also enhances selection pressure of virulent pathotypes and overall reproduction of BCN.

Future solutions will be based on a multifactorial approach. Technical advances are being made and will deliver new techniques, for example detection algorithms that use remote sensing technologies in the field to delimit high-risk spots at early symptom development (see also Chapter 58, this volume) instead of sampling large areas. Future approaches like automatic detection and phenotyping of BCN in soil extracts using machine learning methods (Akintayo *et al.*, 2018; Chen *et al.*, 2020) will probably represent new key technologies for nematology that also potentially provide high throughput technologies. Technical development will enable faster and more complex calculation processing, which will be accompanied by the provision of complex models considering biotic, abiotic and technical patterns simultaneously for the provision of user-friendly decision support systems.

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SECTION VII:

Emerging Technologies

56 Let's be inclusive – the time of looking at individual plant parasitic nematodes is over, and new technologies allow for it

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Introduction

Parasitism is a popular life style among members of the phylum Nematoda. Around 46% of the 27,000 described nematode species use either a plant or an animal as a primary food source. A couple of years ago a paper written by John Jones and Roland Perry entitled 'Top 10 plant parasitic nematodes in molecular plant pathology' was published (Jones *et al.*, 2013), and yes, it is true that plant parasitic nematodes cause tremendous crop yield losses. However, it should be kept in mind that probably 95% of these losses are caused by about two dozen nematode taxa. We realize this statement requires some more detailing in order to avoid raising of eyebrows. Just like in the Top 10 paper, we consider root-knot nematodes, cyst nematodes or lesion nematodes (etc.) as a single taxon (harbouring one or, at most, two genera). So, two dozen nematode genera are responsible for the by far major part of the damage inflicted by this group of plant pathogens.

Over the last two decades, molecular phylogenetics has aided in deciphering patterns of evolution and diversification among plant parasitic nematodes. Alignments comprising over 5000 nearly full-length small subunit (SSU) ribosomal (r) DNA sequences (each approximately 1700 bp) with a fairly good coverage of all extant nematode families allowed us to pinpoint patterns with regard to the appearance of plant parasitism. It is justified to label the Trichodoridae (clade 1, for clade delineation see Holterman *et al.*, 2006) as the most basal plant parasite family. Trichodorids have an unusual stylet-like device, an onchiostyle, and one of the peculiarities of this onchiostyle is that it does not have a molecular weight cut off. Trichodorids are unique in that they can ingest relatively large particles such as whole plastids and mitochondria. Outside this lineage, no other plant parasitic nematode is able to do this. The next major branch in which plant parasitism arose is clade 2. The family Longidoridae arose and diversified within the order Dorylaimida. This family is mostly known as a vector of plant viruses (genus *Nepovirus*). It is noted that these two lineages are the only plant parasite harbouring

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branches among the former class Adenophorea. In terms of plant parasitic nematode diversification, clades 10 and 12 are more successful. Clade 10 plant parasites arose relatively recently, and it gave rise to a number of very destructive parasites. The pine wilt nematode *Bursaphelenchus xylophilus* (position 8 in the plant parasitic nematode Top 10) and the red ring nematode *B. cocophilus* are tree parasites vectored by insects that kill their host. The primitive nature of this interaction is illustrated by the fact that no nematode-induced re-differentiation of plant cells takes place. By far most plant parasitic nematodes can be found in clade 12 (mainly order Tylenchida). Within this clade we see a gradual evolution from facultative plant parasites (they also feed on fungi) to sedentary endoparasites. It is worth mentioning that plant parasitic nematodes with a sophisticated and durable interaction with their host are among the most successful ones in terms of proliferation and abundance. The Top 3 of the plant parasitic nematodes according to Jones *et al.* (2013) – the root-knot (*Meloidogyne*), the cyst (*Globodera* and *Heterodera*) and the lesion (*Pratylenchus*) nematodes – all reside in the most distal parts of clade 12 (e.g. Smant *et al.*, 2018). Hence, from the enormous economic and social impact of plant parasitic nematodes worldwide, we should not conclude that we would wish to control nematodes, or even plant parasitic nematodes. We rather should strive to manage specifically a very small subset of plant parasitic nematodes – actually approximately 1% of the total plant parasitic nematode biodiversity. But sure, this is more easily said than done.

State of the art

A large part of the biological diversification patterns described above stem from molecular data. Also here we would like to emphasize there is no principle difference between 'classical' morphological and morphometric data on the one hand, and molecular data on the other. We could summarize this with a very short statement: 'characters are characters'. In fact, it is all about numbers. With molecular data it is pretty straightforward to generate 1000 characters from a single individual nematode. On the other hand, it is more difficult, and maybe even impossible, to generate 1000 morphological characters from a single worm. Another advantage of the use of molecular data is the time efficiency. It is easy to amplify one of multiple fragments within half a day and send them out for DNA sequencing. A more fundamental advantage of the use of molecular data is that one can avoid the effects of convergent evolution. Convergent or parallel evolution has obscured our view on nematode systematics and evolution dramatically. It is hard to find a single morphological characteristic that did not arise at least twice in evolutionary history (Holterman *et al.*, 2017). We think it is fair to state that extensive convergent evolution within the phylum Nematoda is the very reason why nematode systematics has been unstable for decades.

Nematode identification

Currently, we see two kinds of molecular approaches for the identification and (quantitative) monitoring of plant parasitic nematodes. There are focused approaches such as real time (RT) PCR. Using large and taxonomically diverse alignments as a starting point, it is most of the time possible to define species-specific DNA motifs. It is technically not overly demanding to design species-specific PCR primers, even for groups of nematodes that are notoriously hard to distinguish such as plant parasitic *Aphelenchoides* species (see e.g. Rybarczyk-Mydłowska *et al.*, 2012). It is noted that for each species a calibration curve that establishes the relationship between C_t value and the number of nematodes needs to be generated. But once this is done, RT or quantitative PCR is a powerful and affordable technique to identify and monitor plant parasitic nematodes. However, qPCR-based detection technologies are by definition focused. One will never see things that one is not looking for. To see the unexpected, another more open approach should be chosen. Lysates from nematode suspensions can be used as a substrate for the amplification of gene fragments of nematodes in general (or even Metazoa). Most of these approaches focus on variable regions within the SSU rDNA, and the V5–V7 regions are quite popular (Capra *et al.*, 2016). Such a meta-barcoding approach is completely open and allows, as such, for the discovery of unexpected nematodes. However, this comes at a price: the molecular signal present in these V5–V7 regions allow for identification at genus level at best. Moreover, the results of such a nematode community analysis are in essence semi-quantitative.

The soil biome

As already implied by the title, the latest tendency in applied soil ecology is to take a more holistic approach. We no longer focus on a single bad guy – the pathogen threatening our crops – but we rather try to map the biotic environment of the pathogen. In other words, we no longer concentrate on a harmful plant parasitic nematode species, but consider the nematode community as a whole, and even include the bacterial, fungal and protist community. Nowadays it is technically possible to map and monitor the complete soil biome. This approach allows us to map the nematode-suppressive potential of a soil, and experimentally verify whether this potential can be boosted and maximized.

Methodology

Nematode identification in soil

If we really want to assess plant parasitic nematodes in their biotic environment we have to change gears in relation to our methodologies. First of all, the extraction. Currently, techniques to isolate nematodes from the soil matrix differ from the protocols used to extract microbial DNA and RNA from soil. Nematodes will be extracted by Baermann or Oostenbrink elutriation, or any other technique, from >100 g, while microbial DNA/RNA is extracted directly from <2 g of a homogenized soil sample that represents a certain area in a field. This will remain the standard procedure because the size and consequently population density of nematodes determines the soil volume to get a representative sample of the community. The characterization of the nematode community is the part most of us are familiar with. This can be done microscopically, but researchers generally now prefer RT PCR-based methods or meta-barcoding as these methods are easily scalable, time-efficient and do not require as much nematological expertise (see e.g. Quist *et al.*, 2019). With ever decreasing sequencing and data processing costs, PCR-free high-throughput sequencing will replace the at most semi-quantitative nature of meta-barcoding and give access to eco-functionally more relevant transcripts of soil biota.

Microbial community identification in soil

Microbial communities are mostly analysed from rhizosphere or bulk soil. An exciting other niche that should not be overlooked is the microbe community attached to the cuticle of plant parasitic nematodes. These often-non-pathogenic bacteria and/or fungal spores were recently shown to activate the plant innate immunity system (Topalovic *et al.*, 2020), and in that sense contributing to the self-defence of the plant *against* root-invading nematodes. With regard to the soil itself, it is important to know that soils act as a microbial seedbank; only a small part of the soil microbiome is active and the largest fraction is present but inactive ('dormant'). This is especially relevant for bulk soils, where typically 80% of the cells, and 50% of the operational taxonomic units are inactive (Lennon and Jones, 2011). Hence, it is crucial to discriminate between the active microbial fraction of soil – the fraction that potentially interacts with the nematode community – and the resident community that comprises all biodiversity (active and inactive). However, it should be kept in mind that the 'dormant' part of the community partially gets activated by encountering signals from roots or nematodes. Moreover, it is noted that some 'dormant' stages like endospores contain substantial amounts of ribosomal RNA to speed up this activation. Another advantage of targeting ribosomal RNA instead of DNA is the high copy number of ribosomes in active nematodes that allows for their detection within RNA extracts from roots (Topalovic *et al.*, 2020).

Activity of microbiome

RNA from the soil or nematode cuticle can be used to map the active microbiome, whereas

DNA is used to provide an overview of the resident community (Ofek *et al.*, 2014; Harkes *et al.*, 2019). Subsequently, fragments of the 16*S* or 18*S* ribosomal DNA are amplified, and the resulting complex amplicons are labelled. Currently, paired end 2 × 300 bp MiSeq sequencing-based analysis is frequently used. In a single run about 22 million reads from nearly 100 samples can be generated to characterize a taxonomic fraction of the soil biota. Curated databases with taxonomic information or sequence similarities within the dataset are used to translate these rather large data sets into amplicon sequence variant (ASV) tables (a matrix that gives the number of reads per sample per ASV). Such an ASV table can be used to check for the presence and activity of known nematophagous fungi and/or nemato-toxic microorganisms. The overall aim of this approach is to have a methodological framework to map the actual and the potential nematode suppressiveness, as well as a tool to verify the validity of any tool of management practice that is suggested to boost this very wanted soil characteristic.

Pros and cons

We are convinced that the combined use of host plant resistances, smart soil management practices (including pathogen-informed (cover-) crop rotation schemes), and an optimal exploration of the soil nematode-suppressive potential is key to future-proof plant parasitic nematode management. In this scientific brief, we paid most attention to endogenous soil suppressiveness as it is the least well-characterized of the main control options. That is no wonder: the soil microbiome is highly complex, and only in recent years have the DNA sequencing costs been dropped to a level that we can use and explore for agroecological purposes. Nevertheless, we currently are able to handle and analyse the literally tens of millions of DNA reads that are typically produced by microbiome monitoring studies. Moreover, we are able to pinpoint the effects of various soil management systems on the active and resident microbiome in association with increased levels of soil suppressiveness against plant parasitic nematodes.

What are currently the major cons? It is work in progress, it is quite complex, and we are only just starting to understand the underlying mechanisms. The following can be said about this complexity: currently we are reasonably well able to map soil biodiversity and monitor management-induced changes. On several occasions we were able to link desired traits to specific bacteria or fungi. A logical next step is to search for ecological characteristics of these organisms. The crux is in this last step: not always but regularly it appears that literally close-to-nothing is known about the ecological functions of these soil inhabitants. So ecological characterization of soil inhabitants is lagging behind our dramatically increased capability to map soil life. Soil is no longer a black box, but the functional understanding of interesting and probably relevant community shifts is currently the limiting factor in our understanding of the soil biome.

Outlook: a vision of the future

One thing is for sure – future durable plant parasitic nematode management is much more knowledge intensive than it was in previous times. To illustrate this: in 'soil fumigant times' the nature of the nematode problem did not matter at all. In the end it even did not need to be a nematode. With the application of, for instance, systemic acetyl cholinesterase inhibitors, it became a bit more subtle, but not too much. After all, most nematodes use acetyl choline as a neurotransmitter. The current approaches – the combined use of host plant resistances, smart soil management techniques and soil suppressiveness (in combination with biocontrol) – require in-depth knowledge about the biological system. Fortunately, this is happening as we speak: reference genomes have been or are currently generated for the most important plant parasitic nematode species, and molecular pathotyping will allow for a much more effective use of resistance genes. Moreover, we will soon be able to pinpoint the actual and the potential endogenous nematode-suppressive potential of soil in agroecosystems, and help applied science explore and boost this potential and breed crops with a high capacity for the induction of defence by associated microbiomes to achieve tolerance to plant parasitic nematodes in managed soil systems.

An aspect of concern is the accessibility of these knowledge-intensive approaches. It is of utmost importance that we use our host plant resistances in a more durable way as prolonged and uninformed use will unavoidably lead to the appearance of virulent plant parasitic nematode populations. It is also clear that exploring and boosting the soil suppressive potential will be a major additional tool in the foreseeable future. Let us be clear: these approaches are under development and there are no practical applications yet. However, this will not take long and it would be a shame if only farmers that happen to live in countries with an excellent knowledge infrastructure could benefit from it.

Last, but not least, we would plea to value the biodiversity and the functioning soil living community as an intrinsic asset of that soil. So, soils would not only be valued by their physical and biochemical characteristics but also by the condition of the soil biota. This would imply that farmers that invest in soil biological functioning will see a return on their investments in terms of better market values of their acreage. We know this sounds far-fetched, but we are convinced we should strive for this in order to create a healthy economic basis for soil management that includes the durable exploration of soil biodiversity.

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57 Nematode management through genome editing

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Introduction

Plant parasitic nematodes are among the most destructive plant pathogens, causing an estimated US$78 billion yield losses globally. Although approximately 3000 species of plant parasitic nematodes have been described, most of the damage is caused by a small group of root-infecting sedentary endoparasitic nematodes that include root-knot nematodes and cyst nematodes. In fact, a recent expert-based assessment of crop health lists cyst nematodes among the most damaging pests and pathogens in potato, soybean and wheat (Savary *et al.*, 2019). Both root-knot and cyst nematodes induce the formation of elaborate feeding sites inside host roots, which are the only source of nutrients for nematodes throughout their weeks-long life cycle. The formation of the feeding sites is accompanied by massive changes in host cell structure, functions and physiology. A wide range of nematode secretions (effectors) have been implicated in modulation of host pathways for feeding site development.

State of the art and methodology

Sustainable management of nematodes during crop production relies on some combination of crop rotation, cover crops, trap crops, soil solarization, fumigation, biological control agents, chemical nematicides and resistant plant varieties. However, some key management strategies are not effective or available for most crop production systems. Nematicides are generally highly toxic to other organisms, and their use is strictly limited due to environmental concerns. Soil solarization does not work under suboptimal climatic conditions. Natural crop resistance is often ineffective or unavailable, and the application of microbial biocontrol agents has inconsistent results.

Recent progress in genome sequencing technologies, together with advances in approaches to genome editing, has opened the door to the development of crops with the desired traits, including nematode resistance. Given that previous literature amply reviews the breadth of biotechnological methods for the control of plant parasitic nematodes (Lilley *et al.*, 2014; Ali *et al.*, 2017), this chapter will briefly touch on long-standing biotechnological methods but focus on recent progress in, and long-term promise of, the use of CRISPR technology for introducing targeted modifications into host genomes with the goal of enhancing resistance against plant parasitic nematodes.

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Host-induced gene silencing (HIGS)

RNA interference (RNAi) is a conserved biological process in eukaryotes in which RNA molecules silence the expression of target genes through mRNA degradation. RNAi was first utilized to perturb gene expression for investigations of gene function in the free-living nematode *Caenorhabditis elegans*, but has subsequently been widely applied in various plants and animals, including in a range of plant parasitic nematodes. Soaking second-stage juveniles in double-stranded (ds) RNA corresponding to the gene of interest has been shown to reduce transcript levels in several plant parasitic nematode species. HIGS is a RNAi-based process and involves expression of dsRNA constructs targeting vital nematode genes in transgenic plants. Once transcribed, resulting dsRNA or their plant-derived products (small interfering RNAs, siRNAs) are ingested by nematodes, leading to silencing of the target gene's expression. HIGS has been effective for reducing the expression of nematode genes expressed exclusively in the later stages of development, and generally allows more stable silencing of nematode genes. Although a number of studies have shown the utility and effectiveness of HIGS for reducing nematode success, including *Arabidopsis*, tobacco, tomato, banana, soybean and rice, its effect has been more pronounced when targeting a nematode gene that was essential for core cellular processes (e.g. ribosomal proteins, splicing factor, major sperm protein, spliceosomal protein). While it is generally accepted that targeting plant parasitic nematode-specific genes (e.g. the effectors) minimizes the risk of off targeting and may therefore facilitate regulatory/public approval (Danchin *et al.*, 2013), the central dogma dictates that targeting individual effectors is unlikely to be robust because they are already the targets of the plant immune system and so under selective pressure to diversify. Recent improvement in the availability of genomic and transcriptomic resources for several plant parasitic nematode species provides a comprehensive catalogue of suitable candidate genes for RNAi experiments (reviewed by Kikuchi *et al.*, 2017).

Expression of Cry toxins from *Bacillus thuringiensis*

Bacillus thuringiensis is a Gram-positive, spore-forming bacterium that produces crystals of proteinaceous δ-endotoxins called crystal proteins or Cry proteins. Development of transgenic plants expressing genes that encode Cry proteins was one of the first applications of gene modification technology for pest management. Insects that feed on such transgenic plants ingest Cry proteins that are activated by the gut's alkaline environment, binding to the receptors in the membrane of the insect's gut, creating pores and leading to the insect's death. A number of transgenic crop plants expressing different variants of Cry proteins have been registered for use in the US, including maize, cotton and potato. Most of these variants are highly effective at combating phytophagous insects such as the European corn borer, corn rootworm, and bollworm.

In contrast, registration of crop plants expressing Cry proteins to combat plant parasitic nematodes has been limited, despite demonstrable toxicity of various Cry proteins to free-living and some parasitic nematodes (Ali *et al.*, 2017). A major constraint is the relatively large size of the Cry proteins. Root-knot and cyst nematodes feed by ingesting the host cell assimilate through a structure known as a feeding tube. Feeding tubes act as molecular sieves, allowing uptake of certain molecules but excluding others, primarily on the basis of size (Eves-van den Akker *et al.*, 2014). Feeding tube structure and size exclusion limits differs between origins of biotrophy, making it challenging to develop transgenic plants that are resistant to a broad range of plant parasitic nematodes (Lilley *et al.*, 2014). Most notably, cyst nematode feeding tube size exclusion limit is below Cry proteins.

Interestingly, the US Environmental Protection Agency recently approved the registration of transgenic soybean GMB151 targeting the soybean cyst nematode. GMB151 expresses a new, and presumably compact, variant of Cry protein, Cry14Ab-1 (GENBANK accession number KC156652), which damages the gut of soybean cyst nematodes when they ingest it. Over the long term, Cry-14Ab-1 is being proposed for breeding with commercial soybean varieties that have natural resistance traits, such as PI 88788 and Peking (Oslon, 2020).

CRISPR: genome editing for nematode resistance

The application of clustered, regularly interspaced short palindromic repeat (CRISPR) technology has revolutionized the field of biotechnology and genome editing by enabling technically simple and sequence-specific modification of genomes. These short repeat sequences were originally discovered as a component of an adaptive immune system in bacteria and archaea that enable them to defend against invading bacteriophages and plasmids. During the past few years, the CRISPR system has been engineered to introduce targeted genome modification in a range of living organisms including many crop plants (Wang *et al*., 2019).

Fundamentally, the CRISPR system consists of two components: a Cas DNA endonuclease (most commonly Cas9) that is guided to a specific genomic sequence by a guide RNA molecule (sgRNA). Various adaptations of the CRISPR system (either using the Cas9 endonuclease as is or fused to other 'effector' domains) can be used to generate four classes of CRISPR-based genome editing: nucleases, base editors, transposases/recombinases and prime editors (Fig. 57.1; reviewed in Anzalone *et al*., 2020). The majority of genome editing in plants uses the first class of genome editing: Cas nuclease and sgRNA are delivered into plant cells to generate double-strand breaks at the targeted region, after which cells repair the breaks by activating

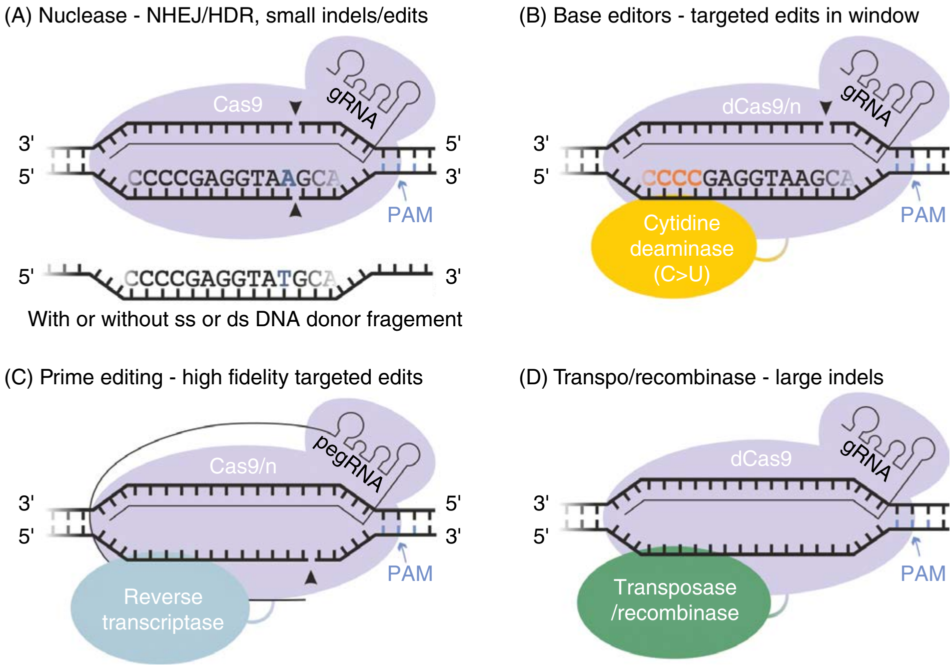

Fig. 57.1. Four classes of CRISPR-based genome editing. **(A)** Nucleases, the most common to date, requires double-stranded cutting of target DNA and repair by either non-homologous end-joining (NEHJ) or homology-directed repair (HDR) to introduce small indels and edits. **(B)** Base editors, variously fuse cytidine or adenine deaminases to Cas proteins to enzymatically convert endogenous bases (with or without various inhibitors of suboptimal repair pathways and nicking non-deaminated strand to promote repair using the edited strand as template) to introduce a very specific set of edits within a narrow window. **(C)** Prime editors, fuse reverse transcriptase to Cas nickase protein and include a longer modified guide to prime reverse transcription using the nicked strand, thereby creating a template for HDR to introduce high fidelity edits. **(D)** Transposases/recombinases, a recent (and not yet deployed in plants) approach to fuse various transposase or recombinase domains to Cas proteins to induce larger indels/structural rearrangements. PAM, protospacer adjacent motif; gRNA, guide RNA; pegRNA, prime editing guide RNA. Author's own figure.

the error-prone non-homologous end-joining (NHEJ) DNA repair pathway, leading to random insertions/deletions or substitutions at the target site, thereby often disrupting the function of the target gene. Alternatively, the availability of a template DNA (single stranded or double stranded) homologous to the region around the target site can lead to homology-directed repair (HDR), giving rise to lower efficiency but more precise genome modification that can include insertions, deletions and single base-pair substitutions.

Since its inception in 2013, CRISPR technology has been employed to target various traits including disease resistance (Langner *et al.*, 2018; Wang *et al.*, 2019). For example, a recent study used CRISPR to target the tomato *MLO* locus, which contributes to susceptibility to powdery mildew. A resulting deletion of 48 base pairs in edited plants led to a powdery mildew resistant phenotype without any pleiotropic effects. Remarkably, whole-genome Illumina sequencing of edited plants showed that they were transgene free and indistinguishable from naturally occurring mutations (Nekrasov *et al.*, 2017).

While CRISPR-based genome editing has shown considerable potential towards crop disease resistance, its application in nematode management is still in its infancy. To date, CRISPR genome editing has been used to interrogate plant–nematode interactions, rather than deliver resistance. For example, soybean hydroxymethyltransferase gene (*GmSHMT08*) has been identified to play a role in resistance to soybean cyst nematodes (Liu *et al.*, 2012). Kang *et al.*, (2016) used CRISPR to disrupt the function of *GmSHMT08* in nematode resistant soybean. Nematode infection assays found that knocking out *GmSHMT08* significantly increases the nematode infection compared to empty vector control roots. In another study, the CRISPR system was used to mutate two syntaxin genes, individually and in combination, in the SCN-resistant Peking and SCN-susceptible Essex soybean lines. Interestingly, Peking roots with mutation in syntaxin genes showed a significantly reduced resistance to nematodes, confirming their critical role in SCN infection (Dong *et al.*, 2020).

The impact of CRISPR technology on the management of plant parasitic nematodes is not restricted to *in planta* application: CRISPR technology also has the potential to impact integrated nematode management by catalysing fundamental research on the parasites themselves. However, functional genetic tools have eluded the plant parasitic nematology community for decades, for a number of reasons. Firstly, and perhaps most important, the physiologies and life cycles of the most important plant parasitic nematodes are generally those least conducive to the technical steps required for genetic modification; for example (i) second-stage juveniles are accessible but without a developed germline; (ii) males are the only accessible life stage with a developed germline (females are necessarily sequestered inside/on plant roots and thus opaque) but have extremely tough cuticles and are under high internal pressure; and (iii) the life cycle of sedentary endoparasites is weeks to months at best, making experimentation slow. Secondly, the lack of benchmark progress has meant little dissemination of what has – and, crucially, what has not –worked among numerous independent attempts.

The recent formation of the ‘TransPPN consortium’, an international initiative spanning 21 individuals from 15 research institutes across 5 countries, offers a path for breaking free of these constraints by focusing efforts across the community. Although stable germline transformation remains elusive, the first output of the consortium has publicly documented some of the major challenges associated with developing functional genetic tools in plant parasitic nematodes and, notably, has demonstrated promising routes forward (Kranse *et al.*, 2020). Most notably, it was discovered that by encapsulating mRNAs in lipid bilayers (liposomes) and ‘forcing’ juveniles to ingest the liposomes by bathing them in a neurotransmitter (octopamine), reporter proteins may be transiently expressed throughout the bodies of tens of thousands of nematodes. The ability to transiently express exogenous mRNAs in plant parasitic nematodes using a technically simple and readily adopted method without specialized equipment would enable several experimental approaches that have previously been impossible or prohibitively difficult for plant parasitic nematology: for example, in vivo protein–DNA interaction studies (ChIP seq), *in vivo* protein–protein interaction studies (Co-IP, BiFC, FRET, etc.), and perhaps also targeted genome editing. Providing a new suite of tools for interrogating nematode biology, and thereby expediting research that could lead to novel control solutions, is important in its own right and may also pave the way for stable transgenesis, further accelerating progress.

Outlook: a vision of the future

The recent progress in understanding plant–nematode interactions and the emergence of genome editing tools provide new opportunities for sustainable and broad-spectrum management of nematodes using biotechnology. One strategy to deploy CRISPR-driven nematode resistance would be to generate mutations or deletions in known susceptibility genes as was done for MLO or other plant genes required for successful parasitism. A few susceptibility genes have already been identified in model plants such as *Arabidopsis*. Mechanisms underlying fundamental biological processes such as nematode susceptibility are often conserved in closely related groups of organisms, making it possible to translate discoveries in one species to other species. Such conservation can be exploited by identifying and editing orthologous genes in crop plants using CRISPR. We predict that susceptibility genes will be the prime targets for genome editing over the next 5–10 years. Multiple susceptibility genes can be edited and stacked with classical resistance genes in a single genetic background to help provide durable nematode resistance.

Another promising avenue for future development is establishment of genome editing tools for plant parasitic nematodes. Although what has been achieved without functional genetics is itself remarkable, we propose that arming an able field with new functional genetic tools will accelerate progress in understanding plant–nematode interactions and thereby in achieving solutions for global food security. We predict that expanding reverse genetic approaches beyond RNA interference, using low-cost, technically simple and efficient transformation (transient or stable) will be the single most important advance in the field in some years. The impact should be immediate and long-lasting. However, predicting the timescales for development of these technological resources is an uncertain practice. There is a clear pathway for optimizing transient expression of exogenous genes in plant parasitic nematodes to the point of utility, with the potential for expansion to other nematode groups (no obvious biological barriers to deployment in any plant parasitic nematode), over the next 3–5 years. Whether this technology, or others, will lead to stable transgenesis of sedentary endoparasites within or beyond that timeline is unclear, but we are cautiously optimistic. Stable transgenesis is most likely to be first achieved in those plant parasitic nematodes that have the most conducive biology (e.g. *Bursaphelenchus*, *Radopholus*). The long-term effects on integrated nematode management will necessarily follow the development of an efficient protocol for either stable or transient expression, something unlikely before the end of the decade.

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58 Emerging technologies for integrated nematode management: Remote sensing or proximal sensing as a potential tool to detect and identify nematode infestation

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Introduction

Remote and proximal sensing in plant science

Remote or proximal sensing defines the use of optical sensors, in combination with a carrier platform, to obtain information from objects in a non-invasive manner. Optical properties of plants provide valuable information on the health status, vitality or developmental stages of plants. The difference among remote-sensing and proximal-sensing technologies is mainly characterized by the distance between the measurement system and the object of interest.

Satellites or air-borne carrier platforms enable measurements of crop stands on the field level. Proximal sensors are used in a close distance of below 2 m between the measurement system and the crop stand or plant. With remote-sensing technologies, a bigger area can be measured in a short time frame, whereas proximal sensing is more time consuming but can provide detailed information from the object of interest. Remote and proximal sensing of crop stands to assess infestation with pest, diseases and nematodes by satellite or air-borne data have proven their potential in different systems. The recent progress in sensors and carrier platforms enables new options for agricultural investigations and operations.

Nowadays, unmanned aerial vehicle (UAV) platforms, combined with camera systems, are widely available and easy to operate. Furthermore, simple optical sensors are ubiquitous in mobile telephones, carried by all of us nearly all the time and at every place. In plant pathology and nematology, these techniques can be utilized to assess nematode infested crops. However, since the sensor techniques assess the upper plant parts and not infested root parts directly, technical setups have to be critically evaluated and data interpretation demands the expertise of a nematologist (Hillnhütter *et al.*, 2012).

During the plant breeding process, plant phenotyping in the field is highly relevant. In breeding for nematode resistance or tolerance this process is rather complex, using a recurrent

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selection over a series of generations, combined with soil and root samples to identify changes due to a nematode infestation and to assess the tolerance. In contrast to common visual rating and detection methods, optical sensors are able to measure pathogen-induced changes in the plant physiology non-invasively and objectively. Among different sensor types (thermography, chlorophyll-fluorescence, RGB, multispectral and hyperspectral), multispectral and hyperspectral sensors have significant potential and several advantages for monitoring plant diseases, pests and host–pathogen interactions (Mahlein *et al.*, 2018). For reliable results, the entire system pipeline, consisting of the type of sensor, the platform carrying the sensor and the decision-making process by data analysis has to be tailored to the specific problem. This is a hurdle not only for researchers and companies, but especially for farmers. A practical context in the field for precision agriculture for nematode management is still challenging, but not impossible. In particular, state-of-the-art remote-sensing approaches, like the European Space Agency´s (ESA) Copernicus programme, enable access to satellite data with relevant information for agriculture.

Symptom development: physiological reactions influencing optical characteristics in nematode infested plants

Symptoms in plants infested by plant parasitic nematodes mostly are unspecific. Above-ground plant parts usually show symptoms like stunting, wilting, discoloration and deformation of shoots, stem, leaves or seeds. With the exception of root knot nematodes producing distinct galls, symptoms are even less specific in roots. Thus nematode damage is difficult to detect and usually remains unrecognized especially at early symptom development. Plants remain stunted and develop nutrient deficiency symptoms later in the season. Depending on climatic conditions, a disturbed physiological reaction in nematode infected plants usually induces foliage wilt that can be detected as a nematode focus in crops. Water deficiency in plants trigger stomatal conductance of leaves to reduce transpiration. Consequently, leaf surface temperature in nematode infected plants increases and thus was shown to correlate positively with nematode infestation (Joalland *et al.*, 2017). The plant physiological reaction towards nematode infestation is also associated with reduced chlorophyll contents of leaves, and a reduced stomatal conductance leads to a reduced photosynthesis rate and a reduced plant turgor pressure. These reactions are visible as symptom more or less clearly delimited from non-infected areas in a crop. Stunting of plants might occur very early in the season but is best visible after formation of leaves and before the plant canopy is closed due to the fact that background soil produces an easily detectable contrast towards the non-infected surrounding.

Plants have a species- or even cultivar-specific trait in tolerating damage by nematodes. Tolerance towards nematode infestation appears to be a non-specific adaption towards water deficiency in plants like the development of a deep rooting system (Haverkort *et al.*, 1992). Thus tolerant plants probably mask infestation by nematodes as they do not develop clearly visible symptoms. The same physiological wilt reaction in plants also occurs in shallow soil areas, in compacted soil or in connection with light soil types that only provides limited water availability. Early attempts in the 1980s to 1990s in Germany (Kochs, 2000) to use aerial infrared pictures for detection of beet cyst nematode foci in sugar beet crops worked successfully but required detailed interpretation to exclude artefacts like shallow hill tops and artificial embankments. From these experiences it also became evident that symptoms detected by infrared filter could be best identified after periods of rainfall in warm and sunny weather conditions during summer where non-infested field areas were well watered but beet cyst nematode infested plants showed wilting.

State of the art

Remote sensing with satellites: the opportunity for retrospective analysis and field planning

Controlling nematode diseases by the application of chemicals is difficult, and in many countries prohibited. Currently, resistant or tolerant cultivars and crop rotation strategies are common to control nematode infestation. Over the years, adapted nematodes could be selected, due to continuous growing of resistant cultivars with the same genetic resistance. It is highly relevant to develop further methods and applications, which can be integrated into the current control strategies that are environmentally friendly and sustainable. In the age of digitalization, the precise detection of primary infection sites and disease dynamics are fundamental to make a decision for a subsequent management practice.

Since the European Space Agency's Copernicus programme and the launch of their satellites SENTINEL-2A in June 2015 and SENTINEL-2B in March 2017, multispectral remote-sensing images are freely available in a sophisticated resolution for agriculture. The spatial resolution is up to 10 m per pixel. The spectral range is from 442–2200 nm with a resolution of 12 spectral bands. Besides environmental monitoring and vegetation observation, they enable monitoring for crop diseases and pests. Free data access is possible using different commercial software as well as with no-charge browser solutions like the EO Browser by the ESA (https://www.sentinel-hub.com, accessed 30 July 2020). The image frequency depends on the revisit frequency of each single SENTINEL-2 satellite, which is 10 days and in combined constellation 5 days. On a cloudless day, the image will be perfect to examine the field of interest. If clouds are present and cover the field of interest, between 5 and 10 days will pass before the image can be captured again (Fig. 58.1). For some plant diseases and pests this time span is critical and short-term applications in the field cannot be conducted, which is currently the main drawback of the free satellite data available. Nevertheless, for research investigations of plant breeding processes and retrospective field assessments, spectral images from a satellite are a real benefit to map landscapes with relevant crop and cultivation parameters, identify vulnerable spots, assess the vegetation period and the conducted measures for future precision field management.

From the perspective of plant protection, farmers of North Rhine-Westphalia and Norway can already use the H_2Ot-Spotmanar (http://synops.julius-kuehn.de/synops-2/#/dashboard, accessed 30 July 2020) to calculate the environmental risk for waterbodies and their living organisms due to specific plant protection measures, based on updated satellite data. Such applications indicate the manifold opportunities to use satellite data even with a resolution that cannot represent a single plant. Plenty of commercial field management programs that use satellite data are available. In these programs, farmers give access to their field data or their whole field index. These data are combined with weather and satellite data to give the farmer a complete overview and information (e.g. about plant nutrition, water status, plant healthy status and necessary protection) around their crop growing. In the near future, these programs could be trained to generate 'computer-based solutions' and consulting before and during the vegetation season.

Robots and drones: flexible in-field assessment

Recently, unmanned ground-based or air-based vehicles (UGV and UAV, respectively) equipped with sophisticated cameras have become increasingly relevant and available. These platforms are able to screen a field site automatically, with little human intervention (Fig. 58.1). This offers huge potential also for small-scale farmers. The main advantage of UAV or UGV applications compared to satellites is a comparatively higher spatial resolution. Due to the low distance between the object and the camera, the ground pixel size can be below 1 cm, which means that data can be assessed to the leaf or single plant level. This might help to differentiate between causal agents by changes in optical properties. UAVs may be so-called fixed wing or copters. Fixed-wing UAVs are able to fly over a higher area in short time, whereas copters offer the ability to hover across a specific area to do more detailed investigations. Regardless of the vehicle, in all cases the combination of the platform and the camera is crucial. UAVs and UGVs can be equipped with thermal cameras, simple RGB cameras or multispectral or hyperspectral cameras. For the assessment of stress and symptoms caused by nematodes, thermal and multispectral imaging offers huge potential, in combination with an adapted analysis pipeline.

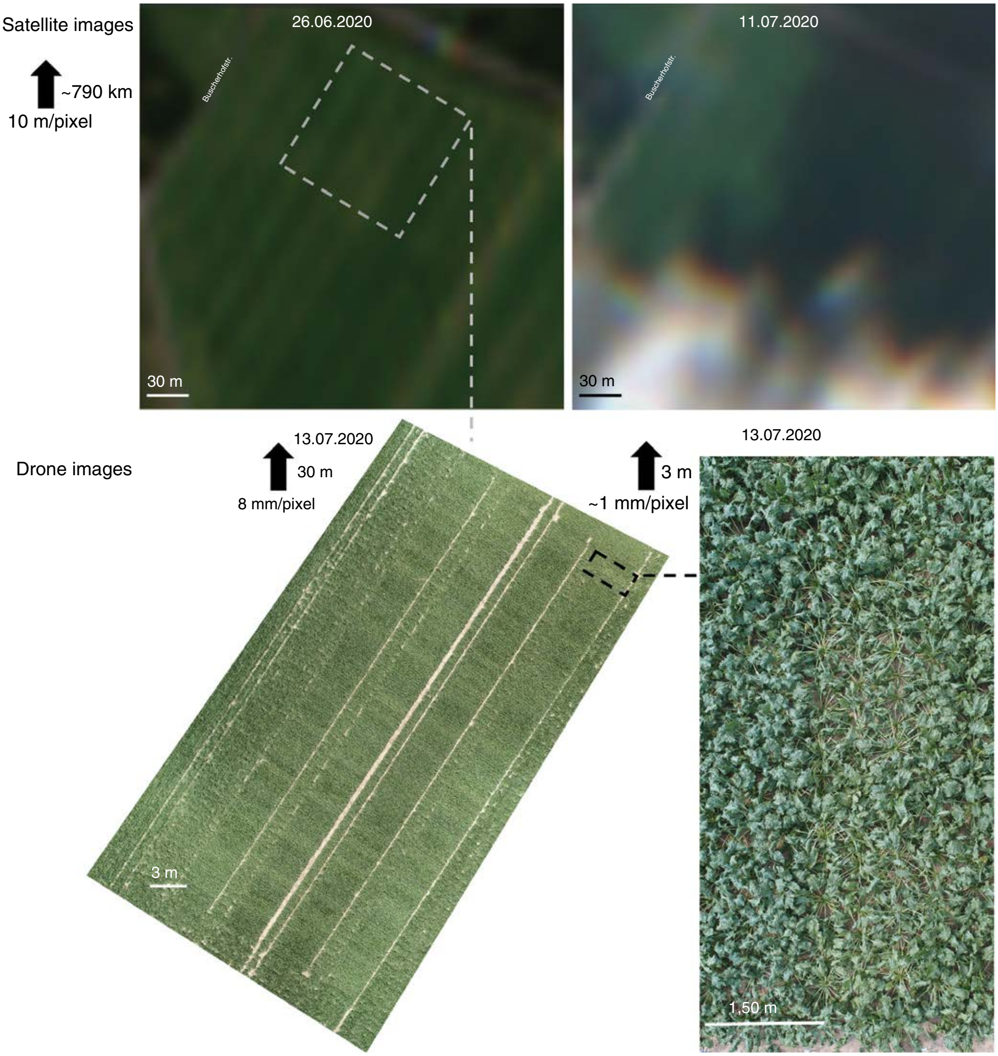

Fig. 58.1. A 7-hectare area with sugar beet in the Grevenbroich region of Germany. In contrast to the satellite image from 11 July, the image from 26 June can be used to examine the field of interest (EO Browser v3.0.20, ESA). In the marked area, sugar beet varieties with different tolerances to cyst nematode, *Heterodera schachtii*, are tested. The images below were taken with a drone (DJI Phantom 4, 12.4 megapixels 4k camera) from 30-m height over approximately 7 minutes. The spatial resolution is higher and enables an analysis in detail. Furthermore, the altitude of the drone can be changed to examine spots of interest. Arrows indicate the altitude of the sensor carriers and the spatial resolution per pixel. Author's own figure.

Analysis and interpretation of remote-sensing data

Multispectral and hyperspectral images record the electromagnetic spectrum that is reflected from crops and the environment. The optical information summarizes the plant compartments, type of leaf, the surface texture, the leaf age, and so on. To extract relevant information on the crop status, the reflectance signature needs to be analysed and characterized. This can be done manually with a high human effort and expertise. Data-driven and machine-learning approaches can reduce the labour intensity and could enable the detection of attributes on the images such as pre-detection and allocation of diseased crops. Among machine-learning approaches, unsupervised and supervised methods for classification and clustering can be applied. Unsupervised machine learning tries to find key patterns in the data without additional manual input. In contrast, supervised machine learning requires a set of labelled training data, which consists of described examples, e.g. image annotations and pixel allocations.

Further opportunity to visualize differences in the field is the combination and calculation of narrow or broad wavelengths ratios. These were developed to establish relationships of multispectral and hyperspectral reflectance signatures to plants and their biophysical variables in remote sensing. These are described as spectral vegetation indices and result in a reduction of data dimension. The Normalized Difference Vegetation Index (NDVI) is a common spectral index to assess plant vitality by the green biomass and chlorophyll content from remote sensing. The NDVI is a normalized difference calculation of reflectance from the near infrared (NIR) and from the red range (NDVI = (NIR − Red)/(NIR + Red)). During the last decades, further spectral vegetation indices were developed and adapted for plant sensing approaches. The calculation of single indices as well as a combination can be used for a fast identification of nematode infested crops in multispectral and hyperspectral images. In addition, the characterization of susceptible and tolerant sugar beets against *H. schachtii* can be characterized in the field (Joalland *et al.*, 2018). In common analysing software a pre-set of indices is given, which calculates indices by a cursor click. Machine-learning algorithms can be implemented into open-source software such as R or Phyton. A database for remote-sensing indices and satellite sensors can be found at https://indexdatabase.de (accessed 30 July 2020). This database is a useful tool to find the required index, sensor and application.

Case studies

The pine wood nematode (PWN), *Bursaphelenchus xylophilus*, is involved in the pine wilt disease (PWD) complex which is fatal to conifer trees primarily belonging to the genus *Pinus*. The PWN enters mature trees via feeding sites of *Monochamus* spp. beetles. Nematodes rapidly invade large areas inside the tree which results in cavitations of the xylem vessels and rapid wilting, accompanied by physiological break down and distinctive discoloration of the foliage, which finally leads to complete defoliation. After invasion of PWN, susceptible mature trees die within a couple of weeks enhanced by higher temperatures and restricted water availability. Due to the fact that PWN is considered an invasive species, it is regulated via the EU eradication programme (2012/535/EU). Hence early detection is a major target to identify infested trees and delimit high-risk areas. An EU pilot study was conducted to establish the feasibility of the remote-sensing-based detection of trees affected by PWN in the 2.2 Mha buffer zone established along the border between Portugal and Spain (Beck *et al.*, 2015). By analysing hyperspectral bands from aerial and satellite images, this study identified five wavelengths that are suitable for calculation of spectral indices to detect progressive stages of canopy decline: 450 nm, 490 nm, 670 nm, 710 nm and 800 nm. These indices include two reflectance band ratio indices, the (re) Normalized Vegetation Index, and a blue-green index. The study showed that repeated image acquisition at high resolution is required to distinguish trees with PWD from other damage as PWD shows rapid progression within weeks. Images should be georeferenced to spot out individual trees. Yet, current satellite image resolution restricts detection to trees with complete defoliation or canopy diameter of less than 2 m.

The sugar beet cyst nematode (BCN), *H. schachtii*, is a major pest of sugar beet with high damage potential (see Chapter 55 in this volume). In several studies, physiological leaf symptoms of different sugar beet cultivars were detected using hyperspectral signatures (HSS) and the data was correlated to initial population densities (*Pi*) of BCN after processing via the Nemaplot-population model (Schmidt, 2015). *Pi* densities were pre-set by cultivation of resistant and susceptible catch crops prior to the sugar beet crop, resulting in *Pi* ranges between nearly zero and >5000 eggs and juveniles per 100 g of soil. HSS were detected using a hand-held sensor connected to different spectrometers (Agrospec, ASD FieldSpec 4) at different times throughout the vegetation period between April and September. Wavelength data from HSS was transformed to numeric parameters and fitted to the Nemaplot model. Transformed data were analysed by multivariate methods as principal compound analysis and general linear modelling. A sufficient correlation (r = 0.74–0.84) was found in sensitive, but not in tolerant cultivars, between population densities assessed by the model and ground truth data. Effective wavelength was identified to be higher than 1100 nm. Due to the multiple interactions between BCN population densities, cultivar, year and vegetation period, measurements of HSS over time did not yield consistantly good correlations at different vegetation stages.

Outlook: a vision for the future

The above-mentioned technologies offer huge potential for future nematode control. A new research project in co-operation between the Julius Kühn-Institute and Nemaplot is in development which aims to progress the Nemaplot model to detect the development of new virulent pathotypes of *Globodera pallida* early in the field, using reference data from the *Heterodera schachtii* model. This will be realized by correlating Nemaplot model processed data of hyperspectral signatures of various severely infected plants and plant stands with factors of climate, management and nematode population as a variation in time and space. Hyperspectral bands will be contributed to a databank for a collection of various host–pathogen systems which should serve as a source for further development of applications. Studies on detection of plant symptoms even in field scenarios by using remote-sensing technologies have demonstrated basic feasibility for many nematode pests like *Globodera pallida*, *H. glycines*, *H. schachtii*, *B. xylophilus* and *Rotylenchus reniformis* as well as *Meloidogyne incognita* both in cotton. However, most of the concepts are not yet established or integrated into decision support solutions and are still in their infancy or are prototypes. Depending on the crop, specific approaches need to be developed. An interpretation with agricultural expertise is a prerequisite. Detailed investigations and specific analytics of soil probes cannot be substituted by these technologies. Since tools for direct control of nematodes are limited, air-borne and imaging technologies can support the breeding process or field planning process.

For implementation into agricultural practice, the basic requirement is an intuitive data input, control, analysis and output. This is a hurdle for many farmers because of the manifold and intensive labour during the vegetation and the state-of-the-art technologies that need to be developed and performed by scientists and trained experts. It is more likely that these technologies become available to farmers via companies, start-ups or the advisory service. Small and simple solutions, as mobile phone-based services, e.g. farmerJOE or Xarvio for field management or the ISIP Leaf Scan for foliar disease identification, are already available. Satellite images are to some extent available for free, but standardized and easy analysis pipelines are still missing for the farmer. Nevertheless, the progress in digital technologies is extremely fast and digital transformation, where software development is only one aspect, is entering agriculture rapidly. We are curious how the invention of remote and proximal sensing in a farmer's everyday work will proceed.

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59 Implementing precision agriculture concepts and technologies into crop production and site-specific management of nematodes

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Introduction

Over the last 20 years the adoption of precision agriculture and precision agriculture technologies has increased worldwide. Precision agriculture is defined as a management strategy that gathers, processes and analyses temporal, spatial and individual data and combines it with other information to support management decisions according to estimated variability for improved resource use efficiency, productivity, quality, profitability and sustainability of agricultural production (ISPA, 2020). This includes a wide range of technologies, many of which are linked to geographic information system (GIS) technologies used to analyse spatial location and organize layers of on-farm data (ESRI, 2020).

Southern root-knot (*Meloidogyne incognita*), reniform (*Rotylenchulus reniformis*), Columbia lance (*Hoplolaimus columbus*) and sting (*Belonolaimus longicaudatus*) nematodes are significant problems on cotton in the US. Collectively they are responsible for mean annual yield losses on cotton of greater than 5%. Each species can cause losses that exceed 25% in individual fields. Cotton cultivars resistant to these nematodes have not been available and the wide host ranges of these species have limited the use of crop rotation as a control. Cotton cultivars have recently been released that are resistant to the southern root-knot nematode. Granular and fumigant nematicides have provided control when applied at uniform rates across fields pre-plant in-furrow or at-plant in-furrow at costs of US$148 and US$74 per hectare, respectively. Site-specific variable-rate (SSVR) technologies offer producers the potential to move away from uniform application rates and apply these nematicides only to specific management zones in a field. The goal is to sustain yield levels while minimizing nematicide applications and thus increasing economic returns.

Strategies for the development of management zones

The success of SSVR nematode management schemes depends upon the quality of the management zones created. These zones should define areas where nematode densities exceed damage thresholds or areas of the field with high sand content that predispose plants to moisture and nu-

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trient stresses which are exacerbated by nematode damage to the root system. In the southern US, for many nematode species these areas correspond to areas of coarse textured, sandy soils. Columbia lance and sting nematodes are present only in soils with greater than 75% coarse textured sand while reniform and root-knot occur in soils with a wider range of sand content.

Historically, nematode management zones were often defined by soil texture since it was easier to measure than the presence and density of nematode species. Collection of quality soil texture information in a cost-efficient manner is one of the keys to developing and delineating management zones. Historically, soil texture data was generated by dividing a field into a uniform geometric grid pattern that ignored any obvious inherent variability in the field (Fig. 59.1). Samples were manually collected from these geo-referenced grids and sent to a lab for analysis. Costs for the collection and analysis of soil samples varies with grid size. For example, for 2 ha grids the cost is approximately US$59+ per grid. Grids often range from 0.2 ha to 4 ha. In general, the smaller the grid size the more accurate the management zones created. An additional US$20 per grid was required to collect nematode samples used to 'ground truth' the presence of nematode species. Overlaying historical yield data on top of the soil texture map strengthened the quality of the information, but the potential still existed for immense within-grid variations in soil texture and nematode densities.

Over time, novel, more cost-effective methods have been developed to define and delineate management zones. The most prominent are the use of soil electrical conductivity (SEC) and imagery obtained from either unmanned aerial vehicles or satellites via the internet. SEC is a measure of the electrical conductivity in each soil texture (Fig. 59.2). Soils with high clay contents have higher SEC values than soils with high sand content. SEC is measured and mapped by pulling a sensor cart that logs global positioning system (GPS) position (Veris Technologies, Salina, KS) across the field, with coulters on the cart that introduce electrical current at a set voltage into the soil (Fig. 59.3). Additional coulters on the cart are responsible for measuring the drop in voltage as the current passes through the soil. SEC is highly dependent upon soil moisture content, clay content, soil salinity, organic matter and soil temperature. SEC values typically range from 0.5 mS/m in the sandy coarse textured soils in the south-east US to 50 mS/m in the heavy shrink-swell clays of the mid-southern region of the US. Soils that are subject to saltwater intrusion are also subject to high SEC values. SEC has a strong correlation to soil texture changes within a field; however, comparisons between fields with major differences in soil type cannot be made. These SEC-generated soil texture maps can then be used to create nematode management zones if some level of ground truthing of the nematode species present is done.

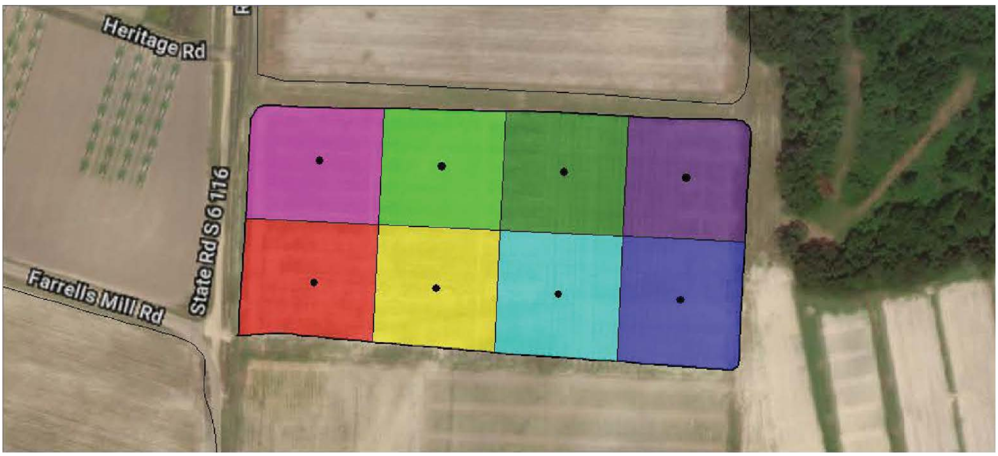

Fig. 59.1. Soil sampling grids overlaying a field. Author's own figure.

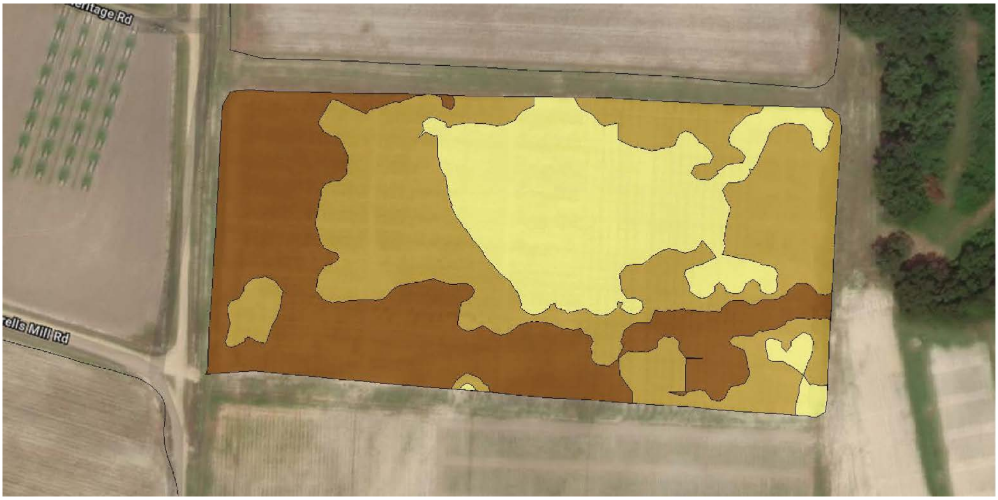

Fig. 59.2. Soil electrical conductivity map with three divisions. Map colours represent differences in soil texture. Author's own figure.

Fig. 59.3. Veris 3100 sensor cart used for collecting soil electrical conductivity data to make soil texture management zones for site-specific applications (Veristech.com). Photograph courtesy of Veristech.

Regression models for predicting relative differences in soil texture within a field from red, green and blue imagery generated by unmanned aerial vehicles (UAV)-mounted inexpensive cameras or satellite imagery commercially available on the internet can be coupled with GIS software. This would provide farmers, consultants and other agricultural practitioner apps a cost effective and efficient way to develop management zones for SSVR management of nematodes and other crop inputs. In a 50-hectare field with 20 grids, generating management zones with laboratory analysis of soil textures and nematode densities would cost approximately US$1375. Utilizing SEC technology at US$20 per hectare, the cost would be approximately US$1000 plus

US$400 for the cost of nematode samples. The same maps can be generated with UAV photos for less than US$500 and utilizing web-based imagery would be closer to US$250 for the same field. A significant part of the costs is associated with hiring a consultant to manage the data and development of a prescription application map. For all these technologies the zones created would be useful for at least 10 years, so the costs could be depreciated over time.

Evolution of application technologies needed for SSVR applications

The first and most popular precision agricultural technologies with producers were GPS-referenced guidance and autosteering on tractors. These allow farmers to collect spatial data with sub-inch accuracy while performing field operations as well as increase field efficiency and precision placement of inputs such as fertilizers and pesticides. Effective utilization of nematode management zones requires technology to allow geo-referenced variable-rate applications of preplant fumigant and at-plant granular and liquid nematicides. Over the last 5 years the development of precision liquid, granular and planting application technologies has increased rapidly. Producers can now implement very precise SSVR applications of granular and liquid pesticides and fertilizers. Granular products, such as aldicarb, were previously applied at uniform rates across a field using ground or electrically driven applicator boxes mounted to the planters. These boxes were difficult to accurately calibrate, incapable of changing rates within the field, or toggle on/off while on-the-go. New technologies allow granular applicator boxes to be independent of the mechanical drive transmission on the planter, allowing for easy rate changes, calibration and GIS-referenced on/off operations. In-furrow spray placement technologies have been developed where liquids can be directly applied to the seed, under the seed, over the seed, to either side of the seed, or in-between the seeds with the use of pulse width modulation (Capstan Ag, Topeka, Kansas). The utility of these advances has yet to be explored for application of nematicides.

Planter technologies have also been recently developed that allow improved accuracy of planting operations and seed placement. Multi-cultivar split-hoppers utilizing independent row drives and row clutches allow producers to switch varieties on-the-go based on prescription maps loaded into a GIS-referenced control terminal on the tractor. Each cultivar is placed in the desired locations within a field in one planting pass (Fig. 59.4). Varying seeding applications include, but are not limited to, nematicide-treated

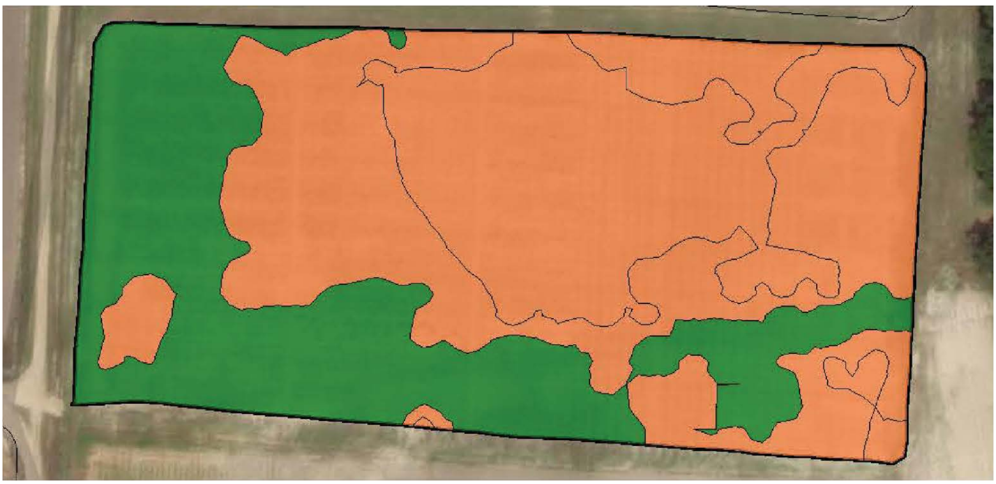

Fig. 59.4. Multi-cultivar planting prescription. Cultivar A planted in the green areas of the field and cultivar B planted in the orange areas of the field. Author's own figure.

versus untreated seed or susceptible versus nematode resistant cultivars. In many instances, nematode resistant cultivars have exhibited a yield drag. It is not as profitable to plant them in nematode ‘free’ areas of a field as higher yielding, susceptible cultivars. Root-knot nematodes are typically present in damaging levels in less than 50% of a given field. Resistant cultivars could be planted in zones designated at high risk for nematode-induced yield losses. The remainder of the field could then be planted to southern root-knot nematode susceptible cultivars that may yield 10–20% higher in the absence of the nematode than the resistant cultivar. For fields infested with Columbia lance nematode for which there is no resistance, more expensive nematicide-treated seed could be planted in high-risk zones to minimize losses.

Assessing nematode damage from multispectral images

The use of UAVs in agriculture has allowed for quick field scouting, crop data collection, and data-filled imagery to be produced in ways that have not been available previously. With UAVs equipped with hyperspectral and multispectral cameras, images can be produced showing details that go unnoticed by the human eye. Imagery containing data such as Normalized Difference Vegetative Index (NDVI), the degree of greenness or ‘plant health’, is becoming a viable and useful option for detecting crop damage from many pests, including nematodes. Using these GPS-coordinated images, management zones can be created to utilize SSVR nematicide applications or multi-cultivar planting schemes to help manage nematodes. This technology is still early in its development and needs significant improvements in the software needed to create a prescription application map from the initial image.

Field experiences with site-specific nematode management

Successful SSVR nematode management programmes exhibit some or all the following traits. There must be a strong relationship between nematode densities and yield losses. Variations in soil texture should be readily apparent and a close relationship between soil texture and nematode population density or nematode-induced yield losses should exist. An economically viable and consistent control method must be available, and the technology must be available to implement the control measure in a site-specific and possibly variable-rate manner.

Cotton production in the southern US fulfils all the criteria listed above. Coastal plain soils contain more than 60% coarse textured sands, so they are conducive to infestation by root-knot and Columbia lance nematodes. These species are easily detected and have reliable damage thresholds that occur in easily detected ranges. Damage from root-knot is most severe in the sandiest soils (Monfort *et al.*, 2007). Variable-rate technologies for application of the most commonly used nematicides, aldicarb and 1,3-dichloropropene (1,3-D) are available.

In South Carolina, Mueller *et al.* (2010) demonstrated 5% greater yields using variable rate versus uniform rate applications of either aldicarb or 1,3-D for controlling Columbia lance nematode. Total nematicide applied was decreased by 34% with variable-rate aldicarb and 78% in the variable-rate 1,3-D plots. They obtained similar results in tests involving root-knot nematodes. In Arkansas, Monfort *et al.* (2007) had similar results utilizing variable-rate applications of 1,3-D to control root-knot in cotton. Mean yields for their uniform rate were 113 kg/ha greater than for their non-treated control, whereas yields for the variable application were 143 kg/ha greater than the non-treated control. The variable-rate application utilized 40% less 1,3-D compared to the uniform rate. Other field trials evaluating control of root-knot on cotton have had mixed results. In Georgia, in similar soil textures, variable-rate applications of aldicarb in-furrow at-plant did not show yield or economic advantages compared to a uniform application (Baird *et al.*, 2001). In the same test fields, variable-rate applications of 1,3-D produced similar or greater yields than the uniform rate and the increase in revenue resulting from lower nematicide costs offset all costs associated with the variable-rate process. Trials in Texas compared variable-rate applications of aldicarb in-furrow at-plant to uniform rate applications chosen by growers for each field (Wheeler *et al.*, 1999). Variable-rate applications provided increased or equivalent yield to a uniform rate in three of eight tests. In three other tests variable-rate applications used more aldicarb but resulted in lower yields than the uniform rate that the grower selected. In the other two tests, there were no yield differences. In this case the problem was probably that damage thresholds were not defined well enough to make functional rate prescriptions across this wide range of soils and environmental conditions.

Examples from attempts at nematode management in potato production further illustrate when SSVR applications are and are not successful. In Idaho, a simple grid sampling system was utilized for detection of the Columbia root-knot nematode and implementation of a site-specific application of 1,3-D on 640 ha of potatoes (King and Taberna, 2013). Nematicide usage was reduced by 30% with a production cost savings of US$209 per ha. In the UK, an attempt to use grid samples to build a spatial nematicide application prescription to control potato cyst nematodes (PCN) on potato was less successful since the damage threshold for PCNs are less than the detection level (Evans *et al.*, 2002).

The use of several layers of historical yield data, coupled with an index of potential nematode damage for specific zones along with SSVR nematicide applications, resistant cultivars, crop rotation and precision application technologies is currently the most effective approach to implementing cost-effective nematode management. One of the most important aspects of all these studies is that with prescription variable-rate applications, when equivalent or greater yields are obtained than with uniform rates, the environmental impacts from nematicide applications have been reduced.

Economic importance of precision agriculture technology

The economic impact of the implementation of current and future precision agricultural technologies goes far beyond the economic benefits of reducing yield losses due to nematodes. The core technologies used to remotely collect the geo-referenced data used to create nematode management zones are expensive but can also be used to map soil textures related to fertilizer applications, soil moisture status to control irrigation application, plant stress related to pest pressure, moisture or nutrient deficiencies, and crop growth stage and yield potential. Some of the technologies, such as variable seed planters, are relatively expensive and others such as UAV-mounted cameras used to generate multispectral data, are relatively inexpensive. The more extensively the technologies are used across a wide range of productions processes, the more the cost of the initial technology and overall concept can be shared across crops, enterprises and inputs, rather than for nematocidal applications alone.

Future research requirements

As with any new emerging technology, validation that the technology appropriately and repeatably works is needed. Currently with precision agriculture, our data collection capabilities have exceeded our decision-making (data interpretation) capabilities. We no longer want to make specific blanket recommendations for uniform rate applications across a field. We need to learn how to implement appropriate technologies across different fields or farms to address the multitude of pests and agronomic challenges facing crop production. The strength of precision agriculture is that it can generate site-specific data almost down to the plant level. Its weakness is the time it takes to develop accurate treatment prescriptions for this almost infinite set of conditions. We can apply any rate of nematicide to any spot in a field. However, we have only a crude understanding of the rate response curves for nematicides against the multiple species of nematodes present in a field. We need in-depth information on the levels of yield drags, if any, associated with resistant cultivars to assess the validity of switching to a more susceptible but higher yielding cultivar in some management zones. Additional work is needed to expedite the collection of soil texture data, nematode sampling, prescription development and implementing the prescription through variable-rate capabilities.

Outlook: anticipating future developments

Agriculture faces the continuing challenges of reducing production costs, responding to emerging pests and reducing environmental impacts while producing the high-quality products consumers demand at low prices. By incorporating precision agriculture into cropping systems and using precision technologies to help collect on-farm data to make and implement decisions, farm efficiency should increase. Anticipated future developments include utilizing satellite imagery to measure crop health through NDVI, determining soil texture and variability across fields, and accurately estimating yields across a field. Some of these concepts and practices are still in the developmental stage. One trait they all share is that the data generated can be very costly and require skilled individuals to process and present in a useable format for producers. As the cost of GPS technology decreases and GPS accuracy increases, collecting sub-centimetre accuracy, site-specific data will likely become the standard for more applications across farms. Utilizing cell phone networks, towers, GPS receivers and cameras in smartphones, and the ability for accurate data collection could be realized across farm scales, growing regions, cropping systems and production practices worldwide.

The ability of precision agriculture to reduce inputs, production costs, and environmental impacts while maintaining or increasing yield make it one of the prime candidates for future nematode management programmes in row crop production systems.

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60 Decision support systems in integrated nematode management: The need for a holistic approach

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Farmers' decisions regarding the best approach to take in managing a nematode problem are often made just before the planting season begins and then at different stages in crop growth (Sikora and Roberts, 2018). This decision is usually determined by a combination of past experience, anticipated market prices and recommendations from both public and private extension services. In many cases an integrated nematode management (INM) system with multiple components is not considered due to lack of knowledge of the severity of the nematode problem, or the absence of an acceptable management tool such as a suitable resistant or tolerant cultivar, an appropriate non-host rotation crop or a suitable biocide for the situation.

Seldom, if ever, are data on nematode population densities and distribution patterns across a field taken into consideration in the decision-making process. This haphazard approach to INM is comparable to what could be called a 'take a chance' or 'shot in the dark' tactic which often results from the absence of effective decision support tools (DST) or decision support systems (DSS).

The development of INM strategies is knowledge intensive, bringing together host status, crop sensitivity, damage thresholds, nematode density and distribution, with known effectiveness of individual management measures. The vast majority of DSS were developed in countries with modern agricultural structures by scientists working at universities, extension services or agricultural base companies. In some cases, government agencies have played a major role in developing or suggesting management programmes where invasive pests are concerned.

In this chapter, we attempt to present presently available information on different forms of DST and DSS and discuss developments that are required to streamline and improve INM. Obviously, we will not be able to discuss all DST and DSS that have been developed over time, therefore, this chapter is not all inclusive.

Governmental databases

Databases for quarantine nematodes have been developed in many countries. For example, that of the European Plant Protection Organization (https://gd.eppo.int/, accessed 20 October 2020) as well as by other national plant protection organizations around the world. These DST are aimed at preventing the introduction and spread of important nematode quarantine pests both between and within countries. They are

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also designed in some cases to limit nematode spread on export/import planting material. Their focus is clearly on diagnosis and regulation.

Knowledge databases of universities and extension services

These databases are basically knowledge banks containing quality information on nematodes and INM approaches. They are open-access links that can be used to find and select management options across a wide range of nematode/crop interactions. There are a large number of websites and databases developed by universities and extension services around the world. This is especially true for the US where most university extension services have a website that covers nematodes and their management in their state (Table 60.1). These websites can be accessed not only locally, but also by growers on a global scale for information on nematode diagnostics, symptomology and methods of INM. These sites vary in size and detail depending on the importance of nematodes to agriculture in the region as well as by the number of extension specialists available to focus on nematode issues at that location. They are in most cases updated regularly by experts of the organizations involved and are therefore important as a first-line DST in obtaining information on INM. Due to the number of websites available we have limited the list to a few examples that cover a broad array of crops and nematode problems (Table 60.1).

We want to highlight the DST Nemaplex site (http://nemaplex.ucdavis.edu/Uppermnus/topmnu.htm, accessed 15 October 2020) of UC Davis University in the USA which offers an extensive amount of information to students, farmers, advisers and applied nematologists. The topics range from teaching nematology to supplying nematode management options.

Two well-developed commercial knowledge banks with extensive and up-to-date information on the most important plant parasitic nematodes on a global scale are marketed by CAB International in the UK:

- CABI Crop Protection Compendium (https://www.cabi.org/cpc, accessed 10 November 2020; and
- CABI Plantwise knowledge bank (https://www.plantwise.org/knowledgebank/, accessed 10 November 2020).

Decision support tools

Decision support tools are important building blocks in support systems used for INM. They generate information on specific abiotic factors that influence nematode behaviour in the field and can be utilized to improve INM.

Soil sensors

One of the most important DST available to nematologists are temperature and moisture monitors. They have been used in different forms for many years to measure abiotic effects on crop growth and nematode and disease behaviour. They are now more accurate and convenient for use in research and for INM. These monitors supply the user with automatic real-time monitoring of temperature and moisture levels in the soil that influence nematode population development over short and long periods of time. They generate data that can be used in the development of INM programmes designed to time the use of treatment options such as: post-plant nematicide treatments, application of biocontrol agents in standing crops, estimating population development, as well as for timely destruction of trap crops before egg laying begins. These types of monitors, coupled with advanced models for nematode population development over cropping sequences, could be used to improve INM in many crops.

Two of these monitors are discussed below.

Nematool

This DST was developed by BayerCropScience (https://nematool.com, accessed 12 November 2020) in close collaboration with nematologists in Spain to determine the progression of root-knot nematode development in the field under a host crop, and thereby determine the optimum time for application of the biopesticide Bioact in an established crop. The system collects temperature data in the field and sends information

Table 60.1. A partial listing of websites available from universities, extension services and government agencies offering integrated nematode management information on a wide array of nematodes and crops worldwide.

Institution	Website[a]	Information coverage
Sociedade Brasileira de Nematologia/Brazilian Society of Nematology	https://nematologia.com.br/ (in Portuguese)	General nematology, diagnostics and management of nematodes on amongst others: soybean, cotton, maize, potato, rice, wheat, sugarcane, coffee, vegetables and fruit crops
NemaDecide, Wageningen University	http://www.nemadecide.com/	Gives real-time results of soil sampling for specific nematodes on potato along with GPS coordinates to aid in site specific management. Mainly potato cyst nematodes but also lesion and root-knot
University of Florida Cooperative extension	https://sfyl.ifas.ufl.edu/search-result/?q=nematodes	Covers all major fruits, vegetables and field crops grown in Florida. Gives pertinent information on nematodes, symptoms, control tools and management recommendations. Updated regularly by Florida nematologists
University of Florida Entomology and Nematology Department	https://edis.ifas.ufl.edu/department_entomology_and_nematology	Provides in-depth profiles of insects, nematodes, arachnids and other organisms and integrated management tools
Nemaplex, University of California Davis	http://nemaplex.ucdavis.edu/Uppermnus/topmnu.htm#	Extensive information on diagnostics, management, methods, ecology, including spreadsheet tools for INM and making economic threshold decisions
University of California Agricultural & Natural Resources - IPM	https://www2.ipm.ucanr.edu/agriculture/	Pest management information on 44 crops or groups of crops including nematodes. Chapters updated every 3–5 years by faculty members
Australia Department of Primary Industries and Regions	https://pir.sa.gov.au/search?collection=PIRSA-web&query=nematodes	Used in Western and South Australia to determine the presence and levels of *Pratylenchus thornei, P. neglectus, P. penetrans* and *P. quasitereoides*
UK, Agriculture and Horticulture Development Board	https://ahdb.org.uk/(A('xTcrINcvSX))/Search?q=nematodes	Covers management of a number of nematode problems including migratory, cyst and leaf nematodes on a number of crops in the UK
Indian Agricultural Research Institute Nematology ICAR	https://iari.res.in/index.php?option=com_content&view=article&id=72&Itemid=161	All India coordinated research project on plant parasitic nematodes with integrated approach for their control
Louisiana State University Cooperative Extension	https://www.lsuagcenter.com/portals/our_offices/departments/plant-pathology-crop-physiology/nematode-advisory-service	Coverage of nematodes on cotton, turf, vegetables, soybean and others
University of Georgia	https://plantpath.caes.uga.edu/search.html?q=nematodes&cx=008984291200700708817%3Abqxfpp1iz4w&searchScope=siteOnly	Disease clinic provides diagnostic analysis or pests and diseases including nematodes and recommends appropriate management strategies

Continued

Table 60.1. Continued.

Institution	Website[a]	Information coverage
Lucid, Australia	https://keys.lucidcentral.org/keys/v3/crop_rotation_plant_parasitic_nematodes/	Crop to be used in rotations and their resistance to plant parasitic nematodes
Best4Soil	https://www.best4soil.eu/	A network of practitioners, for sharing knowledge on prevention and reduction of soil-borne diseases in 22 European languages. Offers a tool to develop unique crop rotation sequences to take into account nematodes and soil-borne diseases

[a]Websites accessed 1–15 November 2020.

on degree days, along with information on application timing directly to the grower's computer or smart phone in real time.

LoRa Soil sensor

The practical application of this sensor was co-developed by Valenco and Syngenta. It gathers information on both soil moisture and temperature. It generates data that can be used to correctly estimate the stage of nematode development using degree days. Wireless data transmission also allows real-time availability of information (Fig. 60.1).

Remote sensing

Remote sensing is a rapidly developing DST for nematode detection, visualizing field clustering and estimating crop damage (see Chapter 59 in this volume). The Normalized Difference Vegetation Index (NDVI) is one of the most widely used indices in remote sensing for vegetation observations. NDVI can reveal where vegetation is thriving and where it is under stress, for example due to pest and disease damage (Oerke *et al.*, 2010). Remote sensing with NDVI can be used to estimate relative yield and supply an end of season plant assessment, as well as nematode distribution in the field during the cropping season. For example, remote sensing has been used to correlate sugar beet damage with *Heterodera schachtii* pre-plant densities (Hillnhütter *et al.*, 2011). It has also been used to measure tolerance to *H. schachtii* (Joalland *et al.*, 2018). Noling and Cody (2014) and Noling *et al.* (2015) used quantitative descriptions of canopy dimension to select integrated nematode management practices, describe yield impacts as well as treatment performance on horticultural crops on an industry-wide basis (see Chapter 26 in this volume). Hyperspectral sensors using NDVI can be

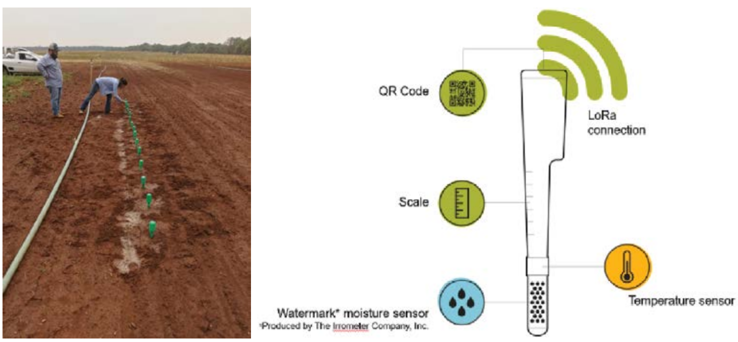


Fig. 60.1. Soil sensors (Valenco GmbH, Switzerland) for monitoring temperature and moisture relationships on nematode population development in microplots. Figure courtesy of M. Goll, Syngenta, Switzerland.

Fig. 60.2. AISA hyperspectral false colour infrared picture at GS 31 and digital map of *Heterodera schachtii* damage clustering. **(A)** Infrared photograph; **(B)** digitalized computer map with colours correlated with final nematode (*Pf*) densities (Hillnhütter *et al.*, 2011). Author's own figure.

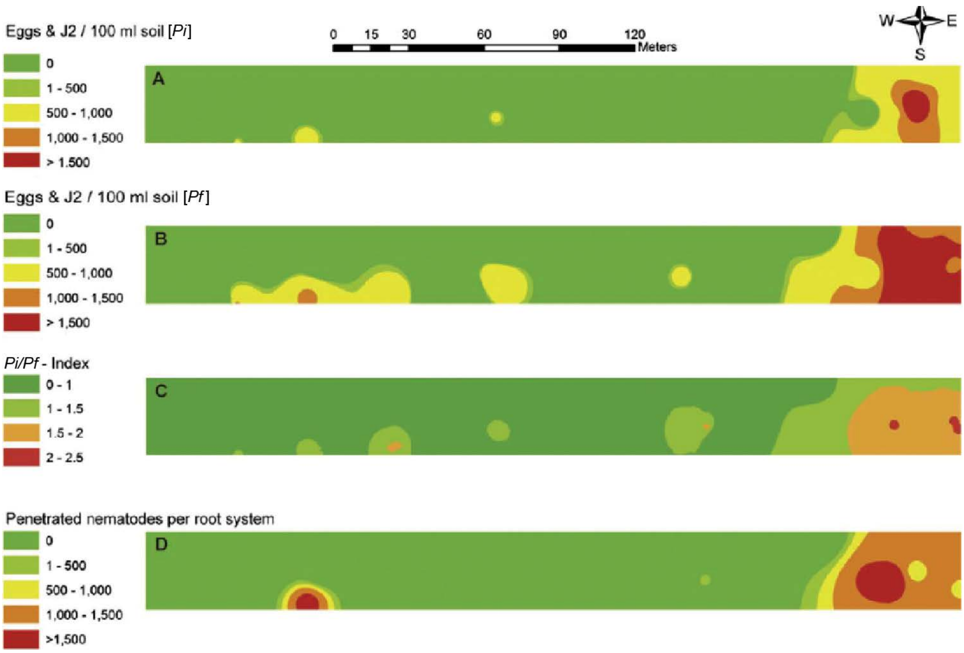


Fig. 60.3. Spectral angle mapper classification of *Heterodera schachtii Pi* and *Pf*, the *Pf*/*Pi* index and nematode penetration/root generated from the AISA infrared picture in Fig. 60.2. Author's own figure.

used to detect clustered distribution by measuring nematode-induced changes in crop canopy chlorophyll reflection (Fig. 60.2), which can then be related to ground truth data in specific clustered areas in a field (Fig. 60.3).

Correlating NDVI remote sensing with soil temperature sensor data can be used to predict nematode population dynamics over time in crop rotations in different parts of a field. This information can then be used to develop computer models for decision making at the farm level (see Nemaplot below). Once this relationship is established INM can be optimized for various nematode–crop interactions.

Predictive models

Predictive models could have a major impact on INM in the future. They will become important as climate volatility impacts INM decision making and alters nematode population dynamics from year to year, making decision making complex. Nemaplot is an example of a predictive model developed to predict yearly oscillations in *H. schachtii* population densities in rotations of varying lengths and crops (Schmidt *et al.*, 1993). Figure 60.4 shows how a population fluctuates over a 3-year period as influenced by crop cycle, ambient temperature and intercropping management. Decreases from initial pre-plant densities as influenced by non-host crops and the natural antagonistic potential is extrapolated from the literature and research data. Using the initial population density determined by sampling before sugar beet and this natural decline data, the *Pi* before the next sugar beet crop can be estimated by the model and used to support grower selection of management options. In Sweden, SBN-Watch offers comparable functionalities to control sugar beet nematodes (Omer *et al.*, 2019). This type of model could be expanded to other plant parasitic nematodes to improve INM decision making where ground truth data and past research data has been or will be generated in the future.

Decision support systems

Advanced DSS are designed to aid growers in making real-time decisions on how to manage farms and in some cases nematode problems. These programs combine information on many different aspects of farm management.

There are many DSS available developed by industry and government agencies for agronomic aspects of farm management. However, none of these DSS have components that focus on INM. The systems discussed below have been developed for use in the EU and could be used to develop similar programs for other plant parasitic nematodes in appropriate cropping systems wordwide.

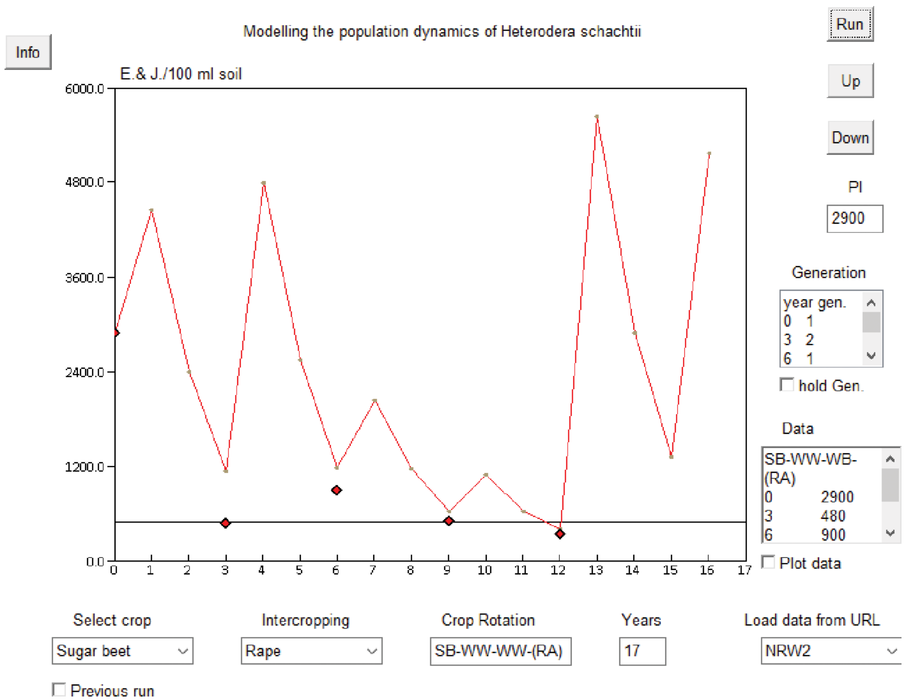


Fig. 60.4. Graphic representation of the population fluctuation of *Heterodera schachtii* in a 3- year rotation with intercropping in Germany as calculated by the Nemaplot program compared to long-time observations (red dots). Figure courtesy of K. Schmidt, Bonn, Germany.

Akkerweb/FarmMaps

This is an open-access DSS providing information required for smart farming application, including weather data services, satellite crop images, soil maps, crop polygons and more (Van Evert *et al.*, 2018). The program also provides visualization tools, a DSS digital store, task map generator and an array of models. Examples include models for calculating water availability (Watbal model), potato crop growth (Tipstar model), late blight infection (Blight module) and nematode management (NemaDecide) for individual fields or within a field. Other models are available for variable-rate application of herbicides, fungicides, nitrogen top-dress application in potato and potato haulm killing. Akkerweb has over 30 apps. FarmMaps, released in 2021, is the new version of Akkerweb and has a new data repository and management system as well as a more intuitive dashboard design (Fig. 60.5).

NemaDecide

NemaDecide is a DSS for the management of plant parasitic nematodes (Been *et al.*, 2005). It was developed for INM of nematodes in potato (*Globodera* spp., *Pratylenchus penetrans* and *Meloidogyne chitwoodi*). Models for population dynamics, yield reduction, partial resistance, soil sampling, chemical control, etc. are included in the system. It combines several data sources and models to enable strategic and operational decisions at the farm and field level. The quantitative information system provides growers with the possibility of estimating risks of yield loss, population development, probability of detection of foci by soil sampling, calculation of cost/benefit of control measures, as well as providing advice for farmers to optimize financial returns. Farmers can also compare cropping scenarios and ask 'what if' questions (Fig. 60.6).

This DSS offers the opportunity to compare different INM designs and can be used for general agricultural practices and certification schemes, as well as for educational purposes. Gaps in knowledge related to INM recommendations are pinpointed in cooperation with the agricultural sector, user groups and extension services. Thus, the most problematic questions receive priority in research programmes, which are now better focused on practical problems.

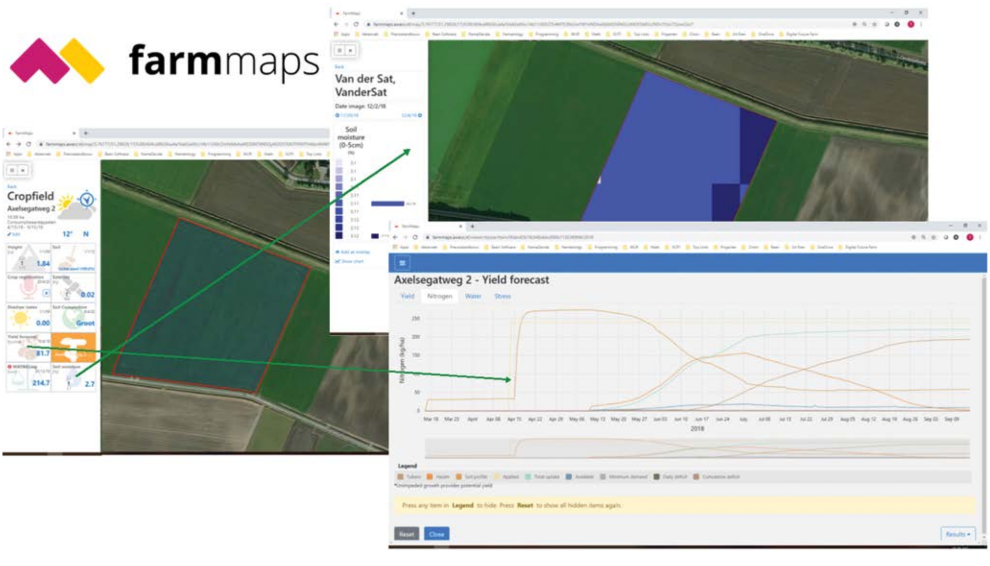


Fig. 60.5. An impression of the dashboard of FarmMaps. All information of a field can be reached via the widgets. In this figure soil moisture and the yield forecast of potato are highlighted. Figure courtesy of Wageningen University & Research, Field Crops.

(A)

(B)

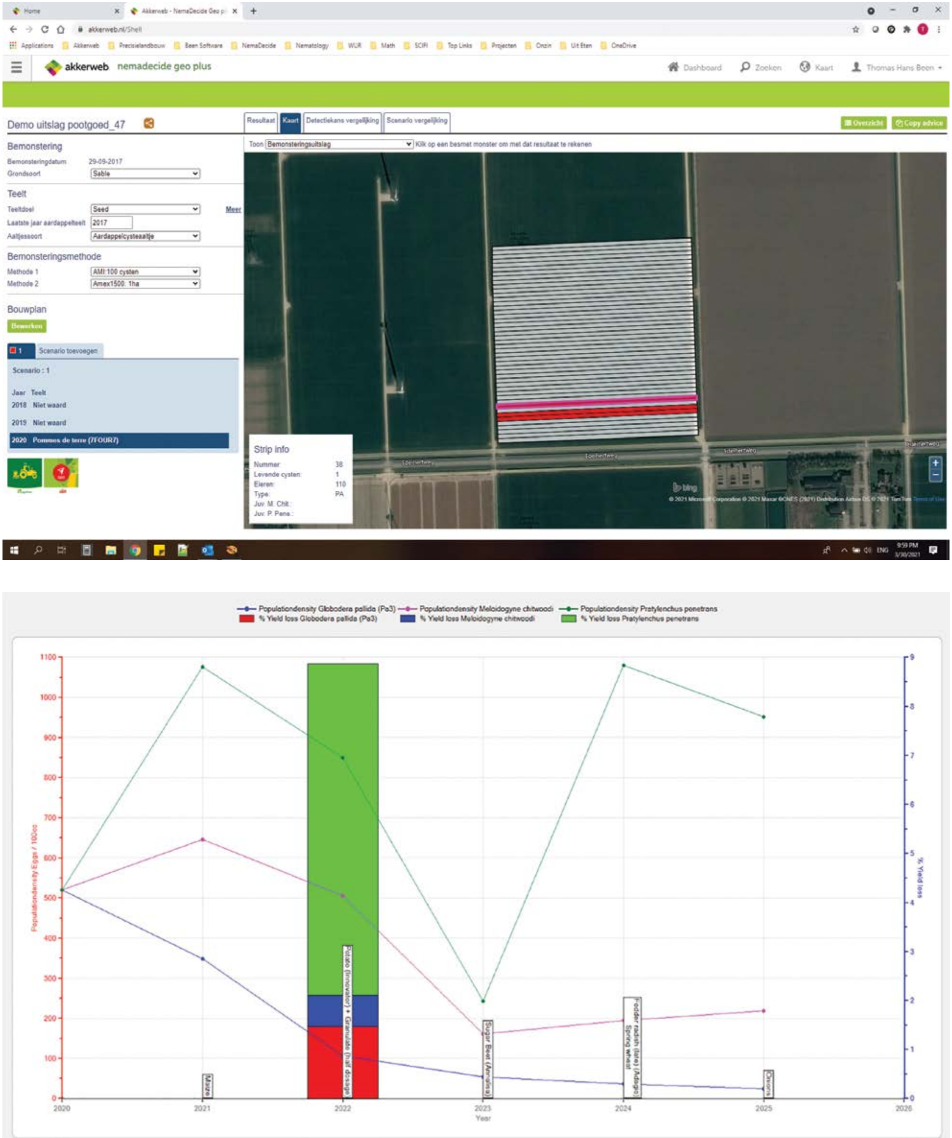
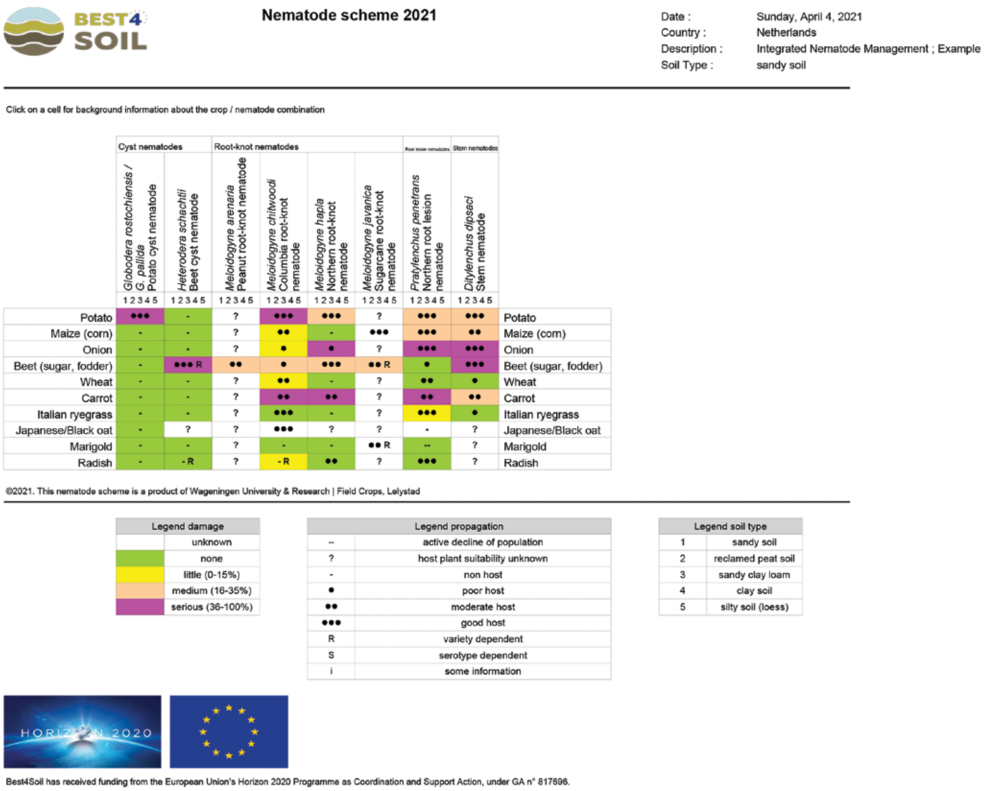


Fig. 60.6. **(A)** Screenshot of NEMADECIDE GEO application on Akkerweb, with on the right a map of the field with the sampled strips and the red strips in which a nematode infestation has been detected. The data from the infested sample strips (red) can be uploaded via the nematicide button and the comparison of scenarios can start. **(B)** The lines show the population dynamics of *Globodera pallida, Pratylenchus penetrans and Meloidogyne chitwoodi.* The bar expresses the percentage yield loss in potato (innovator + granular nematicide half dosage) caused by each species. Figure courtesy of Wageningen University & Research, Field Crops.

Best4Soil

This is a European network DSS developed in co-operation with practitioners for sharing knowledge on prevention and reduction of nematodes and soil-borne diseases. The program provides an INM system by developing what we term 'clever crop rotations' (www.best4soil.eu/database, accessed 15 February 2021). The approach and design is based on the Dutch program www.aaltjesschema.nl (accessed 15 February 2021) which has its origin in 1968



BEST4 SOIL

Nematode scheme 2021

Date : Sunday, April 4, 2021
Country : Netherlands
Description : Integrated Nematode Management ; Example
Soil Type : sandy soil

Click on a cell for background information about the crop / nematode combination

	Cyst nematodes		Root-knot nematodes				Root lesion nematodes	Stem nematodes	
	Globodera rostochiensis / G. pallida Potato cyst nematode	*Heterodera schachtii* Beet cyst nematode	*Meloidogyne arenaria* Peanut root-knot nematode	*Meloidogyne chitwoodi* Columbia root-knot nematode	*Meloidogyne hapla* Northern root-knot nematode	*Meloidogyne javanica* Sugarcane root-knot nematode	*Pratylenchus penetrans* Northern root lesion nematode	*Ditylenchus dipsaci* Stem nematode	
	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	
Potato	●●●	-	?	●●●	●●●	?	●●●	●●●	Potato
Maize (corn)	-	-	?	●●	-	●●●	●●●	●●	Maize (corn)
Onion	-	-	?	●	●	?	●●●	●●●	Onion
Beet (sugar, fodder)	-	●●● R	●●	●	●●●	●● R	●	●●●	Beet (sugar, fodder)
Wheat	-	-	?	●●	-	?	●●	●	Wheat
Carrot	-	-	?	●●	●●	?	●●	●●	Carrot
Italian ryegrass	-	-	?	●●●	-	?	●●●	●	Italian ryegrass
Japanese/Black oat	-	?	?	●●●	?	?	-	?	Japanese/Black oat
Marigold	-	-	?	-	-	●● R	--	?	Marigold
Radish	-	- R	?	- R	●●	?	●●●	?	Radish

©2021. This nematode scheme is a product of Wageningen University & Research | Field Crops, Lelystad

Legend damage	
	unknown
(green)	none
(yellow)	little (0-15%)
(orange)	medium (16-35%)
(purple)	serious (36-100%)

Legend propagation	
--	active decline of population
?	host plant suitability unknown
-	non host
●	poor host
●●	moderate host
●●●	good host
R	variety dependent
S	serotype dependent
i	some information

Legend soil type	
1	sandy soil
2	reclamed peat soil
3	sandy clay loam
4	clay soil
5	silty soil (loess)

HORIZON 2020

Best4Soil has received funding from the European Union's Horizon 2020 Programme as Coordination and Support Action, under GA n° 817696.

Fig. 60.7. Nematode scheme of the Best4Soil Decision Support System. The colours express the sensitivity for damage, the dots the host status of the crop. Figure courtesy of Wageningen University & Research, Field Crops.

(Hijink and Oostenbrink, 1968). An example of the nematode scheme is given in Fig. 60.7.

Pros and cons

We contacted a large number of colleagues worldwide and were surprised at the low numbers of DDT and DSS available for use in INM. Decision support systems work as crystallization points of knowledge. Loose chunks of knowledge are brought together with local data from the growers so that the best INM measures can be followed over the short and long term. In this way, knowledge improves INM and impacts practical agriculture at the farm level. Conversely, it becomes clear where 'black holes' exist in our knowledge of nematode–crop interactions and how our research agenda should be prioritized.

The implementation of DSS demands a high standard of knowledge and education in farming, extension services and research. Many countries/regions cannot meet these requirements. For example, access to DSS requires Internet with sufficient bandwidth and availability at many levels of decision making. This high level of Internet availability is not yet standard in large parts of the world. A prerequisite for using DSS is a link to mobile phone technology. In many countries, telephone networks are often better developed than the Internet such as in Africa and India (Baumüller and Kah, 2020). DSS development for use on laptop computers needs to be adaptable to all digital information carriers and available worldwide.

When switching from chemical treatments to knowledge-driven and complex INM solutions, education and extension are the most important parts of transformation. Baseline information, data sharing and converting data into management tools is a big hurdle to success. As in many areas of science, big data without proper tools ends in a digital traffic jam. An important issue is how to create confidence in the data and tools. The potential of INM is based on data combined with knowledge and this requires ambition and action in the realm of DSS in nematology. A good starting point would be the development of an Internet platform of nematological tools.

There are limitations to all DSS in that they need to be adaptable to conditions as well as the customs and laws in the country targeted for use. This is often a difficult barrier in making them important nationally and globally. Limitations include:

- deficits in Internet coverage;
- grower suspicion of data collection;
- overcoming farmer independence;
- presence of quarantine nematodes;
- resolution of satellite images;
- major data protection problems; and
- need for ground truth verification.

Outlook: a vision of the future

INM will advance in importance and become more knowledge intensive as crop production is influenced by: (i) global food security related to human population growth; (ii) environmental protection issues; (iii) public concern for safe food; and (iv) the ever-present impact of climate volatility on nematode damage. INM using DSS will evolve into highly efficient, tailor-made systems that ensure the production of healthy crops in a clean environment. Some of the expected future developments connected to the main pillars of INM (see Figure 1.1 in this volume) are discussed below.

Prevention

Geo information systems will stack sampling data and historical information to prevent production of 'nematode-free' propagating material in nematode infested fields for both local and export markets. The tracking and tracing of seed and planting material back to the producer will need to be improved due to regulatory controls.

Cultivar choice and crop rotation

Knowledge of the levels of cultivar resistance and/or tolerance and relation to yield loss will be available in databases that make the design of smart rotations possible.

Targeted control

Both the use of nematicides and alternative control methods will be optimized and used when damage thresholds are exceeded. Remote sensing will allow treatment of clusters where infestations exceed thresholds.

Monitoring and remote sensing

The use of multiple soil temperature/moisture sensors coupled with mathematical models will allow exact monitoring of nematode population development over time and allow prediction of *Pi* before the next susceptible crop. This information will be incorporated into DSS programs. The use of remote sensing and NDVI technology will enable exact determination of nematode distribution and coupled with precision mechanization, allows precise placement of chemical and biopesticides.

Molecular soil biodiversity tracking

The development of deep sequencing will allow full scans of soil samples for nematodes and microbial antagonistic diversity and will expand the knowledge bank for use in DSS management programs.

Holistic crop and field management systems

Last but not least, the future of INM lies with holistic approaches to field management. We believe this is where the great leap forward must be made.

Future crop and nematode management must include all the pillars of INM as well as the following: soil fertility, carbon sequestration, water quality, resilience to climatic volatility, biodiversity maintenance, as well as weed and soilborne pathogen management. The process of DSS development will probably begin with high-value horticultural and industrial crops and where IT is highly developed and accessible to farmers. Farmers and extension agents in the decennia will NOT indiscriminately ask themselves what type of management a field requires for sustainable production but will use advanced DSS for their decision-making process.

Building DSS at this level of integration will provide a platform where all disciplines meet and develop interdisciplinary approaches that give the best possible answers to healthy agricultural production based on the best knowledge available.

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61 What does it take to develop a nematicide today and for the future?

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Introduction

There are three major drivers for the development of a next-generation chemical nematicide. The first key element of a new nematicide is strong intrinsic potency against all economically relevant plant parasitic nematodes to ensure maximum protection leading to best return on investment for this measure, in yield, to the farmer. Secondly, the chemical should have an improved human and environmental safety profile in comparison to commercially available nematicides in the market, thus overcoming existing and future global regulatory constraints. The final driver is the ease of use of the product to provide a convenient, simple and effective method of application such as a seed-applied technology. Finding a molecule that fulfils all the above criteria at an affordable cost for the grower is not an easy endeavour.

Building on the history of nematicides

The launch of every new generation of nematicides requires market preparation. Since plant parasitic nematodes are microscopically small, transparent and live in the soil, awareness regarding spread of this pest, symptoms of damage and associated yield loss needs to be conveyed to the grower. Demonstration trials illustrating the yield benefits and corresponding return on investment for the grower have been carried out since the early 1920s. The first chemicals used to commercially control nematodes in the early twentieth century were fumigants, following the discovery of the devastating impact of sedentary nematodes such as *Heterodera schachtii* in sugar beet or root-knot nematodes on tomato and pineapple (Johnson and Godfrey, 1932). The first nematicides were non-selective (biocide) fumigants such as chloropicrin, 1,2-dichloropropane/1,3 dichloropropene mixture (D-D), ethylene dibromide (EDB), 1,2-dibromo-3chloropropane (DBCP) and methyl bromide (MBr) that vaporize when applied to the soil. As gases, they diffuse through the soil, killing not only nematodes but also acting as general biocides. The significant positive impact on yield following the use of a fumigant led to wide adoption of this technology. Due to their high level of phytotoxicity, these could only be applied before planting (Loiseleur *et al.*, 2019). The first non-fumigant nematicides were carbamates and organophosphates that were discovered in the late 1950s. Their clear benefits were relatively low phytotoxicity, which allowed for an application at planting and post emergence, and their improved selectivity towards target

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organisms. They required water in order to move through the soil profile and were typically applied in granular or liquid form either as an in-furrow treatment or a broadcast soil incorporation. In recent years, many fumigants and non-fumigants have been phased out due to their hazardous human or environmental safety profile. The loss of a broad range of chemical nematicides has triggered the need for newer and safer nematicides. Several agrochemical companies have demonstrated their commitment to develop replacement solutions for the grower. Over the past 15 years, nematicides with novel modes of action were developed and introduced into the market. They are in the class of avermectins (abamectin), fluoroalkenyls (fluensulfone), imidazopyridines (fluazaindolizine), pyridinyl ethylbenzamides (fluopyram) and phenetylarylamides (cyclobutrifluram) (Loiseleur *et al.*, 2019). Three of these (abamectin, fluopyram and cyclobutrifluram) have very high intrinsic potency and are applicable as seed treatments. The seed acts as a vehicle to position the active ingredient in the rhizosphere exactly where it needs to be to prevent nematode attack of the root system. The benefit of a seed treatment in comparison to an in-furrow application is the reduction in the amount of active ingredient required per hectare. The first seed treatment developed with these features was Avicta™ (abamectin). It was introduced in cotton in the US in 2006 and expanded to soybean and maize thereafter. With that, a new market segment for field crops was created which was readily adopted by growers who could refrain from applying in-furrow nematicides. The large untapped opportunity for a seed treatment nematicide was recognized by all leading agrochemical companies.

Methodology

What does it take to develop a modern nematicide?

The process of identification of a chemical class, its optimization and the development of a single compound for delivery to the grower takes 10–15 years and comes with an investment of up to US$250 million. Thousands of molecules belonging to hundreds of different chemistries are screened to identify the chemical class that leads to the delivery of a final product. Sources for new chemistries usually come from literature searches, patent monitoring, chemical libraries, serendipity and molecule design.

At Syngenta an internal project was started in 2007 with the aim to find molecules that have high intrinsic potency against all economically relevant plant parasitic nematodes, an improved human and environmental safety profile and a duration of protection of at least 6–8 weeks. Furthermore, the active ingredients need to be applicable as a seed treatment as well as in drench application for use in annual and perennial crops across the globe. For this endeavour a screening platform tailored towards identifying a next-generation seed treatment nematicide was set up across global research sites. To find the right molecule, an adapted screening protocol was initiated. The first step in the screening cascade (Fig. 61.1) is a high throughput in vitro assay that allows for the identification of chemical compounds with a fast mode of activity and efficacy at very low dose rates. This assay is testing against cyst and root-knot nematodes to ensure broad-spectrum control potential. Thousands of compounds are assessed on a yearly basis in this test system. Compounds entering screens are carefully selected by chemists on criteria such as molecular properties steered towards seed application and modes of action that may be relevant. Hits within this test are progressed to seed treatment evaluation which is the central focus of the screening platform. Once potential chemical classes are identified it is key to detect and understand their mode of action, to assess the biological novelty and to support chemical optimization. Thereafter a computational three-dimensional model of the binding pocket in the target site within the nematode may be established. Through a combination of synthetic and computational chemistry, compounds are designed and synthesized to fit the binding pocket thus increasing the rate of the optimization considerably. In this process, preferred lead compounds undergo further greenhouse pot studies which are carried out against all economically relevant nematode genera in key crops. Typically, a few hundred compounds are tested for their seed treatment activity annually. If a high level of seed treatment activity as well as crop safety is proved, the compound is promoted to field testing, given all other functions

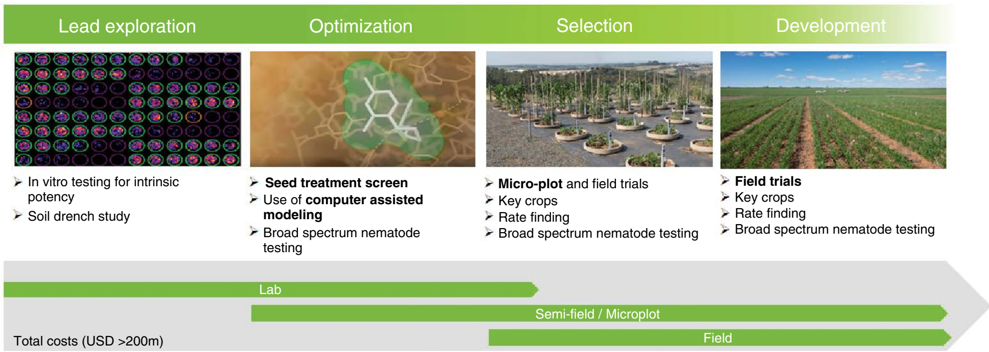

Fig. 61.1. Nematicide screening cascade. Figure courtesy of M. Gaberthüel, Syngenta, Switzerland.

of the project team supported the selection. The multidisciplinary project team consists of members from chemistry, biochemistry, biology research, product biology, regulatory, toxicology, formulation, environmental sciences, intellectual property and business and is required to review potential candidates on a monthly basis for their likelihood of success.

Robustness in the field and regional fit

Selected compounds need to be assessed under natural growing conditions with factors influencing compound performance such as climate, soil type, varietal response and natural nematode infestation within a complex of other diseases and pests. Five to ten compounds are promoted to field testing on a yearly basis. Carrying out field trials to determine nematicidal activity of chemical compounds is not an easy task as nematodes occur in patches in the field and are very heterogenous in distribution. It is difficult to compare performance of compounds in a field site given the variability between replicates of even a single treatment. Thus prior to entering field trials, the field candidates are benchmarked in a microplot trialling platform on a range of different crops and nematode species. Typically, microplots are containers placed outside which are filled with field soil. Microplot trailing allows for a homogenous distribution of a defined number of nematodes at a given life-stage under otherwise natural conditions. In this system it is possible to evaluate nematode damage to the root and assess the final reproduction factor. Its limitation is the inability to obtain conclusive information on yield impact due to the low number of plants per microplot. This system aids in identifying required use rates related to crop and nematode species as well as detecting which compound provides the overall best rate response. Hereafter, one to three of the best candidates are further progressed to global field trialling in order to assess yield benefits on major crops such as soybean, maize, cotton, sugar beet, cereals and vegetables and its corresponding return on investment for the grower. Field trials are carried out by regional field scientists against the most common and economically relevant nematode genera in all major regions of the world to ensure that performance of the molecule is consistent across climates and agricultural systems. In addition, local requirements, farmer expectations, profitability, product concepts and formulations are considered for each crop and country.

Internal education is essential and a key enabler for the development and launch of a new compound

To ensure proper nematicide field trialling in over 100 crops across the globe and the creation of proper biological assessment dossiers for registration of the compound, it is essential to train internal field scientists in general nematology and methodology of nematicide trialling. Trainings are held by internal and external nematologists which are adapted to the regional needs and markets (Fig. 61.2). Important outcomes are the establishment of a network of experts that

Fig. 61.2. Nematology training with field biologists at Vero Beach, USA. Photograph courtesy of M. Gaberthüel, Syngenta, Switzerland.

are well connected and the sharing of know-how and capabilities.

Advice and critical feedback from independent researchers are also an important factor for a successful product development. Contracted work is done with universities not only to gain independent confirmation of internal results, but also to gain a more in-depth understanding of fundamental product features. The standard field trialling methodology is not set in stone but under permanent revision and optimization. As an example, the randomized complete plot design was complemented for field crops by a checkerboard trial design which allows each treated plot to be surrounded by control plots for direct comparison to account for the high heterogenicity of nematode density in field soils (Fig. 61.3).

Final requirements for product delivery to the market

All the information collected on a new compound is reviewed regularly by the global project team. The industry landscape needs to be continuously monitored to ensure the new nematicide will be sustainable, competitive and longevity in the marketplace will be granted in order to guarantee a positive return on investment for the company. Production cost as well as scalability of production of the molecule are important aspects as the final product needs to be affordable for the grower. Also, patentability of the active ingredient and its related chemical class is key to secure exclusivity. Furthermore, the ability to prepare flowable solo formulations of the active ingredient as well as ready-mix formulations with up to eight different molecules (fungicide, insecticide, nematicide) in a single formulation requires specific physico-chemical properties of the active ingredient. All these key factors, in addition to strong biological performance and a favourable human and a sustainable environmental safety profile, contribute to securing a successful launch of a new chemical nematicide.

As a consequence of all development efforts, a new nematicide with a specific formulation and application technology as well as a precise rate recommendation dependent on the crop, region and nematode species will be delivered to the grower. The most recent innovation by Syngenta

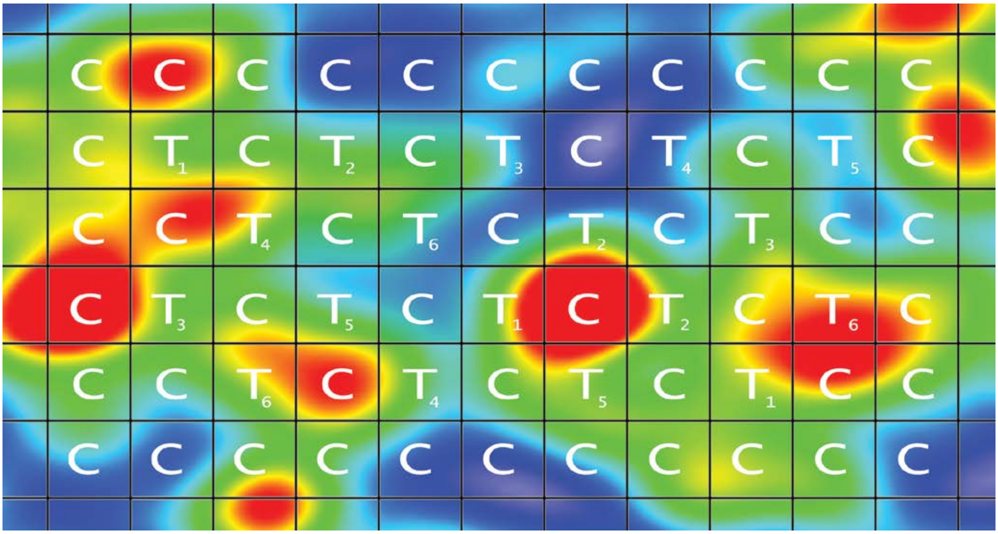

Fig. 61.3. Heatmap displaying nematode densities in a soil (red, high; blue, low). The grid shows the checkerboard design used in trials. Six treatments (T1–6) and four replicates. Each treatment is surrounded by four untreated control plots (C). Figure courtesy of M. Gaberthüel, Syngenta, Switzerland.

is TYMIRIUM™ technology with the active ingredient cyclobutrifluram (Syngenta, 2020).

Pros and cons

Chemical nematicides and integrated nematode management

Chemical nematicides are one important tool in integrated nematode management and can be combined with other tools such as tolerant or resistant varieties, crop rotation and catch crops where these measures are feasible. The use of resistant varieties is an effective option for season-long control of specific nematode species. However, there are limitations as resistant varieties do not exist for all economically relevant nematode species. Also, if multiple nematode species are present in field sites, additional measures such as biological or chemical control should be undertaken. The advantage of chemical control is that it can offer strong activity against a broad range of plant parasitic nematodes and protect the plant during its early and vulnerable stage of plant and root growth. A nematicide application can also serve as protection of genetic resistance traits by delaying the shift in nematode races to those that can overcome resistance traits. Modern chemical nematicides are applied at very low use rates to minimize contamination of soil. Targeted applications such as seed treatments position chemicals precisely where they are needed to protect the plant during early growth.

Visions of the future

Education and awareness about the impact that nematodes can have on yield will remain an important part of nematicide development in the future. It will become more important to also include the public in this process. In this it will be key to make the public better understand what challenges the farmer faces when it comes to pest, disease and nematode control. The recent awareness of what a pandemic outbreak can do to human populations could support an understanding that sometimes chemicals are, amongst others, one important tool for the control of a disease or pest outbreak to fight high losses. Diagnostic tools are the base to understand the danger of an outbreak and are key to take control measures based on educated decisions.

Prediction models, crop rotations, cultivation system adaptation and breeding will support chemical nematicides in nematode management in the future

Nematodes can only be managed, never eradicated. In order to define a nematode management strategy, it remains key to determine whether field sites are infested with plant-parasitic nematodes. Once infestation has been confirmed and specified, further sensor-based technologies can aid to identify, and monitor the spread of nematodes within a field site. Advanced digital technologies combined with the use of prediction models could possibly provide an accurate map on the localization and dynamics of nematode populations under specific conditions, allowing a targeted application of a control measure down to a section of a grower`s field.

Crop rotation will remain important when targeting specific nematodes species with a narrow host range such as cyst nematodes. Growing non-host crops or cover crops can also aid in effectively reducing nematode numbers. Integrated cropping (more than one crop in a field) can become feasible with the advancement of precision farming harvesting capabilities.

Under high-value greenhouse cultivation of fruiting vegetables, growers can replace nematode infested soil with artificial media in order to avoid high yield losses due to nematode contamination.

Plant breeding will stay an important complementary tool to chemical nematode control as well. Newer technologies such as targeted gene silencing (RNAi) and editing (CRISPR) are methods that could lead to rapid advancements in this area.

Application and formulation technology trends

The future use of soil diagnostic tools and the establishment of detailed maps of growers' fields on the occurrence and spread of soil-borne pathogens, soil pests and plant parasitic nematodes will allow growers to locally apply pesticides where specifically required in the field with the appropriate technologies that are currently in development. Direct injection of the chemical formulation into the furrow at planting is such an example.

Tailored formulations designed for a chemical nematicide and specific application are another field currently under investigation. Controlled release that supports maximum activity with minimum leaching and persistence under all environmental conditions is the ideal. All these technologies share the same aim to minimize chemical pesticides applied per hectare.

The compatibility in mixtures with other crop protection products but also with fertilizers to minimize the number of required applications is also an important aspect.

Outlook: nematicide development trends

Key enablers in discovering new nematode control modality in the future will be the application of contemporary technologies developed in life science for discovery such as bioinformatics and cheminformatics. Combined with advancements made in nematode genome sequencing, it will aid in the identification of further starting points in new target sites within plant parasitic nematodes. Modern ways of better assembling relevant compound collections for screening and techniques to efficiently optimize hits will continue to deliver innovative candidates for specific use against the target organism avoiding effects on non-target organisms. With the regulatory and socio-political demand for minimal environmental impact and no residues on crops, an emphasis will be placed on biological control agents (bacteria, nematodes, fungi) and natural products (metabolites, natural plant compounds) that can be used in combination or as an alternative to chemical control agents.

Over the past decades, an abundance of experience and knowledge has been collected by renowned nematologists. It is key to ensure this legacy is carried on in combination with other new development streams in agriculture such as molecular breeding methods. This has and will continue to provide an essential foundation of nematode management strategies.

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62 Critical terms during development and commercialization of microbial agents for the control of plant parasitic nematodes

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Introduction

About a century has elapsed since the idea of using biological control for nematodes was developed, however to this day no robust commercially successful biological control agent for plant parasitic nematodes is routinely used. Soil suppressiveness to plant nematodes is a well-established phenomenon and yet we clearly do not understand the ecology of it sufficiently well to manipulate it in a way that we can predictably control these important plant pests.

The original vision of phytonematodes' biocontrol has been to reduce the pest population by natural enemies with the active involvement of the human role. Two main arguments against the use of chemical control to combat plant parasitic nematodes directed both the consumers and the producers to look for alternatives: health risks to the customers and ecological concerns. At the beginning, there had been many expectations about the potential of biocontrol to substitute, or at least to reduce dramatically, the treatments with chemicals to control phytonematodes. However, problems regarding the techniques and other concerns relevant to different issues of biocontrol resulted in polarizing attitudes toward this discipline. Scepticism by several consumers and producers versus optimism by others has always been part of the biocontrol agenda. The difficulties and restraints faced by the developers, producers and/or the consumers (farmers) include: (i) health and ecological concerns; (ii) production techniques; (iii) efficacy of the product in the field; (iv) field application protocols; (v) shelf life of the final product; and (vi) registration.

Health and ecological concerns

Biological control candidates such as bacteria or fungi produce and secret various metabolites, for example enzymes and/or antibiotics. Although these secondary compounds are usually of minor concern, some may cause health worries to human and/or other animals. In 1993, Oka *et al.* found that *Bacillus cereus* could function as a very efficient candidate to serve as a biocontrol agent against the root-knot nematode, *Meloidogyne javanica*. *Bacillus cereus* has strong proteolytic and collagenolytic activities, which enables it to rapidly and efficiently decompose organic materials to ammonia and to damage the collagen-made cuticle of the second-stage juvenile (Sela *et al.*, 1998). However, *B. cereus* is often related to

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food poisoning as it produces and secretes toxins that cause intestinal illnesses such as diarrhoea and nausea/vomiting. *Bacillus cereus* has also been implicated in infections of the eye, respiratory tract and in wounds (McDowell *et al.*, 2020). This information defined our decision to drop *B. cereus* candidacy as a commercial biocontrol agent (Y. Spiegel, personal communication). An early screening for medical records of a microorganism can help to avoid unnecessary research and development capital and time investments. When, at a first glance, an organism is grouped within a human pathogenic taxon, a comprehensive phylogenetic investigation can result in the description of a new taxon, which is not adapted to humans but rather to the plant rhizosphere (Wolf *et al.*, 2002).

Production techniques

This stage includes ten processes (Fig. 62.1):

1. Selection of a microbial antagonist to serve as a good candidate for a commercial control agent.
2. Long-term preservation technology to maintain genetically stable inoculum.
3. Molecular identification to strain level.
4. Literature review targeting safety records for protection of laboratory personnel and later registration.
5. Preliminary efficacy tests in laboratory conditions.
6. Establish the mass production protocol in solid or liquid media and downstream processing.
7. Screening for the most suitable formulation.
8. Development of quality control protocol.
9. Development of packing and adapted transport logistics.
10. Calculation of production costs.

The procedure is relatively well known and should not present difficulties: strain selection, methods and criteria for selection are already reviewed extensively in the literature (Ravensberg, 2011). Selection cascade for the best antagonistic candidate can be highly dependent on the predicted difficulties in the future: registration procedures for bacteria or fungi antagonists are much more complicated and expensive compared to predatory nematodes, as predatory nematodes are exempt from the requirements of FIFRA (Federal Register, 2007). Therefore, only well-established companies might cope with such expenditures. Highly sporulated fungus or bacteria are lead criteria for additional selection to reach the best candidate; spore production guarantees easier and enhanced mass production of the antagonist. Spores assist longer shelf life of the final product and a better resistance to transportation from the producer to the consumer. Moreover, spores are potentially better candidates to resist pesticides, dryness and extreme soil temperature conditions. A survey of the top ten list of economically important phytonematodes (Jones *et al.*, 2013) revealed that the two main problems are the sedentary endo-type nematodes: root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* and *Globodera* spp.). This fact dictates the type of the organism, that will antagonize the phytonematode life stages that are outside the root: egg-mass, eggs, cysts or second-stage juveniles. Such antagonists are occasionally equipped with chitinolytic and/or collagenolytic/proteolytic enzymes (Sela *et al.*, 1998; Macia-Vicente *et al.*, 2011).

Mass production techniques, though covered well by literature and patents, often decide the potential of a product. Media costs, growth parameters and stability during scaling-up decide whether an organism can be carried further. During downstream processing losses must be minimized. Finding the most suitable formulation (solid or liquid) may well be very ‘tricky’, as the final product should comply with several criteria: (i) maintain a high viable number of the microorganism; (ii) long persistence (shelf life) of the product; and (iii) adapted to agricultural practice, e.g. likely to be delivered via drip irrigation. Quality control is essential both during and after the production process. It is also essential to check for appropriate packing to maintain shelf life after the product has left the factory, when it reaches the farmer and after application in order to check for viability. Finally, an economic calculation of all process steps will contribute to product costing and enable a comparison with available competing products in the market.

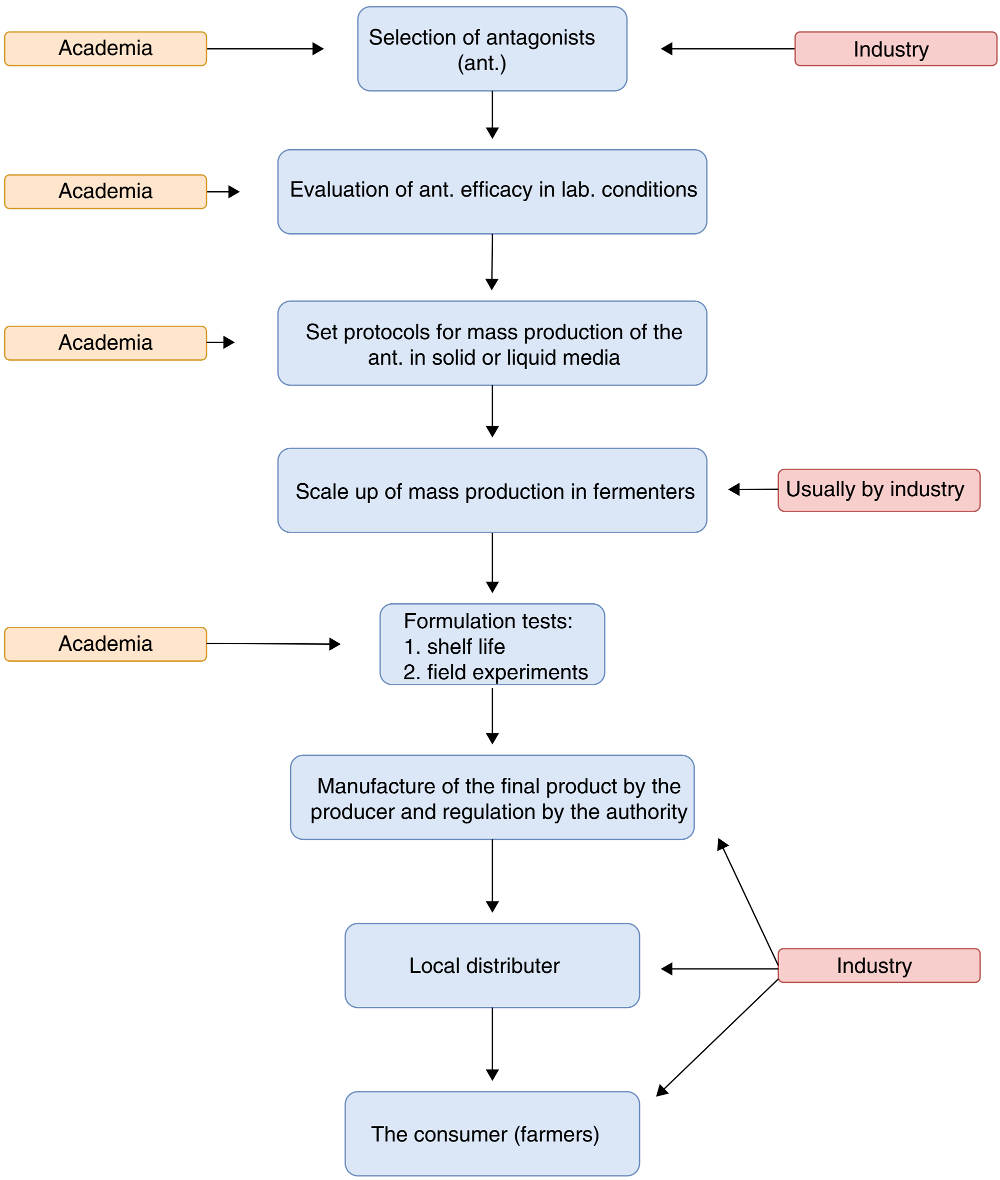

Fig. 62.1. Production techniques flow chart in the development of a biological control agent. Figure courtesy of Y. Spiegel, *Rishon LeZion*, Israel.

Efficacy of the product at field conditions

Efficacy screening in the laboratory indicates the possible potential of the 'active ingredient' (the antagonist microorganism). Field trials with the formulated product are crucial to understanding whether the product fits into farming practice. Several years of trials under different climatic and edaphic conditions will provide information about the general potential and reproducibility in variable conditions. The margin return of the target crop in relation to the possible increase in yield is critical for market introduction. Biocontrol agents tend to have limited spectra of soil conditions to develop their full potential. Soil acts as a massive buffer. Application of a biocontrol agent shifts the stability existing within a soil habitat for a short time. The amount of the antagonist needs to change the equilibrium, while acting efficiently on the pest. The optimum between efficacy and application density must be carefully elaborated as it has a major impact on the application costs. Lesser amounts might be insufficient to achieve high enough control, while greater can be less or even non-economic.

Field application protocols

Application of chemical or biological agents to soil demands specific guidelines to get the most efficient control. Elements such as the optimal amount of formulation, timing and frequency of application (before, at or after planting, or the right combinations of them) and delivery methods (via drip irrigation, soaked speed-seedlings or directly drenching into soil), are the result of reams of trial data cumulating in the development of reliable instructions to the farmers. Variables such as the parasitic nature of the target phytonematode species (e.g. ectoparasite, ecto-endoparasite or endoparasite, or sedentary versus migratory), soil pH, soil characteristics and the variety of the crop, must be taken into consideration and tested. Finally, the product must fit into general agricultural practice and common application technology.

Shelf life of the final product

Shelf life is the major challenge in handling biocontrol agents. Chemical companies can handle the transport logistics of *Bacillus*-based products as these spore-forming bacteria have a long-lasting shelf life. However, the much larger control potential often lies in microbes with limited potential to survive the distribution logistics common in the plant protection market. Sophisticated formulation technology can help to overcome this limitation. Other than with chemical products, which are formulated to improve their handling and performance on the plant, the primary parameter in formulation of biological agents is to ensure their survival at high quality. The wide spectrum of different additives used with chemical pesticides is not applicable as most substances interfere with viability and activity of microbial agents. Major progress is expected from novel micro-encapsulation technology. Sterile packing can also be an approach to prolong the shelf life of liquid formulations.

Registration

Considering the safety of biological control agents, the public perception about chemical pesticides, the on-going increase of resistance against pesticides and their phasing-out, one would expect that government authorities and politicians would promote the further introduction of microbial agents and do everything to accelerate their access to the market. Unfortunately, activities from this part of society have not been much more than lip service when it comes to registration requirements. As a result, the registration process is still the major hurdle in the struggle to bring biological plant protection products to the market. The organization and data requirements for registration of microbial biocontrol agents are a complete disaster, particularly in the European Community, preventing many small- to medium-sized enterprises from access to the market. Consequently, many potential products stay on the shelf of the developers. For example, the European situation is described next.

Unlike the EU, in the USA the handling of products is more professional due to the centralized evaluation in expert departments (Environmental Protection Agency, EPA). In other countries, the rules might be even more stringent. In the EU, the first attempt to agree an EU-wide regulation of plant protection products (Dir. 91/414 EEC) did not include microbial agents on the list. When rules to include biological control agents were developed, the strategy followed the tradition of the risk assessment and management of synthetic chemical compounds. This has not changed much since the introduction of the Regulation EC 1107/2009. Data requirements are not well adapted to the biology and potential risks of microbial biocontrol agents. The many steps necessary to obtain, finally, a national authorization build too many bureaucratic hurdles and authorities hardly ever keep to the timelines.

Proposals for adapted regulation procedures were elaborated within the REBECA Policy Support Action in 2008, but almost all of them were ignored (Ehlers, 2011). On 15 February 2017, the European Parliament adopted with near unanimity a resolution (2016/2903 (RSP)) calling on the European Commission to submit, before the end of 2018, a specific legislative proposal to establish a fast-track evaluation, authorization and registration process for low-risk plant protection products of biological origin. In principle, the idea was not new. It asked for a provisional authorization after the completeness check of the complete data file for only those products complying with the definition for 'low-risk products'. Provisional authorizations already existed during the legislative of the 91/414 EEC. The European Commission (EC) refused to act. Instead, they started a long-term evaluation of the existing regulation (REFIT). On the one side the EC phases out more and more chemical compounds, on the other side they do not facilitate quicker availability of low-risk products for the farmers.

In the meantime, the EC authorities worked on the development of procedures to regulate biostimulants, including products based on microorganisms, within the Fertilizer Regulation (Regulation EC 2019/1009). By definition, biostimulants promote plant growth and increase resistance to abiotic stress. Any claims of biocontrol activity are illegal. Despite the inclusion of only four microorganisms (*Mycorrhiza* fungi, *Azotobacter* spp., *Azospirillum* spp. and *Rhizobium* spp.) to be accepted as biostimulants, many strains of microorganisms are on the market that are in the same genus or even the same species of microbial biological control agents (e.g. *Trichoderma* spp., *Bacillus* spp.). A stringent prosecution, in particular of the many cases in which plant protection activities are advertised, is necessary to avoid this unacceptable situation for the biocontrol industry. Because of exaggerating regulation requirements, which are often considered as not reasonable when compared with other agricultural practice (e.g. liquid manure) and diverse handling of these products in different member states, many companies bring biostimulants, soil amendments, plant strengtheners, etc., to the market to avoid long-lasting and expensive registration. For management (note - not 'control') of plant parasitic nematodes this might be a future route the agriculture industry will take. From the standpoint of the International Biocontrol Manufacturers Association (www.ibma-global.org, accessed 15 January 2021), microorganisms used in agriculture, whether applied as fertilizers, in the food industry, seed treatment, soil amendment, compost additive, silage. etc., should be handled under one regulation, as the risks are more or less the same, independent from how the microorganisms are used. Such a system should regulate the real risks and be handled by experts at a central European agency. However, it looks like we are far away from such a pragmatic approach. Consequently, the registration of microbes to manage plant parasitic nematodes will not change within the near future.

Vision of the future: biocontrol overtakes chemical control

The global biological control market is projected to grow at a compound annual growth rate of 14.7% during the forecast period (2020–2025) (https://www.mordorintelligence.com/industry-reports/biological-control-market,(accessed 27 December 2020). Nevertheless, unless the consumer willingly accepts the concept of sustainable agriculture, where 'drastic' means (chemical control) are not used to combat pests, and therefore will be ready to tolerate some damage to plants, it is anticipated that biological control alone is not adequate to get economically satisfactory agro-products. Several approaches have been developed to reduce chemicals, e.g. integrated pest management and sustainable farming systems (Stirling, 2011), where biocontrol took part of the assembly attitudes. Careful survey concerning the data published as well as an outcome of our experience, reveal that the effectiveness of biocontrol agents against phytonematodes is limited up to lower to medium infectivity level. In the root-knot nematode, an effective biocontrol candidate will be able to reduce galling index (G.I.) up to 2.5 (on a scale of 0–5) to an economical threshold, a point where it can be considered as a realistic alternative to chemical control. Sole treatment with a biocontrol agent in cases of highly infested fields (G.I. 3.5 to 5.0), was doomed to fail (Y. Spiegel, personal communication). However, incorporation of a lower dose of a chemical agent, which alone is not sufficient to achieve nematode control and *a priori* does not harm the antagonistic material, with a biocontrol agent might improve dramatically the usefulness of the control in a field highly infected by nematodes (Y. Spiegel, personal communication). Merging two or more antagonistic candidates may possibly cause a synergistic effect; however, two major concerns should be taken into consideration. Firstly, test whether these two (or more) candidates are not antagonists to each other. Secondly, developing a strategy based on two different biocontrol microbials demands the compilation of two files for registration, thus increasing the product costs.

Epilogue

Over the last twenty years there has been a revolution in mass production of microbial biocontrol agents (Figs 62.2, 62.3 and 62.4).

These innovative developments have opened options for the formulation and application of biocontrol candidates (bacteria, fungi or predatory nematodes) now available. Other processes are beginning to be developed, including extending shelf life, packaging and handling for long-distance delivery, accurate and rapid identification of the microbial antagonist and the development of specific barcodes of these products. The tremendous achievements promise significant development of opportunities for accelerated progress in bringing new and efficient biocontrol agents to the field of integrated nematode management.

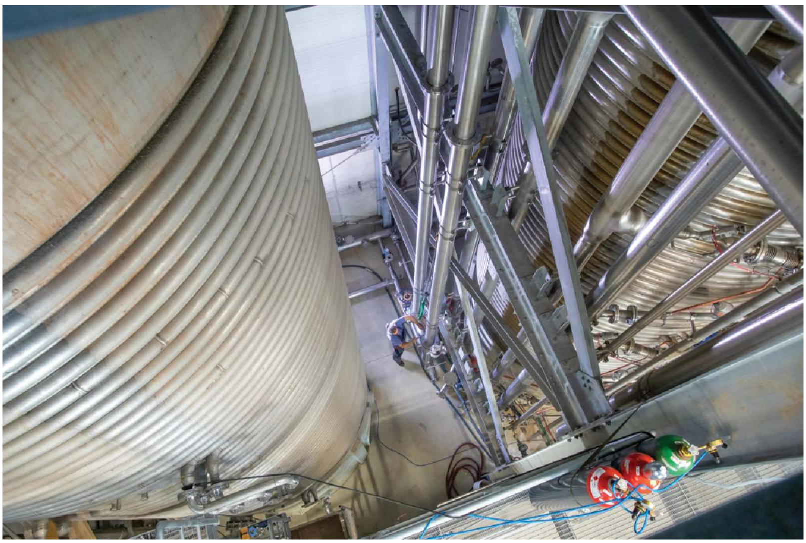

Fig. 62.2. Bioreactor of 100,000 l volume for liquid culture production. Photograph courtesy of e-Nema, Kiel, Germany.

Fig. 62.3. Separator centrifuge for the concentration of liquid broth harvest of microorganisms. Photograph courtesy of e-Nema, Kiel, Germany.

Fig. 62.4. Vacuum drum filter for the harvest of biomass from liquid culture. Photograph courtesy of e-Nema, Kiel, Germany.

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Section VIII:

Constraints

63 Technologies for integrated nematode management in smallholder farming systems: No one-size-fits-all

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Introduction

In this chapter, the need to take a more critical look at the highly precarious and vulnerable situation of smallholder farming systems, the predominant type of the agricultural output worldwide, will be emphasized.

These farmers represent 98% of the farmers in the world that sustain the local production of staple crops such as rice, maize, cassava, groundnut and millet. Although there is some disparity in the figures, recent data estimates that there are between 380 and 500 million smallholder farming households globally (Samberg *et al.*, 2016). From these, 35% are in China, 24% in India and 9% correspond to small family farms situated in sub-Saharan Africa (SSA), similarly to East Asia and the Pacific (Lowder *et al.*, 2016). These farmers cultivate between 30% and 53% of the global agricultural land and produce 70% of the calories consumed in their countries.

Generally, smallholder farmers suffer from a lack of access to markets, supportive government policies, credit and access to knowledge, to mention a few (Sikora *et al.*, 2020). Still, the productivity of the smallholders' farms varies greatly among continents and regions. It depends on soil health and quality, agroecological conditions (irrigated versus rain fed), access to agricultural inputs and new technologies. These last two also critically influence smallholders' ability to manage pests and diseases, including plant parasitic nematodes (PPN). The 'one-size-fits-all' model is not applicable to these farmers. Many of the technologies and integrated systems outlined in the chapters in this volume do not relate to these subsistence farmers. Smallholder farmers do not and cannot practice modern integrated nematode management. This dilemma, which could be called a catch-22, is also stressed in the chapters on rice, banana, maize, tomato and yams in this volume.

Epidemiologic perspective

PPN are consistently overlooked as a devastating pest in the tropical agroecosystems. There has been a meagre investment in research, policy development, training of extensionists, equipment of national research centres and on the development of specific solutions for PPN management targeting subsistence farmers. From an epidemiologic perspective, there is still limited understanding of the distribution of species, both geographically and per crop. The lack of information is exacerbated in countries with no nematological experts and with scarce resources dedicated to conducting basic science. Besides,

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private nematode diagnostic services are often insufficient. If available, these services are typically considered too expensive by smallholder farmers or the species' diagnostics is weakly backed up by tailored technical advice to the farmer on how to manage the farm.

Diagnostics and surveillance

Species identification is critical to deploy effective PPN management strategies, develop national phytosanitary strategies and agricultural policies. A recent survey in Kenya revealed the presence of a new *Pratylenchus* spp. of coffee, and the presence of *Rotylenchus macrosoma* (in coffee), *R. robustus* and *Scutellonema brachyurus* (in soybean) for the first time (unpublished data; Dr Wim Bert, personal communication).

Another example is the recent detection of the *Globodera rostochiensis* and *G. pallida* in Kenya, a quarantine species worldwide. Nematologists demonstrated the widespread nature of the infestation across the country and that this pest could be responsible for up to 50% of the yield losses experienced by potato growers. The case of *Meloidogyne enterolobii* could be somehow similar. Researchers found this species parasitizing African nightshades (2016) and sweet potato (2017) in Kenya, and previously in Mozambique, Malawi and the Democratic Republic of Congo. Yet, this quarantine nematode has received nil attention from the regional phytosanitary services and the extension programmes.

As an outcome of the Kenyan survey, the local plant protection authorities made potato cyst nematode (PCN) inspection compulsory for foreign potato seed consignments and the accreditation of local certified seed farms. Furthermore, the international potato seed industry brought into Kenya new PCN resistant varieties from 2017.

The limited recognition of PPN has environmental consequences as well. Family farms cultivated in open fields suffer from a large number of pests and pathogens concomitantly. Therefore, smallholders affected by the presence of PPN in their crops often watch with concern a decline in their productivity, without being able to dilute the pathogen responsible for it. The untimely diagnostics on the presence of nematodes results in smallholders' over-reliance of chemical fertilizers, excessive watering and indiscriminate use of pesticides. Overall, it leads to the impoverishment of soil health and ecosystem degradation.

Plant resistance

Smallholders differ in their resources and production objectives, and such differences are a critical determinant of the farmers' ability to embrace innovations. Even though there are technologies and methodologies available to manage PPN effectively, these do not necessarily match with the socioeconomic context of the subsistence farming systems around the world. Plant resistance is the most effective and environmentally safe strategy for managing nematodes, and its simple deployment makes it incredibly valuable for smallholders. Still, the use of natural plant resistance (PR) requires particular conditions. PR can be highly species specific and it is not available for all crops. Besides, some of the resistance genes (R) are thermolabile which limits efficacy in tropical conditions. An example is the case of resistance against root-knot nematodes (RKN) in tomato (*Mi 1.2*) and capsicum (*Me 1* and *Me 3*). The two are highly demanded crops worldwide and are important cash crops for smallholders. These *R*-genes have proven extremely useful to manage *M. incognita*, *M. arenaria* and *M. javanica* (Fig. 63.1). But whenever smallholders use such

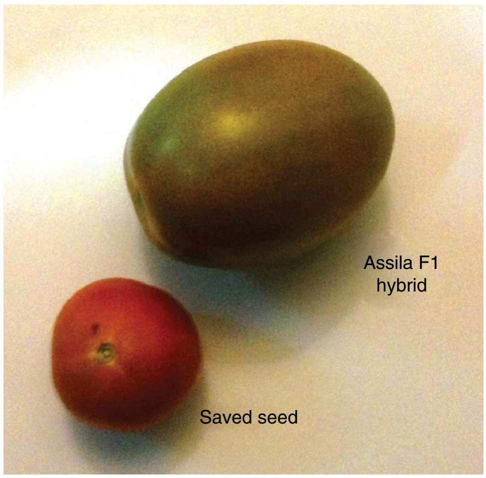

Fig. 63.1. Comparison of the fruit size between a farmer's saved tomato seed (bottom left) and an Assila F1 hybrid, with resistance to virus, fungi and nematodes from a smallholder's field in Somalia. Photography courtesy of Danny Coyne pictures.

RKN resistant cultivars in the presence of other RKN or PPN species, they miss the positive impact in their crops. So, smallholders are frequently unaware of the benefits of using improved cultivars to manage pests and diseases, particularly nematodes. Overall, this situation causes confusion and distrust among farmers and unskilled extension agents on the efficacy of resistant varieties.

Breeding for nematode resistance

Private seed companies have paid little attention to nematode resistance breeding in non-cash and staple crops, such as plantain, yam, cowpea, cassava or groundnut. Thus, breeding and nematode screening for these types of crops have been mainly supported through international research for development (R4D) projects. An example is the Breeding Better Banana Project in which scientists in Uganda and Tanzania are deploying advanced-breeding techniques to select Matoke and Mchare plantain lines resistant to *Pratylenchus* spp. and *Radophulus similis*. Also, a recent screening of NERICA rice in Tanzania identified a handful of resistant cultivars to *Meloidogyne* spp. and *P. zeae* (Nzogela, 2020). This data provides positive perspectives for the local rice industry where heavy PPN infestations cause severe yield losses.

Seed delivery system and clean planting material

Less than 35% of smallholders have access to improved seed in Kenya, <25% in Ethiopia and Nepal, and <20% in Tanzania (FAO, 2015). Frequently, the price and the packaging of the seeds in large quantities following standards of 'foreign economies' does not match smallholders' needs; sometimes the cost of germplasm can peak before the planting season, deterring smallholders' purchases.

At times, the formal seed systems in least developed countries (LDC) also fail to guarantee sufficient varieties and standardized quality. Thus, smallholder farmers tend to use farm-saved seeds and planting material from local cultivars, either to minimize investment risks or for cultural reasons. This type of planting material is exchanged informally within communities, and even across countries. Yet the use of recycled germplasm is one of the primary sources of contamination with nematodes, viruses and bacteria when it is not correctly selected, disinfected and stored. Hence, the use of clean planting material is especially crucial to prevent PPN infestation for vegetatively propagated crops such as banana and plantain suckers, potato seeds or yam tubers. A few international research institutions (e.g. CGIAR) are supporting national breeding programmes to produce cultivars and clean planting material resistant to nematodes, pests and diseases. Such germplasm is adapted to the local agroecological conditions, and to the gender and socioeconomic demands of the smallholders.

Another way of cleaning vegetative planting material is by delivering a targeted nematicide application (if registered products are available) by which farmers dip yam tubers in chemical solutions before planting, to eliminate PPN (Fig. 63.2). For banana and plantain, the disinfection of suckers by immersion in hot water and the peeling

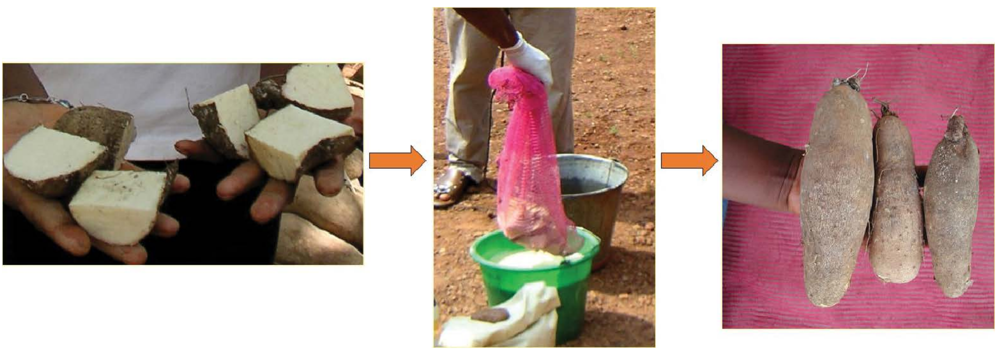

Fig. 63.2. Yam mini tubers infested with plant parasitic nematodes (left) treated in a nematicide-dipping solution (centre) produced healthy and big-sized tubers (right). Author's own photographs.

and burning of roots is very effective to eliminate PPN species during replanting.

The use of healthy tomato and capsicum seedlings primed with endophytes to manage species of *Meloidogyne* and *Helicotylenchus* significantly reduced the use of nematicides; it also increased the number of marketable fruits, even during drought, in smallholders' fields in Uganda (D. Coyne, personal communication). Likewise, grafting resistant rootstocks to enhance seedling vigour and effectively protect solanaceous crops from PPN and soil-borne pathogens also has enormous potential. However, despite its effectiveness, smallholders need access to credit and specialized nurseries to acquire these technologies and perform the grafting process, 5–6 weeks ahead of the planting season. Whenever climatic conditions are erratic, agricultural insurance is unavailable and smallholders have no marketing prospects, these tend to limit the adoption of new technologies to minimize financial constraints ahead of the season.

Alternate host and infield spread

Due to the costs of installation and maintenance of the drip irrigation systems, smallholders in LDC usually obtain water directly from a natural watercourse for irrigation, without any previous sedimentation process into water storage tanks. This approach is one of the main entry points of nematodes to the farms. Also, weeds remain critical reservoirs of PPN because weeding is yet another challenge for smallholders, due to the high labour costs associated with the manual removal of the weeds (Fig. 63.3).

Land availability and rotations

Land availability influences smallholders' ability to practice fallow and rotation for nematode management. Farm size varies significantly across continents: while the smallest are in Asia (e.g. 0.24 ha in Bangladesh, 0.32 ha in Vietnam, 0.5 ha in Nepal), in SSA these are slightly bigger (e.g. 0.47 ha in Kenya, 0.9 ha in Ethiopia); and in Latin America farms range between 2 and 5 ha, with some exceptions (FAO, 2015). Expansion of the urban areas and massive population growth causes high competition for land and irreversible loss of agricultural soils. Therefore, most smallholders cannot afford to leave uncultivated land for a season to suppress nematode populations.

Thus, the limited availability of land for cultivation has further implications on the type of crops that are cultivated, both in time and space. This constraint is particularly relevant in the farms with reduced portions of land (<0.4 ha), because these dedicate the lowest percentage of their agricultural products for market sales. Hence, those crops that can be used as cash crops but that can also ensure food security at the household level (e.g. banana, potatoes) are repeatedly cultivated. Inevitably, the continued cultivation of land leads to increased incidence of soil-borne pathogens, soil degradation and nutrient depletion. Unfortunately, farmers tend to counteract their yield gaps by increasing the use of chemical fertilizers, exacerbating the problem of soil exhaustion even further. The lack of fallow or of rotation periods at all has noticeable detrimental effects on the management

Fig. 63.3. A smallholder tomato crop in Somalia. Left: a tomato crop affected by parasitic weeds (*Orobanche*); centre, the same crop from a distant perspective; right, tomato plants are stacked using acacia branches, which hampers pest scouting and crop husbandry. Author's own photographs.

of smallholders' farms where PPN populations build-up above the economic damage threshold.

Trap crops

Non-food crops, such as *Solanum sisymbriifolium* and oilseed rape, are used as trap crops in other parts of the world to manage cyst nematodes; nevertheless, they are of limited applicability for smallholders in LDC because these are neither food nor fodder crops. Noteworthy, indigenous leafy vegetables remain mostly unexploited as locally suitable options for PPN management, and scientists and funding programmes should pay more attention to their applicability as trap crops for sustainable intensification programmes. Recent studies have shown that *S. vilosum* and *S. scabrum* are promising trap crops for PCN in the context of subsistence agriculture in SSA. These leafy vegetables are highly nutritious, and smallholders can easily incorporate them into their diets with appropriate awareness and demonstration campaigns.

In humid tropical conditions, the planting of short-cycle crops (lettuce, radish, Chinese cabbage) was sufficient to reduce the incidence of RKN in the soil and to increase the yields of subsequent solanaceous crops; these types of rotations should be more studied to determine their effectiveness for smallholders considering the degree day accumulation of the target PPN species in different agro-climatic conditions.

Cover crops for nematode suppression

Alternative cover crops can help to diversify livelihoods in most of the rural homesteads, including the agropastoral communities where farmers keep livestock. Therefore, the use of grasses and other cover crops, such as sudangrass and/or *Mucuna* spp. (e.g. *Mucuna utilis*) can be an excellent option to help smallholder farmers manage PPN as these can be directly fed to livestock or sold as fodder. Even so, farmers tend to prefer dual-purpose crops to nourish themselves and their livestock (e.g. sweet potato) and to use the crops' leftovers (e.g. banana stems, maize and sorghum stalks and leaves) to feed their animals, rather than growing grasses for livestock feeding only. Where access to land and fodder is constrained, the use of mulching as a way to enhance the biodiversity of the soil food web to suppress PPN conflicts with the need to feed animals and obtain animal proteins in low-input and low-output farming systems. It is also the case with the incorporation of brassicas as green amendments for biofumigation of soils.

Currently, researchers from Ibadan University, Nigeria, are screening the germplasm collection at IITA of Bambara groundnut in response to RKN. From the accessions screened so far (n = 50), 8% were consistently resistant to *M. incognita* and open a promising door for breeding programmes (A. Claudius-Cole, personal communication). This leguminous crop is mainly grown by female smallholders for household consumption but it is also used to feed livestock. It contributes to naturally fix nitrogen in the soil, and it is a drought-resilient crop that grows in marginal lands with minimal inputs.

Nematicides

Chemical control is not a suitable solution for smallholder farmers. Governments in LDC often do not have the ability to enforce safety standards for transport, storage, labelling and use of such chemicals. In some of these countries, the new-generation nematicides have also arrived (e.g. Kenya and Ethiopia), but only for particular upmarket niches, such as the flower industry or the crops for export markets. The high quality of these products, backed by international tests and regulations, often makes the price of these products prohibitive for small-scale farmers. Companies should look at repackaging their products and reduce the quantities to make them safer and suitable to the size of local farms. More robust phytosanitary policies are needed in LDC regarding commercialization, storage and disposal of pesticides.

Biological control

Biological control agents, such as antagonistic fungi and bacteria (delivered as seedcoat treatments) and endophytes, have entered the market on a large scale for nematode control (Sikora and Roberts, 2018). Seed treatments to protect the seedlings from early root penetration and damage is effective in reducing yield loss in maize and soybean. Fungal endophyte-priming technology is also used with tissue culture banana plants. When primed with endophytes, plants are more resistant to *R. similis*, *Pratylenchus goodei* and *Helicotylenchus multicinctus* as well as the banana weevil and produce higher yields. Nevertheless, the adoption of this technology is again somewhat limited. Companies should create more awareness about their efficacy, and producers need to adjust the products' commercialization to the needs of smallholders too.

Outlook: a vision for the future

Smallholder farmers in the least developed economies are bound by countless challenges that prevent them from accessing information, knowledge, inputs and services to increase agricultural production and ensure a better living. Many of these options have been outlined in this volume. Just as the challenges facing small farmers appear to be limitless, the diversity of PPN that affect agroecosystems is also vast. Thus, there is no one-size-fits-all solution to mitigate the impact of these pests in smallholding farming systems, and the management strategies necessarily have to be multiple and very context specific.

Therefore, more human and financial efforts must be diverted to enhance the capacity of the extension services and their diagnostics capabilities. Famers should understand what nematodes are, recognize their symptoms and have access to factual information to make decisions at their farms. The lack of sufficiently equipped and trained extension services to assist smallholders is a recurrent obstacle in the LDC. Hence, sensitization is needed to generate awareness among policymakers on the importance of recruiting nematologists and skilled personnel in quarantine stations for routine inspections, and also for enforcing safety regulations regarding the trade and use of hazardous pesticides. Likewise, nematology as a discipline needs further support at the donors' community level to develop basic and applied research, including epidemiologic surveys.

New areas worth further exploration are the use of indigenous and underutilized species that can also be used as resilient sources of fodder and food, and also act as non-host rotational or trap crops (e.g. grass pea). The future also lies on elucidating the plant–microbe interactions in the rhizosphere from a biological perspective, with the discovery of new microorganisms in the soil with nematicidal activity; but also from exploiting the plant–nematode interactions from a chemical ecology point of view (Ochola *et al.*, 2019).

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64 The unpredictability of adapting integrated nematode management to climate variability

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Introduction

The decision to add a chapter to this volume on the interrelationships between climate and integrated nematode management (INM) was based on the number of statements made in the chapters concerning the future importance of climate change and variability on nematode damage and integrated management. The areas of concern mentioned include:

- shifts in the distribution of nematodes;
- stimulation of additional generations;
- increased reproductive potential;
- development of more severe nematode–pathogen complexes;
- inability to monitor with remote sensing populations over multiple seasons;
- negative yield due to nematodes and reduced soil moisture levels;
- adapting INM to highly volatile interannual fluctuations;
- loss of organic matter and soil antagonistic potential;
- lack of an effective in-season plant curative pesticide;
- enhancement of cumulative multi-species impact; and
- inactivation or loss of plant resistance to nematodes.

In this chapter, we will reflect on some of the above points and how long-term climate change and increasing climate variability may impact nematodes, crop losses and potential modification of INM under climate change induced risk.

It is important to understand that both long-term (multi-decadal) climate change and seasonal and interannual climate variability, which is partly a manifestation of anthropogenic climate change, will both impact INM. For those working in nematology, climate variability will have a greater immediate impact on nematodes and INM research than will long-term climate change. In addition to climate factors, a combination of geographic region, cropping regimes and plant nematode complexities will determine what INM strategies need to be modified to offset crop loss.

Climate change and climate variability in the context of INM

Climate change (CC), refers here to multi-decadal temperature and precipitation trends associated

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with anthropogenic warming. The presence of CC has been clearly observed in the heating of the oceans, melting of glaciers and loss of ice in polar regions. It is also seen in the increase in average yearly global temperature impacting terrestrial, freshwater and marine systems (Scheffers *et al.*, 2016).

Climate variability (CV) refers to interannual and seasonal changes in weather patterns locally or regionally. CC is increasingly considered a factor in the extreme variability and volatility in weather conditions that are and will impact agricultural production in the near future (Sikora *et al.*, 2020). For instance, a shift towards more intense rainfall observed in some regions, the reduction in winter temperatures in other areas, and more extreme events overall (i.e. droughts, floods and heatwaves) suggest that climate change is increasingly influencing climate variability (Bathiany *et al.*, 2018; Li *et al.*, 2021).

The extent to which CV impacts agricultural production also depends on a farmer's ability to modify their production systems. Climate adaptability will vary across geographic regions and is greatly influenced by environmental, social and economic factors (see Chapters 7, 24 and 63). Generally speaking, smallholder farmers cannot adapt to CV with the speed of large holder farmers due to their: (i) <1.5 ha landholdings; (ii) lack of land ownership; (iii) degraded soils; (iv) inadequate mechanization; and (v) poor or non-existent access to credit for crop and pest management inputs.

Climate impacts on agricultural crops

Climate change has the potential to significantly undermine efforts to both achieve food security and sustainably manage the natural resource base of agriculture. Rising temperatures, increased frequency and severity of extreme climatic events, and changes in the distribution and timing of rainfall will have strong negative impacts on crop, fishery and livestock production, and could further compound the already substantial challenges facing agriculture (Shukla *et al.*, 2019). According to the Intergovernmental Panel on Climate Change (IPCC),

> yields of some crops (e.g. maize and wheat) in many lower-latitude regions have been affected negatively by observed climate changes, while in many higher-latitude regions, yields of some crops (e.g., maize, wheat, and sugar beets) have been affected positively over recent decades. Warming compounded by drying has caused large negative effects on yields in parts of the Mediterranean and climate change has affected food production in the drylands, particularly in Africa, and the high mountain regions of Asia and South America.

As shown in Fig. 64.1, climate change is projected to significantly impact crop yields negatively within near-term time horizons, particularly in some regions of the global south where food security is already a significant challenge. These decreases in yield are to some extent determined by the inability of farmers to adequately adapt to climate change. In temperate regions, yields will probably increase due to more favourable climatic conditions. Climate change in both regions will require additional modifications of INM.

Critical climate change hotspots

A general indication of where CC hotspots are most critical is shown in Fig. 64.2. In some cases the effects are already manifested and will intensify over time in these regions. Climate change and CV is having a major, though erratic, impact on crop production as seen in the heat and drought stricken semi-arid subtropics and tropical regions in the southern USA, central and southern South America and in most of Africa, India and Australia. This figure provides those working in these hotspot areas an indication of the crops and nematodes that need to be considered in climate relevant research both short and long term.

Rain-fed crops are clearly more sensitive to climate risks than irrigated crops where temperatures and transpiration in the former will drastically increase drought conditions. However, water use efficiency in irrigated crops will also become more complex. Many tropical and subtropical crops in high temperature hotspots will be simultaneously affected by increases in the combined damage caused by multiple nematode species present on important food crops such as groundnut, cowpea, rice, banana and maize that are grown in continuous relay systems. In some hotspots there will be shifts in species dominance, for example, *Heterodera*, *Globodera* and

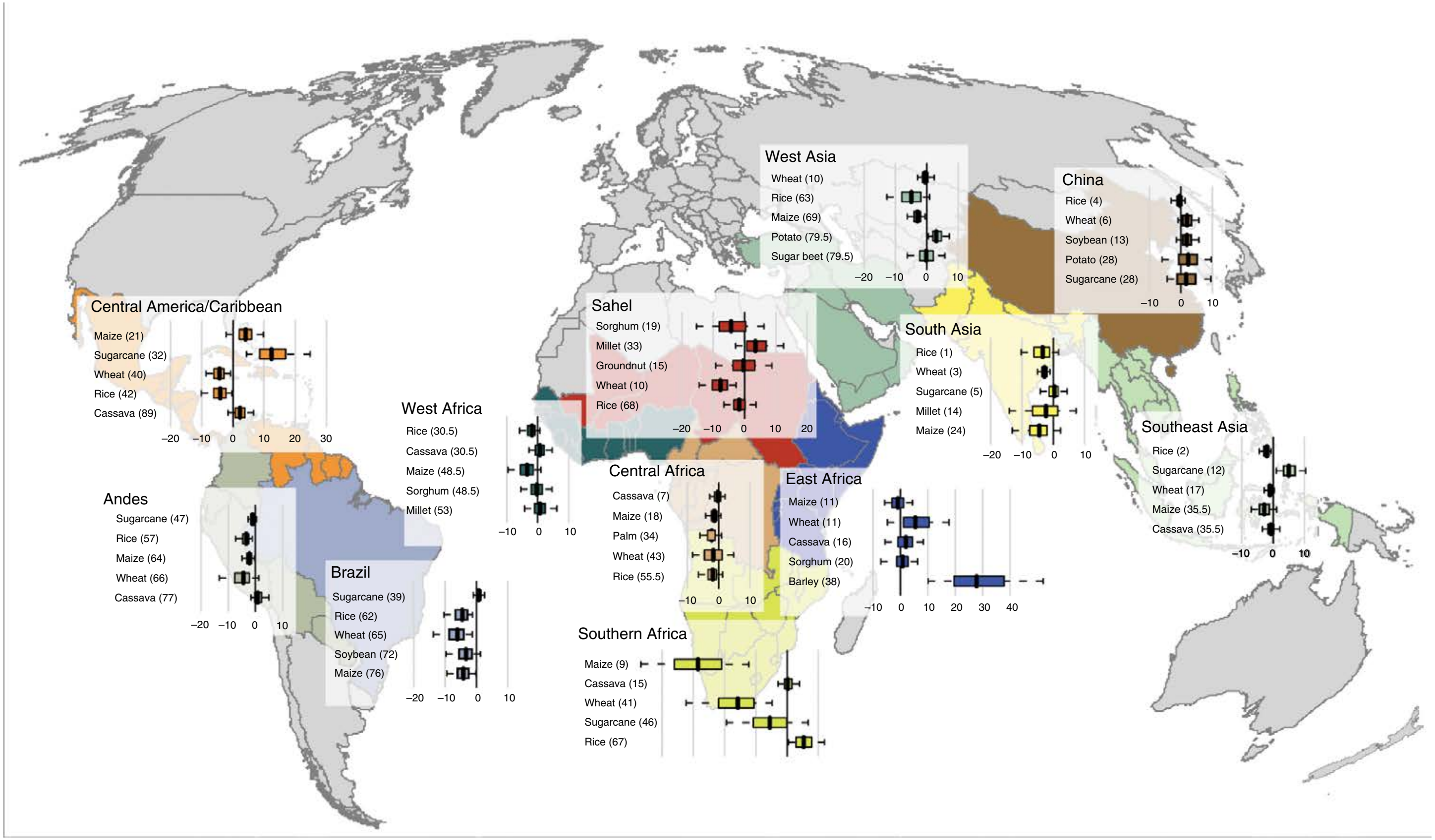
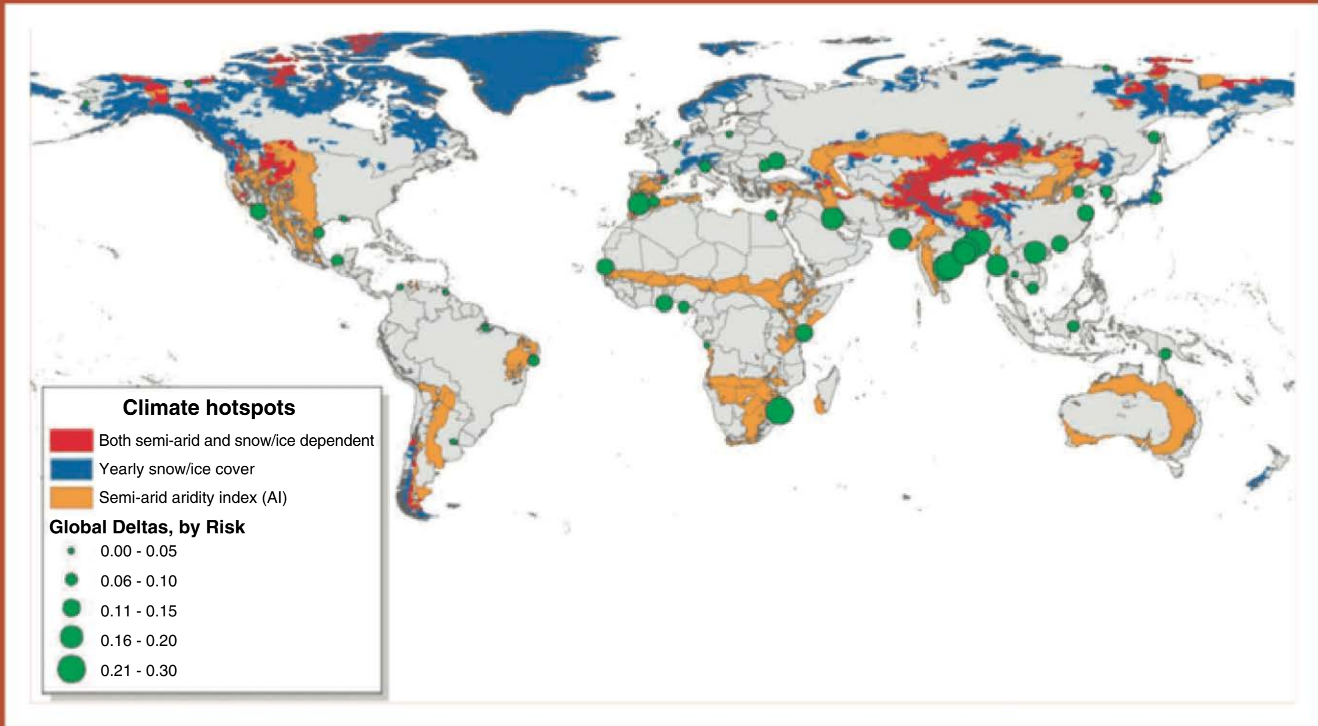


Fig. 64.1. Projected impacts of climate change by 2030 for five major crops in each region. For each crop, the dark vertical line indicates the middle value out of 100 separate model projections, boxes extend from 25th to 75th percentiles, and horizontal lines extend from 5th to 95th percentiles. The x-axis represents per cent yield change compared with the 1980–2000 baseline period. Number in parentheses is the overall rank of the crop in terms of importance to food security, calculated by multiplying the number of malnourished in the region by the per cent of calories derived from that crop. The models assume an approximate 1°C temperature rise between the baseline (1980–2000) and the projected (2020–2040) period (Lobell, 2008). Figure courtesy of David Lobell, Stanford, USA.



Note. The three major types of climate hotspots used in the proposed multiscale SDG framework are shown, including (1) major gbbal delta locations (green dots), varied according to contemporary risk due to sea-level and anthropomorphic factors; (2) semi-arid regions (orange) where an Aridity index (AI) fails between 0.2 and 0.5; (3) snow and ice runoff-dependern basins (blue), delined as basins with average yearly snowlice cover ≥25%; and (4) overlapping areas with both semi-arid AI and snowfice runoff dependency (red).*

Fig. 64.2. Climate change hotspots requiring focused attention using the Sustainable Development Goals (SDG) indicator framework. (Szabo *et al.*, 2016, open access.) Figure courtesy of Taylor & Francis Ltd (www.tandfonline.com).

Pratylenchus species on wheat, potato and banana, respectively. As water becomes a limiting factor in hot spots where rice predominates, there will be changes in production toward direct seeded upland rice which will lead to new nematode problems such as *Meloidogyne* coming from rotation crops as discussed in Chapters 8 and 9 (Padgham, 2009).

Climate and nematode biological processes

Temperature and moisture are the main abiotic drivers that influence nematode and plant development. The near-term effects of increasing seasonal and interannual climate variability of both soil temperature and moisture will obviously be extremely important in appropriately modifying INM. Moreover, given warming trends (particularly the greater rate of increase in minimum temperature compared with maximum temperature), changes in nematode distribution over time and population dynamics need to be assessed through long-term government pest monitoring programmes (where available and resourced). However, measuring the expected impact of CC on soil nematodes is complicated. While soil temperature positively affects the development of most soil nematodes, soil warming also induces drying which negatively affects soil nematodes. The changes in distribution and damage potential of plant parasitic nematodes due to climate warming will therefore be the result of the integrated effects of the resulting soil temperature and moisture changes in relation to the length of the growing season and the effects on winter decline.

Soil temperature optimums for a broad array of plant parasitic nematodes were compiled by Norton (1978). Temperature, however, also influences soil moisture and thereby nematode survival, hatch, penetration and development (Wallace, 1973). Optimum temperature for hatching differs between subtropical and temperate climatic regions. It is also well known that differences in hatching exist, e.g. between *M. incognita* and *M. hapla*/*M. chitwoodi*, but also *Globodera rostochensis* and *G. pallida* (Van Gundy, 1985). Optimum temperature for embryogenesis is considered to range between 25°C and 30°C for tropical and subtropical root-knot species and slightly lower for temperate parasites. Increases in temperature will affect hatching behaviour and therefore early root damage in many crops as soils heat up earlier in the season and overwintering temperatures rise.

With longer term multi-decadal climate change, elevations in soil temperature will cause thermophilic nematodes (comfortable at >41°C) to expand their distributions into previously cooler zones and cryophilic species (comfortable at −20°C to +10°C) to expand and survive in ever more northerly regions as agriculture expand into areas with warming temperature. This means the ranges for root-knot nematodes in the USA will be altered from what we recognized in the past (Fig. 64.3). These shifts may not always be immediately predictable, as nematodes are very adaptable. For instance, northern temperate nematodes like *M. hapla* and *P. penetrans* have become very common in the warm soils of Florida, especially in the major strawberry growing region near the middle of Florida's west coast. It is suspected that these nematodes have been introduced over the years from northern states or Canada where the strawberry nurseries are located (each year millions of live plants are transported to production fields in Florida). Possibly, root-knot and lesion nematode populations have increased in these northern nursery regions as a result of warmer soil temperatures, combined with loss of soil fumigants.

Soil moisture regulates root growth and all stages of nematode development as well as root exudates that are important for attraction and nematode hatching (Norton, 1978; Van Gundy, 1985). Similarly, soil moisture is also important for the germination, movement, development and parasitism of soil antagonists that regulate nematode densities. Nematodes require 40–60% moisture in the soil for migration. Therefore, reduced soil moisture will cause changes in nematode behaviour and antagonistic potential that will alter nematode impacts on plant growth and ultimately yield. Increased temperatures and reduced soil moisture in the upper soil horizons could cause nematode populations to expand in deeper regions in the profile. In some temperate regions, increases in temperature, rainfall and soil moisture will ultimately result in higher infestation levels. Temperature increases at planting time would also increase early root penetration and seedling root damage that has effects throughout the season. In semi-arid regions of the world where irrigation is introduced as an adaptation response to climate change, the subsequently more favourable soil moisture conditions could intensify nematode damage to crops. Crops grown at higher elevations will also be affected as temperature changes increase the number of generations per crop season. This could affect *Ditylenchus dipsaci*, *D. destructor* and *Bursaphelenchus xylophilus* in the northern hemisphere and shifts in species of lesion nematodes damaging many important food crops.

Degree-days and nematode development

Temperature effects on nematode development are best monitored using degree-days. Degree-days have been calculated for a number of nematodes and can be used to determine stages of development at multiple sites using computer-monitored soil temperature probes (see Chapter 60). Using computer-controlled temperature probes, the timing of early root penetration, the initiation of follow-up generations and even egg production can be determined. These probes and data generated can be used for timing both biocontrol and chemical control applications as well as for trap cropping (see Chapter 60). More importantly, the data could be used to estimate *Pi* and optimize INM in the next season (see Chapter 58).

Plant parasitic nematodes as research models

While it is obvious that changes in climate affect temperature and moisture, the speed with which CC is impacting ecosystems and agricultural regions is real and immensely important on a global scale. Furthermore, it is important to recognize that targeted research designed to transform INM systems to deal with increased CV risks, such as extreme events in hotspot regions, requires modification of INM.

The following sections are based on the authors' own interpretations of how climate risk will impact nematodes and INM. The lists below are intended to identify topics to stimulate research. Nematodes have a limited ability to spread, they are simple to detect, and their population densities can be easily monitored. This makes plant parasitic nematodes unique models for the study of climate change in soil ecosystems and gives them an advantage over other more mobile pests when trying to adopt integrated management strategies to CC and CV.

Some of the key characteristics that we believe make nematodes excellent models for research on CC and CV in the soil ecosystem are:

- They are poikilothermic with body temperatures regulated solely by ambient temperature.
- Nematodes are omnipresent, thereby allowing comparisons between regions of the world.
- They are mainly active in the soil profile to a depth of 30 cm.
- Nematodes have limited mobility, migrating 10–20 cm both upwards and downwards depending on soil type, in sandy soils vertical migration can be higher.
- Nematode development, as influenced by soil temperature, can be easily monitored.
- Plant damage can be monitored by remote sensing.
- The microbial antagonistic potential that limits nematode densities can be monitored.
- Nematode maturity indexes exist to monitor total soil communities.
- A wide array of management options exist for rearranging management approaches.

In a review of abstracts published between 2000 and 2020 using CAB Abstracts, the articles dealing with CC and plant parasitic nematodes dealt mostly with shifts in nematode community structure in natural areas and grassland ecosystems and with modelling changes in potential nematode distribution and damage impact. Some of the conclusions from that literature and from the chapters in this volume were:

- *Bursaphelenchus xylophilus* will spread to north-eastern regions of Germany.
- *Globodera* infestation levels need to be reduced by up to 40% to negate yield loss in the UK.
- Reduce status of *G. pallida* in favour of *G. rostochiensis*, especially in the southern UK.
- Increased cyst and lesion damage to wheat in North Africa/West Asia (see Chapter 3).

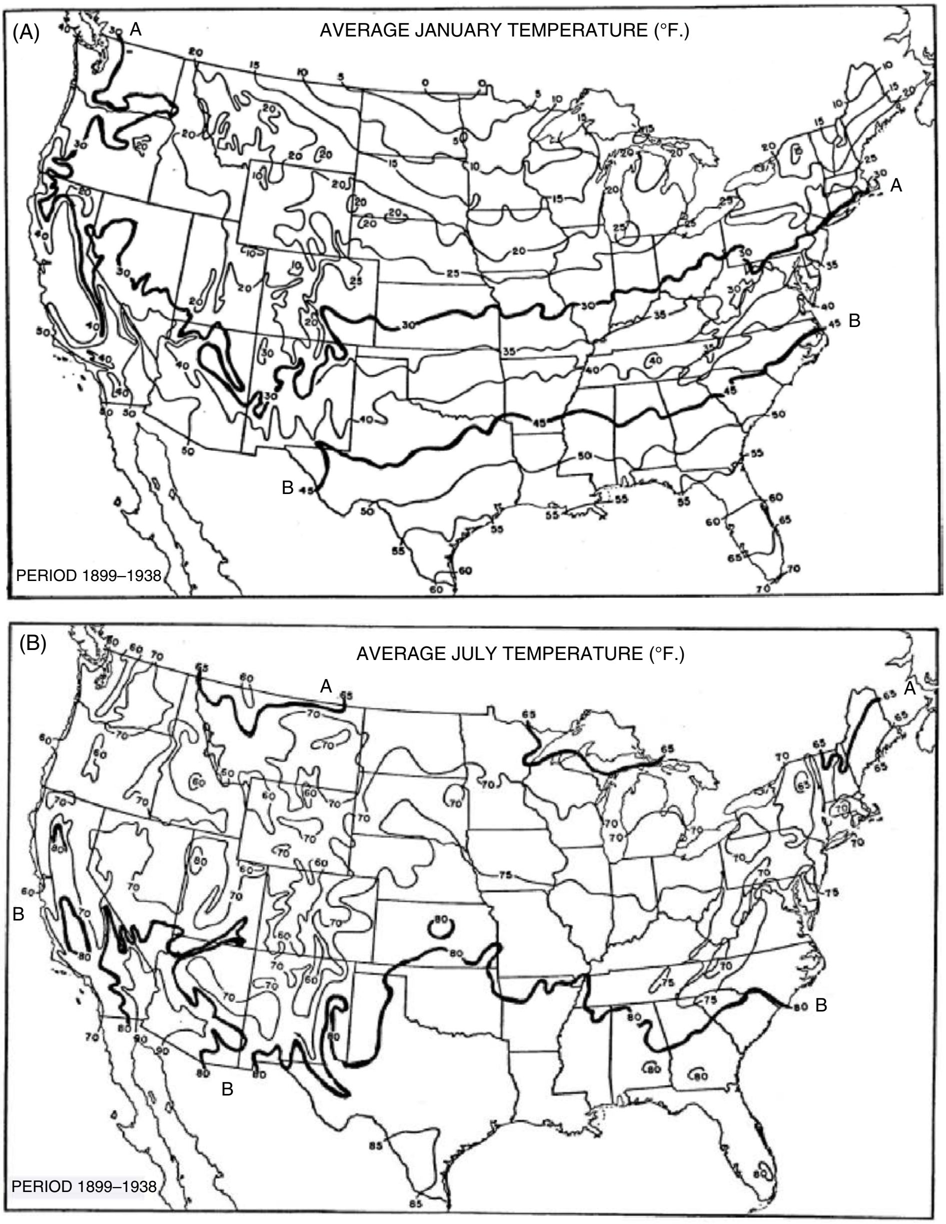


Fig. 64.3. Distribution of the root-knot nematodes **(A)** *Meloidogyne incognita* and *M. arenaria*, and **(B)** *M. hapla* in the USA (Sasser and Carter, 1985). Figure courtesy of R. Davis for A.L. Taylor and J.N. Sasser, Raleigh, NC, USA.

- Increased temperature could favour *Pasteuria penetrans* parasitism of root-knot.
- Shifts in lesion nematodes on banana will influence yield and breeding (see Chapter 24).
- The move from flooded to upland rice will favour root-knot (see Chapter 8).
- Increases in volunteer potato will help maintain potato cyst nematode inoculum (see Chapter 44).

- Reniform and root-knot could spread north adding to soybean losses in USA (see Chapter 18).
- *Xiphinema* and *Longidorus* problems could intensify in northern Europe.

Immediate priorities for improved near-term climate risk management within INM

Seasonal and interannual climate variability will definitely impact how we conduct INM both now and in the future. The challenge facing nematologists is attempting to redesign INM so that farming systems are resilient to CV risks. How to control a nematode problem in a crop in standard uniform rotations in the next season can be planned. However, planning nematode control as impacted by more erratic weather patterns in non-uniform rotations, random cropping systems and monocultures is complicated. The development of INM systems when unknown changes in climate might occur at planting in the coming growing season is a major challenge but is feasible if we can monitor the nematode's population density. Research on transformation of INM needs to be started now in regions of the world where productivity will be clearly impacted (see Figs 64.1 and 64.2).

Complicating management is the need to devise responses (before, at, or shortly after planting) that are applicable during abnormally wet, dry or warm growing seasons. We believe nematologists, working with agronomists and climatologists, can design resilient cropping systems that can withstand increased CV or with what could be called climate-smart INM.

Some of the questions that could affect those working in INM are the where, when and why of transforming INM to better cope with seasonal CV risks. We need to be prepared for unknowns and environmental unpredictability in our cropping systems. Studying INM under constantly changing climatic conditions is a major challenge. Some of the questions we felt should influence our INM thought processes, and there are many more, were:

- Can adjusting planting dates improve nematode management?
- How will crops used for CV adaptation affect nematode development and damage?
- Will soil moisture management with minimum tillage affect nematode densities?
- Could intercropping with trap or antagonistic crops improve INM?
- Will increased temperature shorten life cycles and promote nematode damage?
- Can we monitor effectively temperature effects on nematodes over multiple rotations?
- How will nematode inoculum be influenced by periodic soil heat/drying periods?
- Will CC or CV alter antagonist activity?
- Can increases in temperature affect resistance genes?
- Will CV influence the timing and magnitude of cyst nematode hatching?
- Would early planting help reduce early root infection of cyst and root-knot?
- Will trichodorids and Tobacco rattle virus increase on potato in the EU?
- Will milder winters in the USA increase survival of SCN and increase damage?
- As ground water level drops will upland rice see a change in nematode structure?
- Can we find cultivars with combined drought and nematode resistance/tolerance?
- Could we use early maturing cultivars to trap late season generations?
- Will warming in temperate regions affect cyst or root-knot nematode overwintering?
- Have cold/warm temperature species already moved into new areas?
- Can we monitor soil microbial biodiversity and changes in antagonistic potential?
- Can nematode maturity indexes be used to detect CC or CV shifts in communities?
- Will narrower and taller raised beds reduce flood damage and reduce nematicide levels?
- Could cultivar mixtures (resistant, tolerant) influence nematode damage?
- What is the effect of milder winters on cover crop growth and its influence on population dynamics?
- Control of volunteers, e.g. potato, when lack of frost improves their survival?

These are just some aspects of the influence of CC and CV on nematodes that we feel need to be addressed in the near and long-term future in order to plan INM to guard against future yield loss.

Outlook: conclusions

The increasing toll of a changing climate presents profound challenges for all aspects of agricultural systems and food security. Nematodes, as outlined in this chapter, will be influenced by increased climate variability and nematode–crop interrelationships that will cause increased crop loss. We have outlined what we feel are the most important aspects of the interrelationship between climate risk and nematode–crop interactions. The factors we feel are important in developing research on climate variability and change to improve nematode management have been mentioned. Stress obviously needs to be placed on the hotspot regions with emphasis on the semi-arid and arid regions of the world. There is a need for research on a wide array of topics, some of them we attempted to list in the chapter. We could not be all-inclusive but hope our chapter increases interest in the future of climate and INM. As the human population increases and climate change worsens, food insecurity will be more pronounced. Nematodes play a part in yield loss in many food crops (Sikora *et al.,* 2018). Nematology needs to establish a stronger position in climate research and soil-borne pest management as outlined in the chapters in this volume. Finally, many of the methodologies discussed in the chapters on new technologies will be important in adapting INM in the future.

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Section IX:

Conclusion

65 Outlook: A vision of the future of integrated nematode management

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Many of the futuristic visions in the chapters in this book were shared by multiple authors and give us a (pre)view of what integrated nematode management (INM) may look like in the future. Any grower that has dealt with a serious nematode problem can attest to the difficulty of managing them, and while chemical control was the preferred option in the past, the future will require a far more integrated approach. The consequences of non-action regarding improving INM will be severe, including further proliferation of nematode problems and deterioration of soil health.

While it was clear that there is no cookie-cutter tool or one-size-fits-all approach to INM, employing a diversity of management practices and focusing on key concepts such as targeted rotations, intercropping, advanced genetics for resistance breeding, remote sensing to monitor nematode distribution and densities, precision agriculture to target control treatments and molecular tools to measure soil suppressiveness, were repeatedly mentioned by most chapter authors.

The pillars of INM and the wide array of tools they encompass are outlined in Chapter 1. In Fig. 65.1 we have modified the pillars to highlight future anticipated developments in nematode management. In all the chapters it was made evident that the INM building blocks that make up the pillars will need to be modified and expanded upon in order to be able to deal with future changes impacting soil and root health.

In the majority of the chapters, the future impact of climate change was considered a major factor in INM and therefore a chapter (Chapter 64) was included to cover this still poorly understood phenomena on INM. In addition, chapters were included to demonstrate the newest developments in nematological science that could improve and refine INM.

It was clear from the start that when you fill a void you end up with an additional void. Anticipating the future obviously made visible the big hole in knowledge available and solutions needed in the future to improve INM. The innovative technologies and some of the solutions that will influence INM and fill the knowledge gap in the future are presented in Table 65.1.

The table includes the suggestions and projections made by many of the authors when anticipating how INM will need to change in the coming decades. We need to adjust our approaches to offset the drivers of change: human population

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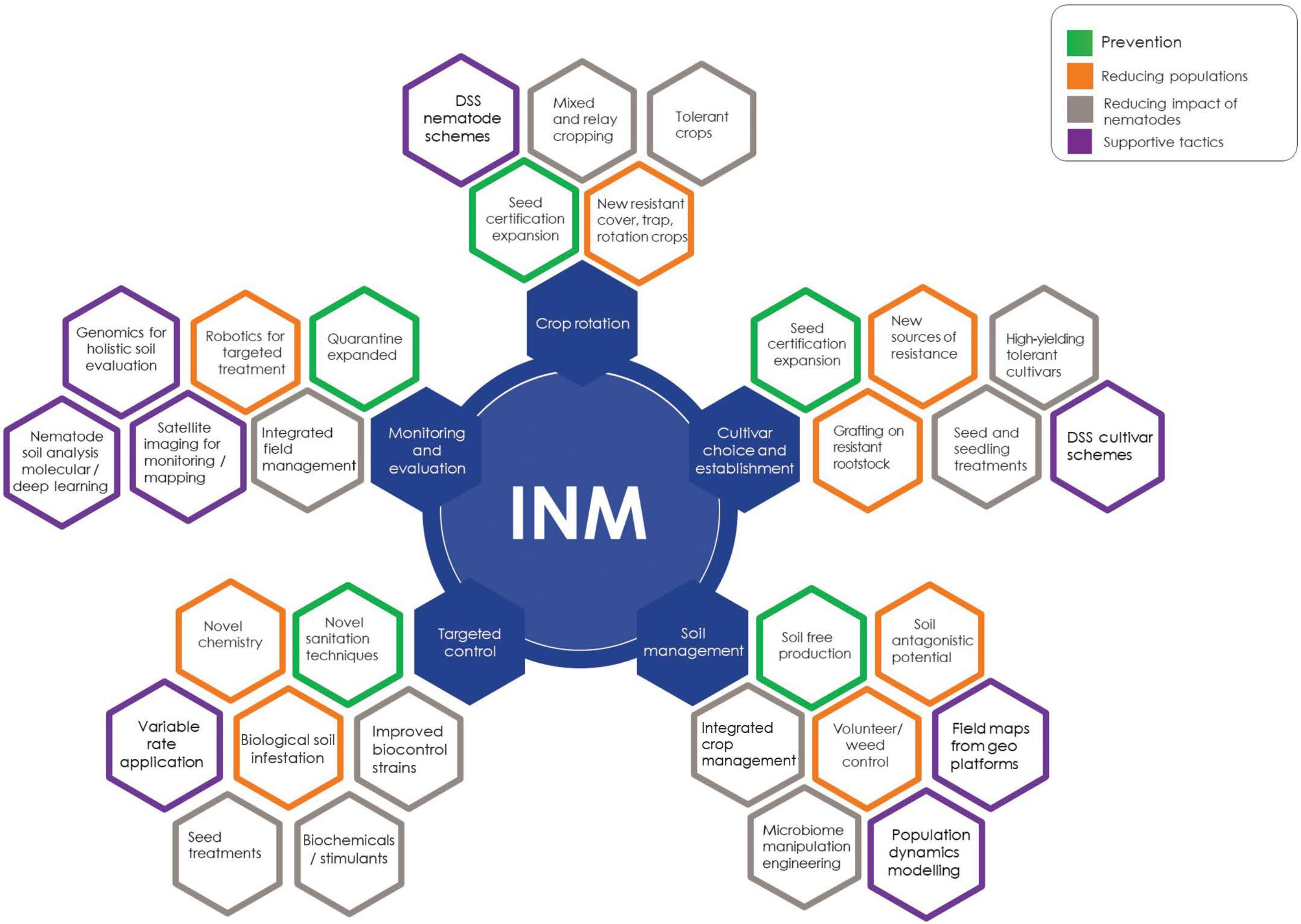

Fig. 65.1. Integrated nematode management (INM) in the decades to come – adapted from Fig. 1.1 in Chapter 1. Figure courtesy of Wageningen University & Research, Field Crops.

Table 65.1. Current and anticipated progress in technologies that will have major impacts on integrated nematode management and soil and plant health.

Technologies	2000–2020	2020–2050
Sanitation	Hot water treatment of plant material Certified seed and planting material but limited equipment cleaning	New sanitation techniques (e.g. controlled atmosphere temperature treatment, steam, accelerated electrons) Seed certification more widespread Quarantine expanding
Rotation and cover crops	Standard but often narrow, decreasing in importance as INM tool Often limited info on specific nematode host status	Clever rotations, intercropping, mixed and relay cropping increasingly designed for nematode management More nematode-targeted crop and cover crop cultivars available
Biological soil disinfestation	Anaerobic soil disinfestation, solarization, biofumigation not widely used and results often inconsistent	Integrated programmes including a biological soil component more common More effective agents and methods (e.g. microbial volatiles)
Soil free production	Substrate production for vegetables (mostly EU and Canada) Ultraviolet radiation treatment of irrigation water	Expanded to include more crops and regions Ozone generators for decontamination of irrigation water in closed systems
Cultivar selection	Resistant cultivars and rootstocks used where available, relatively few crops with nematode resistance Tolerant cultivars often preferred over resistant	New sources of resistance, increasing use of resistant and tolerant cultivars and rootstocks Expanding the success and acceptance of modern genetic editing (e.g. CRISPR)
Seed-based control	Combined fungicide/nematicide seed coating for row crops introduced	Increasing availability of chemical and biological seed treatment options combined with nematode resistance/tolerance
Chemical control	Old chemistry still widely used but being replaced First new reduced-risk nematicides becoming available	Older nematicides and fumigants removed from market New nematicides becoming available (including basipetal treatments) and replacing old nematicides, esp. high-value crops
Biological control	Several products with limited or scattered levels of success, and relatively few field data Effective seed-based treatments still lacking Potential use of biochemical treatments still untested	More competitive agents and strains, including plant- and microbial-derived products Seed and seedling-based treatments becoming standard for many crops Increased use of biologicals for targeted post-plant treatment, and partnered with chemicals and bio-chemicals
Soil antagonistic potential	Presence acknowledged but impact unsatisfactory and not used on a practical scale Organic agriculture still leading promoter Soil microbiome research expanding	Soil microbiome advances leading to targeted methods to stimulate soil antagonistic potential Metagenomics and holistic approaches available for monitoring Holobiont concept used to help monitor nematode populations and improve soil antagonistic potential
Soil sampling	Field scouting, manual soil and root sampling	Remote sensing, drone driven mapping, use of robotics to aid sampling Analysis of samples using molecular and machine-learning tools, including holistic soil analysis approaches

Continued

Table 65.1. Continued.

Technologies	2000–2020	2020–2050
Precision agriculture	Technology developed; field application still limited	Site-specific treatment using sensors and robotics adopted in field and horticultural crops
Modelling population dynamics	Lack of data on population dynamics limits use Initiation of remote sensing for detection of nematode distribution tested	Mathematical modelling available to calculate population development over rotations and *Pi* estimation Remote sensing coupled with practical models used in precision INM Satellite imaging allows detection of nematode hotspots and nematode 'densities'
Other supportive tactics	Limited number of decision support tools/systems (DST/DSS) with low level of implementation	DST with IT and artificial intelligence expanding esp. in horticultural crops High-resolution data platforms providing the farmer/adviser with detailed info and INM recommendations, facilitating integrated crop management

growth, food insecurity, soil degradation, climate change and volatility, and the anticipated increase in nematode impact on soil and crop health.

The new building blocks of INM as summarized in Fig. 65.1 and Table 65.1 that could improve the future of nematode management were emphasized by many authors and will be further discussed below.

A new look at chemical control in the future

Nematicides can be credited for having put the science of nematology firmly on the map. The enormous amount of crop damage and yield loss that plant parasitic nematodes (PPN) can cause was not known until the first trials with nematicides in the 1920s. From the 1950s to the 1970s, the discipline of nematology was booming and research on nematode biology, physiology and management rapidly expanded. This optimism started changing in the 1960s and 1970s, when some of the less desirable side effects of pesticides and nematicides started to become public. In the past decades, regulatory pressure on broad-spectrum fumigant, carbamate and organophosphate nematicides has led to their removal from the market, and as a result renewed pressure on crop protection companies to develop new and safer nematicides (see Chapter 61). New and more selective nematicides are entering the market and are expected to be more compatible with INM programmes and will replace older nematicides. However, for many resource-poor farmers, the new generation of nematicides may not be an option due to the high cost. The same goes for large acreage field crops, unless nematicides can be applied as seed treatments and be cost effective. The main initial use of these new nematicidal compounds will be in high-value crops like fruits, vegetables and cash crops, partially replacing current fumigant nematicides, for which the use will further decline.

Biological control growing up

While biological products may show good effects when tested under highly controlled conditions, they often fail to show high levels of control and consistent results under field conditions. There are many reasons for this: (i) strains are primarily selected for their nematicidal capacities under laboratory conditions; (ii) they often lack studies on strain survival and establishment in the highly competitive microbial soil environment; and (iii) timing of application that ensures early root infection suppression is problematic.

Furthermore, biopesticides do not behave and perform in the same manner as synthetic molecules; they require suitable formulations and application approaches, which are often difficult to develop or are cost prohibitive. The use of biological nematicides, however, has been growing and will continue to do so, but their success will depend on the efficacy and cost of the products. Growers are always looking for 'quick fix' or 'risk-limiting' solutions to prevent potential crop losses when faced with an urgent or unexpected nematode problem. Efficacy for growers is measured in terms of yield, not in terms of reduced nematode populations, which can be highly variable and difficult to measure. Therefore, cost is often the deciding factor as to which product to apply. Organic growers, having more limited options, may be willing to pay more, but conventional growers that use the vast majority of biological agents are much less likely to do so. Public concern about chemical pesticides and governmental regulatory controls will further benefit the growth of biological nematicides, especially when more effective and affordable chemical options become unavailable.

Biological nematicides may also face more scrutiny from regulators, as is already happening in Europe. There has been little research in understanding the long-term impacts of nematode biocontrol organisms. In the case of biological nematicides, little is known of the side effects of fermentation products and shifts in microbial population structure due to botanicals. In addition, not much is known about the active ingredient(s) themselves. Increased scrutiny could slow down the growth of biological nematicides, but as they are in high demand, the expectation is that many more products will become available in the future.

Finally, the holobiont concept opens an exciting and daunting new perspective on the future use of biological agents and INM in general. In brief, a holobiont is defined as the host and all of its microbial symbionts, including transient and stable members (Margulis and Fester, 1991; Bordenstein and Thies, 2015). Therefore, growth and development of living organisms, be it the nematode or its plant host, is influenced by the native microbiota that are naturally associated with them. These associated microbiotas may or may not provide protection against introduced or native biological antagonists (see Chapter 56). The role of symbiotic microorganisms is well known for entomopathogenic nematodes (EPN) but the holobiont concept is just developing for PPN and their hosts. Understanding the interactions between microorganisms from the plant host and microorganisms associated with PPN is a monumental task, but one that will greatly aid INM in the future (Sikora *et al.*, 2008).

From the field to the seed

Due to the increased value of seeds and the desire to protect them from infection, nematicide seed treatments using chemical and biological agents is one of the fastest growing market segments. While the efficacy of seed treatments has not been very consistent, most of the major seed companies now offer soybean, maize and cotton seed not just coated with fungicides and insecticides, but also with a nematicide. Farmers prefer the convenience of seed treatments and for that reason their use will continue to increase. In particular, the availability of biological nematicide and microbial seed treatments is expected to grow significantly in the next decades.

Seed treatments offer many advantages to the farmer by saving time and reducing the overall amount of product applied per field. However, this also constitutes its main weakness as its intrinsic efficacy is limited by the small amount of active product that can be applied, which is why seed treatment efficacy is generally lower as compared to soil applications. Seeds have and will continue to become more and more valuable, equipped internally with proprietary traits and externally with protectants and stimulants. Furthermore, seed treatments will only reduce early root infection processes and increase yield, they will not lead to a reduction in overall population densities.

Lack of and need for resistance breeding

The majority of the authors in this book indicated that breeding for nematode resistance is probably the most desirable and urgent future need. The problem is that except for soybean cyst nematode in the US and potato and sugar beet cyst nematodes in Europe, and in limited cases root-knot on tomato and some green manure crops, nematodes are rarely perceived as the number one priority for resistance breeding. Resistance breeding is a lengthy and expensive process, and requires a significant and long-term investment. The availability of large and diverse germplasm collections is also important, especially traditional locally adapted cultivars. There is some mild optimism that interest in nematode resistance is increasing among seed companies, but for nematode resistance to become more widely available, a much more concerted effort from public and private entities will be needed. For large acreage crops, susceptible rotational crops should be targeted for resistance breeding as well, as this would help to reduce the overall nematode pressure on the main cash crop in the rotation. For instance, a maize cultivar resistant to *Meloidogyne chitwoodi*, would be a very useful rotation crop to help manage this nematode which is an increasing problem for many crops in Europe.

In some instances, tolerance could be an attractive strategy as well. The detection of genes for tolerance to sugar beet cyst nematode led to the development of tolerant, tolerant-resistant and resistant-intolerant sugar beet cultivars. Tolerant cultivars are less costly to the grower than resistant cultivars and yield higher (see Chapter 55). However, the long-term impact of using tolerant cultivars in increasing nematode densities in the rhizosphere is still unknown. The use of tolerance can also cause losses in rotation crops susceptible to the nematode such as rapeseed.

Genome-editing technologies, especially CRISPR/Cas9, provide new and faster avenues for resistance breeding, but societal acceptance for these technologies will remain a major hurdle in much of the world. The emergence of genome-editing tools combined with our progress in understanding plant–nematode interactions could provide many new opportunities for nematode management using biotechnology (see Chapter 57). RNA interference and other genetic approaches could bring broad-spectrum and long-lasting new solutions but predicting the timescales for development of these approaches is about as uncertain as the weather.

Resistance is certainly not a single shot solution, as nematodes will find a way to break the resistance. This is especially true when nematode management relies solely on the use of resistant cultivars without resistance management, again stressing the need for effective resistance management tactics as part of an INM approach.

Suppressive soil and antagonistic potential

Stimulating the always present soil antagonistic potential to improve suppressiveness is something that many authors in this book brought forward as a future resource for nematode management. It was also repeatedly mentioned that rather than looking at one pest or pathogen, there is a need for a holistic approach in which the agroecological system is considered. Significant advances are being made in our knowledge of how complex microbial communities influence root health and plant growth. We know a great deal about the important functions they provide, including outcompeting and antagonizing nematodes and other diseases and in stimulating root defences and plant growth. New analytical approaches employing advanced computing power allow for complex network analysis of soil communities. A better understanding of how these communities interact with one another, with crops, and with the surrounding soil environment will provide predictive power to our understanding of nematode suppressiveness and how it will respond to changes in climate, cropping system and soil management.

The key feature of the antagonistic potential in agricultural soils is the reduction of root damage from soil-borne diseases and nematodes. We need to be able to use INM management tools and modern technologies to monitor and then manipulate this natural biocontrol phenomenon in favour of stable root health (Sikora, 1992). One piece of the puzzle may be the emerging understanding of the role of soil and plant microbiomes and of organisms as holobionts (see Chapter 56). Such research has just started for PPN, but one day it may be possible to predict which functions are mediated by a certain microbiome and which functions must be provided externally by (for example) organic compounds, plant extracts or microorganisms. It is well known that low levels of organic matter reduce natural nematode population regulation. Especially in naturally poor soils, insufficient return of organic matter to agricultural land has led to widespread and severe soil degradation across the world.

Microbiome-based solutions for crop protection are now actively being pursued by many companies. Some of these novel methods to help manipulate whole soil communities include whole soil microbiome plus microfauna inoculation, microbiome engineering and *ex situ* cultivation of soil microbiomes (Ke *et al.*, 2021). As most soil processes are regulated not only by soil microbial communities, but by the whole soil food web, the survival and successful establishment of newly introduced bacteria or fungi to stimulate nematode suppressiveness will depend in large part on the regulation of their abundance by the entire soil community and soil food web after introduction.

Climate change adaption

The impacts of climate change and climate volatility are discussed in detail in Chapter 64. In many areas of the world soil biodiversity will be strongly affected by changes in soil temperature and soil moisture. Increases in temperature will cause a significant loss in organic matter which will have negative effects on microbial communities and the antagonistic buffering capacity of ecosystem services. In particular, rain-fed crops in climate change hotspots will become more stressed and require new management approaches, which offset heat and drought stress as well as nematode root infection that synergistically increases the impact on plant growth of these two abiotic constraints (see Chapter 64). Root health management combined with development of drought resistant crops will be even more critical in the future. With the threat of increasing food insecurity coupled with looming climate change and the need to preserve natural areas and biodiversity, managing these risks will become increasingly critical. Further expansion of the world's agricultural land area, already at nearly 40%, is not an acceptable alternative. This means that food needs to be produced more efficiently and more sustainably on the land now farmed. Improving production will differ greatly depending where on the planet food is grown. Growing practices in places like the EU and the US will be very different from those in Africa for example (see Chapter 63). The difference between the small and large holder farmers also will gravely influence how well they survive as climate in many regions becomes less suitable for agriculture (Sikora *et al.*, 2020).

The high-tech Netherlands-style greenhouse farming has made this country the second largest vegetable exporter in the world. There can be little doubt that this type of vegetable farming will expand in many regions. We may someday see skyscrapers of vertical vegetable farms or even Amazon-delivered food from Mars (?) where farming will be completely independent of the outside climate. This is doubtful but there are dreamers. Nematode management in such systems where artificial growing media are used, will be mostly a sanitation issue. However, in many places this technology will not be economically feasible, and more traditional soil-based farming will continue to prevail.

Regional and site-specific approach

The face of agriculture is changing rapidly all over the world with corporate farming and high-tech greenhouses transforming the way food is being produced, and how nematodes are managed. At the same time, while agriculture is changing rapidly in some regions, this is certainly not the case everywhere. Access to new technology, seed and credit is not available to the majority of smallholder farmers who represent 98% of the farmers in the world (see Chapter 63). These farmers probably suffer more loss from nematodes than anyone else, and it should be clear that future technological advances in nematode management are of no value to them, at least in the short to mid-term. This emphasizes the importance of developing solutions on a regional basis and having nematologists working closely with farmers across the world. So, the answer to the question 'what should we expect in the future?' will very much depend on where this future happens to be. There is a dire need for nematology centres to link more closely with nematologists in food-insecure countries around the world to improve their expertise and access to modern technology as was done in the past in such programmes as the former International *Meloidogyne* Project (IMP) at North Carolina State University USA, The Nematology Initiative for Eastern and Southern Africa (NIESA) of Rothamsted Experimental Station UK and ongoing capacity building programmes, e.g. Nematology Education in Sub-Saharan Africa (NEMEDUSSA) at Ghent University, Belgium among others, that made and are making major strides in improving nematology around the world (Cortada *et al*., 2019).

Loss of nematology positions

The past decades have experienced a consistent loss of applied nematology positions at universities and plant protection agencies and the scope of research has been narrowed by focusing on advanced technologies, e.g. remote sensing, genetics and molecular aspects. Applied nematology is a time-consuming discipline with no quick solutions. This is especially true for INM which is a systems approach and highly data driven. Therefore, for INM to become successful, more applied nematology positions are needed, and a re-alignment of priorities in nematology science is needed, especially with regard to the training of students in INM.

Furthermore, nematologists will have to become more effective and focused at exchanging ideas and findings. One way to do this is through global collaborative projects that are specifically targeted at nematode management, as was done in the IMP mentioned above and now within NEMEDUSSA. Most collaborative projects tend to have a regional focus, combining different competencies that may or may not include a nematologist. This means that nematologists can easily become isolated and deprived of new insights. Our knowledge and understanding of nematodes and their environment has grown tremendously in recent years, but at the same time there seems to be a massive disconnect between fundamental and applied research. A new global nematology project could help bridge this gap.

Funding is the ultimate bottleneck, and it is sad but true that researchers have to spend an exorbitant amount of time writing and managing grant proposals. Nematodes are rarely on top of funding agencies priority lists, which is why it is imperative that nematologists continue to raise nematode awareness among the general public. Such activities are the domain of the extension nematologist, which is unfortunately becoming an increasingly rare breed. While social media can help to popularize nematodes and increase awareness, it is no substitute for the 'boots on the ground' approach of traditional extension nematology.

INM programmes recommended versus that followed by farmers

INM best practices are developed by researchers – who have to write scientific papers – while farmers run a business and have to remain profitable to survive. This means that recommended nematode management practices have to be practical and economically attractive to the farmer. With nematodes being only one of the many constraints that farmers face, INM recommendations will have a much greater chance of being adopted if they can be easily integrated with other management practices. This is the reason why soil fumigants became popular as they provided weed, disease and nematode control all at once. Similarly, the use of cover crops has been a traditional practice for farmers as it has many benefits in terms of soil fertility and quality, in addition to the potential benefits in terms of nematode management. We have to keep in mind that ultimately INM is not a goal in itself, but just one of the many components of a broader soil, crop and farm management plan as outlined in Chapter 60 on decision support systems (Fig. 65.2).

If INM programmes are to be fully accepted by farmers, it will be necessary for nematologists to work closely with agronomists when developing INM programmes so they become a component of larger integrated crop management programmes. Undoubtedly, more and more INM extension will move to

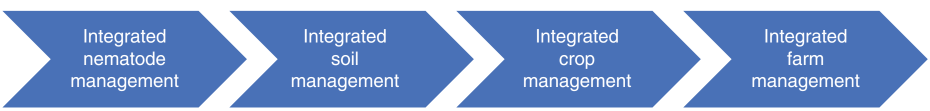

Fig. 65.2. Integrated nematode management as a component of a broader farm management plan. Author's own figure.

online sites, and we need to ensure that such information is easily accessible and understandable. Decision support tools for INM (see Chapter 60) therefore need to be developed on a regional basis and with input from growers, only then will they be acceptable and implemented by farmers and lead to more effective and sustainable nematode management.

Final remarks

INM is not a set of fixed practices, but rather a philosophy, and will have many different shapes and forms depending on the cropping system and region (Sikora and Roberts, 2018). Only when INM is fully integrated in crop and farm management plans will it be acceptable to farmers and able to improve food safety, the livelihood and well-being of farmers, and strengthen the perception of farmers as good environmental stewards.

Nematode management has acquired a new urgency in recent years, not in the least because applied nematology has been vastly neglected and underfunded for decades. This has created a huge gap with few nematology graduates trained in applied nematology and able to connect with industry and farmers. For INM to become a reality, applied nematology needs to be at the forefront of the science of nematology again, and funded accordingly. Only then will the advances in molecular methods, AI and precision agriculture lead to tangible, sustainable solutions for the world's food production.

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The following abbreviations have been used: CCN cereal cyst nematodes, CLN Columbia lance nematode, PCN potato cyst nematodes, RKN root-knot nematodes, RLN root lesion nematodes.

Page numbers in *italics* refer to illustrations.

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INTEGRATED NEMATODE MANAGEMENT

State-of-the-Art and Visions for the Future

Edited by Richard A. Sikora, Johan Desaeger and Leendert P.G. Molendijk

Plant parasitic nematodes cause an estimated US$100 billion per year in damage to crops. They are associated with nearly every important agricultural crop, and have a significant effect on global food security. Future changes in cropping systems, food habits, climate, as well as social and regulatory factors, will affect the options available for integrated nematode management (INM). These factors have already generated a growing interest in new and improved methods of nematode management.

Eighty nematologists from all over the world share their views on INM by taking a systematic crop by crop approach.

This book:

- Outlines the economic importance of specific nematode problems on the major food, feed and industrial crops.
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