Prioritisation of pest species for biosecurity risk assessments: using plant-parasitic nematodes and Australia as examples

Submitted by

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ABSTRACT

Prioritisation of the many potential exotic invasive species is a basic first step in biosecurity screening. This initial step is essential because there are too many species to assess each in detail, and many factors influencing the risks from each. Systematic and transparent methods for prioritisation are required by the World Trade Organization Sanitary & Phytosanitary Agreement (SPS), the overall international governing agreement for biosecurity procedures. Systematic methods are especially important for plant pests because data are often sparse, or variable in quality. Currently, there are no systematic methods for preliminary assessment of risks from plant pests and diseases (as opposed to weeds and animal diseases). This thesis reports studies in developing and testing systematic methods for prioritising the biosecurity risks. Plant-parasitic nematode (PPN) species are used as the exemplar because they have many of the characteristics of both larger insect pests and smaller microbial pathogens, like great diversity, enormous abundance and a wide range of ecological niches, transport pathways and effects on plants. Australia is used as the exemplar geographic location because it is a single political entity which is also well-defined, being an island, and has a wide range of crops and bioclimatic regions.

The biological characteristics, mechanisms for adapting to new environments and pathways for introduction or spread were initially evaluated for all PPN currently regarded as invasive to identify the most important contributors to their invasion potential. Few PPN species are well investigated as invasive species, but several characteristics were identified as common to invasive PPN. These included: adaptations allowing human mediated dispersal; multiple potential entry pathways; microscopic size; large number of propagules; high fecundity; many or cosmopolitan hosts; short life cycles; ability to survive harsh or unfavourable conditions; ability to vary sex ratios; and ability to overcome host plant resistance. Several major knowledge gaps were identified in assessing invasion potential. These were: lack of information on species biogeography; lag times from arrival to recognition as invasive; interactions among PPN or with other organisms leading to competition, co-existence or persistence; impacts on natural ecosystems; and the role of genetic variability in invasion success. These results are used in the development of a multi-criteria scheme for evaluating biosecurity risks in the third stage of this study (the Pest Screening and Targeting or PeST framework).
Initially, all scientifically named PPN species were reviewed and 250 PPN species from 43 genera were selected for further analysis, based on the explicit criteria of: association with economically important crop hosts; ability to act as vectors of viruses or to form disease complexes with other pathogens; and recognition in phytosanitary regulations of any country in the world. Scientific data on these 250 species was consolidated and is presented as a table, together with additional information on the crop hosts and yield losses. This comprehensive dataset provides the basic information for the later stages of this study.

The worldwide distributions of the 250 PPN species were ascertained, and then quantitatively modelled using a self-organising map (SOM) to cluster regions with similar species assemblages and rank species based on their likelihoods of establishment in Australia. Australia as a whole was analysed, together with each of the states and territories separately, and the risks from the different PPN species varied between jurisdictions according to differences in climates and cropping history. The risks identified were consistent with the known biology and ecology of the species. Altogether 18 countries spanning Asia, Africa, North and Central America, Europe and the Pacific had very similar PPN species assemblages to somewhere in Australia. A total of 97 exotic PPN not currently in Australia had high likelihoods of establishing somewhere in Australia. Some of the species identified as high risk are already on high-priority pest lists for quarantine, but some had not been previously identified as risks. The species identified as risks came from many different countries and parts of the world. These results suggest that further investigation of these species is warranted, using criteria other than species distributions, and this is done in subsequent chapters.

The PeST framework was developed to integrate heterogeneous information and data on species biogeography, biotic and abiotic factors into an overall risk index. The framework is based on expert opinion obtained from a survey of expert nematologists and the criteria they use to identify biosecurity risks, plus the review of the characteristics of invasive nematodes and other organisms presented in Chapter 2. The PeST framework evaluates semi-quantitatively information on the host range, pathogenicity, emerging pest status, survival adaptations, pathways, pathotypes, disease complexes, species identification, and uncertainty based on
knowledge base of a species. A weighted averaging method is used to combine the scores from the multi-criteria evaluation with the species establishment likelihoods from the SOM analysis. The PeST framework was used to evaluate the 97 PPN species and to rank them based on the overall risk index. Of the ten criteria used in the PeST framework, emerging pest status, pathogenicity, host range and the SOM index (based on species biogeography) most strongly influenced overall risk. The species identified as greatest risks included both currently-recognised and previously unrecognised species. The former included *Heterodera zae*, *Meloidogyne graminicola*, *M. enterolobii*, *M. chitwoodi* and *Scutellonema bradys*, while the latter included *Bursaphelenchus xylophilus*, *Ditylenchus destructor*, *Globodera pallida*, *Heterodera glycines* and *H. filipjevi*. By explicitly evaluating uncertainty, and comparing with the risk index, species where further research to fill in knowledge gaps would be most beneficial were identified.

Three species were chosen for CLIMEX modelling to cross-validate their biosecurity risks to Australia. Two of the species—*Heterodera zae* and *Meloidogyne graminicola*—were the highest risks identified by the PeST framework, and the other—*Hirschmanniella oryzae*—had the highest SOM index but ranked only 20th using the PeST framework. The models were carefully parameterised with species phenology and global distributions obtained from peer reviewed literature. Rain-fed and irrigation scenarios were included to simulate possible different field conditions. The projected species distributions from CLIMEX were concordant with all available experimental and field observations with only a few exceptions. All three species had high eco-climatic and growth index values in Australia (>30), suggesting the biosecurity risks identified by other criteria are real. Maps of host crops (maize, rice, wheat) and irrigation areas in Australia were compared with projected growth index maps of each nematode species to identify areas at risk. Much greater areas were conducive for the growth of the three species under irrigation than under rain-fed conditions, both globally and in Australia. These results show that modified conditions such as irrigation need to be taken into consideration when assessing the establishment potential and biosecurity risks from exotic species.

This study has developed and tested a systematic framework and analytical methods for use on a large number of potential invasive pest species to rank species biosecurity risks to any country or jurisdiction. The process is transparent, open to peer review and explicitly takes into
consideration heterogeneous information and uncertainty, when estimating the biosecurity risks from a species. The methods and the process outlined in this study could be used by biosecurity agencies worldwide to complement methods already in use for gathering information and analysing data for future biosecurity risk assessments on both nematodes and other taxonomic groups. In addition, the datasets on distribution and main hosts of 250 PPN species of potential phytosanitary importance, with periodic updating, may be valuable for nematologists worldwide in assessing the biosecurity risks from PPN.
PREFACE

Chapters 2, 3, 4, 5 and 6 of this thesis are in the format of scientific papers and have either been published, or under review for publication under the following titles. These chapters have been presented in a consistent layout rather than the layout used by the journals to reflect the inter-relatedness and holistic body of work. The text is exactly that as published or under review by the journals, and only the formatting has been made consistent; i.e. cross references have been added in square brackets where citations are to other publications or supplementary information in this thesis and the figure, table and heading numbers have had thesis chapter numbers added for clarity. The in text citations and references for Chapters 2, 3, 4, 5 and 6 are in the format specified by the journals. The references had to be formatted idiosyncratically for each journal, using different numbering and abbreviations, and so could not be made consistent easily.

The multidisciplinary nature of the work required collaboration with multiple co-authors. The contributions of each author for each chapter were as follows:


**Singh SK:** formulated questions for review, carried out literature search, analysed literature, synthesised concepts and wrote the manuscript.

**Hodda M:** contributed to study design, provided comments and suggestions on the manuscript and contributed to revision of the manuscript.

**Ash GJ:** provided comments and suggestions for revision of the manuscript.

**Banks NC:** provided comments and suggestions for revision of the manuscript.


**Singh SK:** formulated the study, carried out literature search, created database on nematodes of potential phytosanitary importance, hosts, yield loss and regulatory status, analysed data and wrote the manuscript.

**Hodda M:** contributed to study design, provided access to database on nematode synonyms, and provided comments and suggestions for revision of the manuscript.

**Ash GJ:** provided comments and suggestions for revision of the manuscript.

**Singh SK**: designed the study, created and populated database on the worldwide distribution of 250 plant parasitic nematode species selected in Chapter 3, analysed data and wrote the manuscript.

**Paini DR**: contributed to study design, implemented Self Organising Map (SOM) algorithms in MATLAB, provided comments and suggestions for revision of the manuscript.

**Ash GJ**: provided comments and suggestions for revision of the manuscript.

**Hodda M**: contributed to study design, provided comments and suggestions for revision of the manuscript.


**Singh SK**: designed the study, gathered data, carried out expert opinion survey and multi-criteria evaluation, analysed data and wrote the manuscript.

**Ash GJ**: provided comments and suggestions for revision of the manuscript.

**Hodda M**: contributed to study design, provided names of experts for expert opinion survey and gave comments and suggestions for revision of the manuscript.


**Singh SK**: designed the study, gathered and geo-referenced data on species distributions, parameterised CLIMEX models, analysed data and wrote the manuscript.

**Kriticos DJ**: contributed to study design, provided CLIMEX software and critically evaluated the models.

**Ash GJ**: provided comments and suggestions for revision of the manuscript.

**Hodda M**: contributed to study design, provided comments and suggestions for revision of the manuscript.
We the co-authors, give consent for Sunil Kumar Singh to present these papers in his thesis for examination towards the degree of Doctor of Philosophy.

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Ash GJ
Paini DR

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Certificate of Authorship

I hereby declare that this submission is my own work and to the best of my knowledge and belief, understand that it contains no material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged. I agree that this thesis be accessible for the purpose of study and research in accordance with normal conditions established by the Executive Director, Library Services, Charles Sturt University or nominee, for the care, loan and reproduction of thesis, subject to confidentiality provisions as approved by the University.

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General introduction
1.1 Introduction

Biosecurity is defined as “a strategic and integrated approach to analysing and managing relevant risks to human, animal and plant life and health and associated risks to the environment” (FAO 2007a). Biosecurity measures have been recognised as increasingly important to protect countries from the exotic pests and diseases which may be associated with the increasing amounts, range and speed of people and all sorts of commodities moving about the world (Mumford 2002; Tatem 2009). These pests and diseases threaten agricultural production, the environment and human health (FAO 2007a).

Dealing with the threats to agricultural production from plant pests and diseases is an important part of assuring food security, which is one of the major challenges of the 21st century (Strange & Scott 2005; van der Graaff & Khoury 2010; Cook et al. 2011; IUCN 2012). Plant pests and diseases can both reduce production and constrain increases in crop production. In coming decades, increasing demand for food from a growing world population will require crop production to increase, become more efficient and intensify. Greater trade in agricultural commodities is also likely (IFPRI 2002). All these trends mean that biosecurity measures and more effective management of plant pathogens assume even more importance as the potential for pests to spread and cause major damage is increased.

Many of the existing economic plant pathogens in many countries are introduced species which have become invasive. Invasive species account for an estimated 82% of plant pathogens in Australia, 65% in the USA, 74% in the UK, 85% in South Africa, 74% in India and 75% in Brazil (Pimentel et al. 2001). They include most of the most damaging pathogens of the major crops, such as wheat, rice, potatoes, maize and soybeans (Oerke 2006). With these sorts of statistics it is no surprise that economic costs and environmental impacts from existing invasive species are huge (estimated at $120 billion/year for the USA: Pimentel, Zuniga & Morrison 2005). The need for biosecurity measures to protect against future biological invasions has thus been widely recognised (FAO 2007a; IUCN 2012).

Biosecurity agencies face ongoing and growing challenges in preventing the entry of exotic plant pathogens and other invasive species because of several factors. The volume of trade and the diversity of goods traded have increased and with it the chances of exotic species arriving and establishing (Waage & Mumford 2008; Westphal et al. 2008; Dehnen-Schmutz et al.)
Increasing economic development has created new trading opportunities and has been linked to the increased rate of exotic species introductions and establishment (Mumford 2002; Perrings et al. 2005; Lin et al. 2007). The speed with which goods and people can move around the globe has also increased with better and faster transport networks which facilitate the transport of exotic organisms to places outside of their native range (Tatem, Rogers & Hay 2006; Tatem 2009; Keller et al. 2011; Paini & Yemshanov 2012; Seebens, Gastner & Blasius 2013). Changing land use (e.g. clearing new land for farming, growing crops under irrigation), intensification of agriculture (growing more crops or new crops on same area of land) and climate change may also create opportunities for exotic species to enter, establish, spread and cause negative impacts (Walther et al. 2009; Keating et al. 2010; Mainka & Howard 2010).

Historically, countries have had quarantine regulations to prevent the entry of pests and diseases since the early nineteenth century (Ebbels 2003; Devorshak 2012). The quarantine measures, methods, terminology and policies have evolved over the years, and continue to do so with technological improvements and with increasing knowledge about pests and diseases (Hockland et al. 2013; Devorshak, 2012). More recently, the term “biosecurity” has been adopted internationally to describe the methods and the processes in assessing and managing the risks to plants, human health and the environment (FAO 2007a).

Biosecurity risk assessments are a vital first step in understanding and mitigating the risks from species which may enter, establish and become invasive in future (Hayes 2003; Champion, Clayton & Hofstra 2010; Koch et al. 2011). There is a formal regulatory structure for biosecurity risk assessments for plant pests. International standards and guidelines for risk assessment are promulgated by the Food and Agriculture Organization (FAO 2011) under the International Plant Protection Convention (IPPC) and International Standards for Phytosanitary Measures (ISPM). These standards and guidelines are mandated through World Trade Organization Sanitary and Phytosanitary (WTO SPS) agreement (World Trade Organization, no date). Under the IPPC, the risks posed by any organism that is directly or indirectly injurious to cultivated or uncultivated plants can be assessed and managed (FAO 2007b). The IPPC is legally recognised by the World Trade Organization, but phytosanitary measures can only be enacted if they can be justified by science-based risk assessment (World Trade Organization, no date). The scientific basis for the risk assessments is required to justify that any phytosanitary measures taken do not constitute unnecessary barriers to trade.
Risk assessments are used for two main purposes. The first is to inform biosecurity policies, such as what should be on lists of prohibited items or what incursion management plans are needed. The second is to prioritise limited resources, for example on the development and implementation of detection, diagnostic and sampling methods (Leung et al. 2002; Lodge et al. 2006; Keller, Lodge & Finnoff 2007; Leung et al. 2012). Pest risk assessments could also be very useful in providing a focus for future research.

A full analysis and assessment of biosecurity risks from any species is a complex task, involving multiple stages, many different methods and multiple stakeholders (e.g. government, national plant protection organizations, academia, industry, farmers and general public) (FAO 2007a; Devorshak 2012). There are huge numbers – thousands, if not millions – of species which could, and perhaps should, be assessed. But it is not feasible to carry out detailed evaluations on every potential pest species because of the complexity of full pest risk assessments. Species must be prioritised. And this prioritisation, like a full assessment, must be based on science.

In practice, species are often prioritized for biosecurity risk assessments based on just a few criteria, and are not always based on the soundest science available. Currently species are often selected for risk assessments without using any systematic criteria and is often based on expert opinion which could be biased to particular species or groups depending on expertise available. Expert opinions are frequently used, or else the identification or perceptions of particular pathways as being high risk. The interception of a pest can change its priority. Priorities can be based on incursions of a species elsewhere, or with the emergence of new scientific information, or as a result of revisions of biosecurity policy (FAO-ISPM-11 2013).

Reliable information on which to base risk assessments is very sparse. Data on pathways and interceptions records are not available for many species, which is a major limitation in the implementation of biosecurity measures (Hulme et al. 2008; Evans 2010; Chapple et al. 2013). More importantly, the absence of information on pathways and interceptions does not mean that pathways and movements are really absent, merely undetected. The lack of detection may be itself a result of low surveillance as a result of a low priority assigned to a species. The low priority may arise from the lack of detections, making the process logically rather circular.
The papers forming this thesis report studies on methods for performing the best science-based preliminary risk assessments on the broadest range of species possible. The methods can be used to prioritise species for very detailed risk assessments and conform to the international standards agreed in IPPC. They use a broad range of data and are robust where reliable data are sparse, as is typical for many of the biosecurity threats to plants. They have also been designed to be useful in developing biosecurity policies aimed at improving detection, interception, incursion responses and general preparedness.

The methods were developed, tested and applied using plant-parasitic nematodes (PPN) as the exemplar organisms. PPN are major pathogens of plants, causing estimated losses of $157 billion per year globally (Abad et al. 2008). More than that, PPN are representative of the many other cryptic and microscopic pests of plants. Such cryptic microorganisms include many damaging and economic pathogens of major food crops, and pose particular biosecurity threats because they are difficult to detect and can move by many potential pathways (Roques & Auger-Rozenberg 2006; McNeill et al. 2011; Bacon, Bacher & Aebi 2012).

Any prioritisation of pest species is also specific to a country. Species environmental preferences, distributions and other locality specific information should be considered if possible. For these studies, Australia is used as the exemplar geographical location. It is clearly defined in extent and is separated from other land masses. It has a wide range of climatic conditions, a diverse range of crops and does not have many pests. Thus numerous pest species currently not present in Australia could pose a biosecurity risk. Furthermore, Australia has well documented nematological knowledge base, and is the country in which the author was based while studying, making data acquisition easiest.

1.2 Aims of the study

The overall aim of the study is to develop and test methods for performing the best science-based preliminary risk assessments suitable for application to the broadest range of plant-pathogenic species and phytosanitary situations.

Specific aims are:

- to review what is known about PPN as invasive species, to identify gaps, and to identify the characteristics contributing to invasiveness;
• to collect all reliable data on all PPN fulfilling the broadest criteria for phytosanitary concern, including the characteristics identified above;
• to estimate the likelihood of establishment of all species of concern (above);
• to develop a scientific framework for evaluating the biosecurity risks from multiple species using the characteristics identified plus expert opinion; and
• to cross-validate the framework using detailed spatial and climatic analysis of the species with the highest biosecurity risks.

1.3 Thesis outline
Chapter 1 presents a general introduction of the biosecurity context, the need for pest prioritisation and the rationale of this thesis. It does not incorporate an extensive literature review because the published papers forming the later chapters necessarily incorporate both brief reviews and discussion of the relevant literature. Repetition of the material in the later chapters is eschewed.

The literature on PPN as invasive species is reviewed in Chapter 2. Since PPN had not been extensively investigated as invasive species prior to this study, the review is different from a conventional literature review and required a synthesis of concepts from invasion biology and evaluation of the characteristics of PPN using a general framework for assessing invasive species. The literature on risk prioritisation and biosecurity risk assessment relevant to the objectives of each paper is reviewed and discussed in the introduction and discussion of Chapters 3-6 in this thesis. Literature related to the general conclusions and discussion of the study is reviewed and discussed in Chapter 7.

The prediction of future invaders at the heart of biosecurity risk assessment requires an understanding of the characteristics of invasive species and the stages leading up to successful invasion. Thus in Chapter 2, PPN are evaluated as invasive species using the propagule pressure, abiotic and biotic factors (PAB) framework. The characteristics of invasive PPN are compared with those of other invasive species. The pathways for introduction and spread of PPN are reviewed. The lack of information and uncertainty on PPN are identified and the biosecurity implications are discussed.

Following the identification of characteristics of invasive PPN, in Chapter 3, PPN species of potential phytosanitary importance globally were systematically selected using broad criteria for
further evaluation. PPN species representative of a wide range of biosecurity risks including well known regulated species and lesser known species were included on the list. Information on the main crop hosts, yield losses, association with disease complex and the regulatory status was gathered for 250 species. This is the first time such a comprehensive dataset has been assembled for PPN, and it forms the basis of many subsequent analyses.

The methods for prioritising a large number of species based on their biosecurity risks to a country are investigated in Chapter 4. The likelihood for establishment of a species in a country is a good preliminary indicator of potential biosecurity risks. The likelihood of establishment of a particular species in a region can be estimated by comparing species assemblages in different countries if one assumes that the areas with similar species assemblages also have broadly similar niches (Worner & Gevrey 2006; Wisz et al. 2013). The need to analyse species distributions led to the compilation of a database on the global distributions of the 250 PPN. These distributions are analysed using a self organising map (SOM) to rank the likelihoods of species establishment in Australia and each of the states and territories within Australia. These likelihoods are based on the similarity of the species assemblages, and allow likely sources of incursions to be identified. Differences in risks to the different states and territories are also identified. Using examples, the use of the SOM analytical technique in prioritising species for biosecurity risk assessments and its strengths and limitations are discussed.

Chapter 5 describes the PeST framework - an integrated framework developed for screening and targeting species for detailed biosecurity risk assessment. The framework was developed based on an expert opinion survey of the criteria experts use to identify biosecurity risks, plus the literature review in Chapter 2. The framework is applied to screen 97 PPN species selected as most likely to establish in Australia based on the SOM analysis presented in Chapter 4. The PeST framework evaluates semi-quantitatively information on the host range, pathogenicity, emerging pest status, survival adaptations, pathways, pathotypes, disease complexes, species identification, and uncertainty based on knowledge base of a species. A weighted averaging method is used to combine the scores from the multi-criteria evaluation with the species establishment likelihoods from the SOM analysis. Statistical tests were used to determine the correlations of the criteria to the overall risk from PPN species. Features of the PeST framework are compared with the frameworks developed for weeds and fish, which incorporate very
different criteria based on the different characteristics of those organisms and their biosecurity risks. Future applications of PeST framework for species prioritisation are discussed.

In Chapter 6, the risks to Australia for 3 PPN species identified in the previous analyses (Chapters 3, 4 and 5) are cross validated using CLIMEX models. *Heterodera zea* and *Meloidogyne graminicola*, ranked as the greatest risks using the PeST framework were selected. The third species (*Hirschmanniella oryzae*) was included for comparison because it had a lower ranking using the PeST framework but was ranked highest by the SOM analysis (Chapter 4). The potential distribution of the 3 species in Australia based on eco-climatic suitability and potential for growth are spatially represented on maps and compared with host crop and irrigation areas. Field observations and experimental data on the species biology and ecology from literature are then compared with the model projections. Based on these comparisons, the usefulness of CLIMEX projections for practical biosecurity decision making are discussed.

Chapter 7 presents an overview of the prioritisation process and compares the strengths and limitations of the methods used. The potential application of the prioritisation process for other pest groups is discussed and potential avenues for future research are recommended.

1.4 Definitions of commonly used terms
Terms from the ISPM glossary and from invasion biology are used in the thesis because of the interdisciplinary nature of the thesis.

**Alien:** an introduced species which is not native to a particular country.

**Biosecurity:** a strategic and integrated approach to analysing and managing relevant risks to human, animal and plant life and health and associated risks to the environment (FAO 2007a).

**Exotic:** species not native to a particular country or ecosystem (FAO-ISPM-5 2012) and not present in a country.

**Interception:** the detection of a pest during inspection or testing of an imported consignment (FAO-ISPM-5 2012).

**Introduced species:** species not native to a particular country or ecosystem and is present in a country (Simberloff & Rejmánek 2011).

**Introduction (of a species or pest):** “release of a species outside of its native range, either accidentally or deliberately” (Simberloff & Rejmánek 2011).
**Invasive species:** an introduced species that produces reproductive offspring, often in very large numbers, and that spread over large areas causing environmental or economic damage (Simberloff & Rejmánek 2011).

**Native species:** a species occurring naturally in an area, whose presence does not result from human activity (Simberloff & Rejmánek 2011).

**Pathway:** any means that allows the entry or spread of a pest (FAO-ISPM-5 2012).

**Pest:** “Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products. Note: In the IPPC, plant pest is sometimes used for the term pest [FAO, 1990; revised FAO, 1995; IPPC, 1997; revised CPM, 2012]” (FAO-ISPM-5 2012).

**Pest risk analysis:** “the process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it” (FAO-ISPM-5 2012).

**Phytosanitary measure:** “any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of quarantine pests or to limit the economic impact of regulated non-quarantine pests” (FAO-ISPM-5 2012).

**Propagule Pressure:** a measure of the number of viable individuals of a species arriving in a region to which they are not native and the number of introduction episodes (Simberloff & Rejmánek 2011).

**Risk:** “the probability of introduction and spread of a pest and the magnitude of the associated potential economic consequences” (FAO-ISPM-5 2012).

**Threat:** a pest species which is likely to establish and have economic consequences. The evaluations of “likely” and “economic consequences” vary with the context, and so there is no universal definition for this term.

### 1.5 Acronyms

- **CABI** Centre for Agriculture and Bioscience International
- **EPPO** European Plant Protection Organization
- **FAO** Food and Agriculture Organization
- **IFPRI** International Food Policy Research Institute
- **IPPC** International Plant Protection Convention
- **ISPM** International Standards for Phytosanitary Measures
- **IUCN** International Union for Conservation of Nature
- **WTO** World Trade Organization
1.6 References


2

Plant-parasitic nematodes as invasive species: characteristics, uncertainty and biosecurity implications

Sunil K. Singh, Mike Hodda, Gavin J. Ash and Natalie C. Banks

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2.1 Abstract
Few species of plant-parasitic nematodes (PPN) are currently recognised as invasive but this is largely because of insufficient investigation. We compared the characteristics of PPN with those of invasive species generally, using the propagule pressure, abiotic and biotic factors (PAB) framework. Most PPN have many of the characteristics of invasive species and hence have the potential to become invasive. The most common characteristics include: adaptations allowing human mediated dispersal; multiple entry pathways; microscopic size; large number of propagules; high fecundity; many or cosmopolitan hosts; short life cycle; ability to survive harsh or unfavourable conditions; ability to vary sex ratios; and ability to overcome host plant resistance. Information is lacking for many characteristics of many species and their impacts remain unquantified, which leaves some important unanswered questions and challenges for assessing PPN as invasive species. However many economically important PPN species have not been recognised as invasive, even when most of the known characteristics and data suggests they should be.

Keywords: propagule-pressure, abiotic, biotic, survival, adaptation, dispersal

2.2 Introduction
Only some species are regarded as invasive (Williamson, 1993; Williamson and Fitter, 1996). Most of these are macroscopic. Very few are microscopic (Litchman, 2010). The question is: are microscopic organisms really less invasive than macroscopic or just less recognised? This paper addresses this question for a group of microscopic organisms (plant-parasitic nematodes: PPN) which are representative in many ways of other microscopic organisms. We consider the specific characteristics normally associated with invasive species and whether PPN have any or all of these characteristics. For the few PPN known as invasives, we examine whether they share common characteristics. As a result of examining the data on invasiveness we identified several areas where a lack of data has led to notable uncertainty.

Invasive species are defined by botanists, zoologists, microbiologists, ecologists and conservationists in many different ways (Litchman, 2010; Ricciardi and Cohen, 2007; Richardson et al., 2000; Simberloff and Rejmánek, 2011; Valery et al., 2008; Hulme, 2003). However, central to all definitions is that reproductive populations establish and spread to areas beyond their native range and then cause a negative impact on the native species or ecosystem. The definitions of invasive species often do not clearly specify the rates of spread or the impacts
required to be classed as invasive, thus there are differences in classification as invasive depending on the organism. For instance, the rates of spread for plants to be classed as invasive (Richardson et al., 2000) can be very different to that for marine or terrestrial invertebrates, which have different mechanisms and rates of dispersal. In addition, the impacts of some invasive species (e.g. weeds, rabbits) are more easily measured than those of invasive microorganisms (e.g. nematodes, bacteria) whose impacts, although important, are difficult to measure (Klironomos, 2002; Litchman, 2010; Simberloff, 2011; van der Putten et al., 2007). Consequently taxa that are easily observed, counted and reported are better represented in the literature on invasive species while invasive microorganisms are not well represented.

Nematodes are mostly microscopic in size and hence their invasiveness is poorly studied and the risks they pose are not well known. Nematodes are a diverse group of organisms which inhabit a wide range of habitats and can greatly affect functioning of ecosystems (Yeates et al., 2009). Nematodes include free-living forms, bacterial feeders, fungal feeders, plant parasites and animal parasites. There are approximately 25,000 described nematode species (Hodda, 2011) but given their wide range of habitats and hosts, nematode diversity is likely to be underestimated by a factor of hundreds (Blaxter, 2011; Hodda et al., 2009). As a result, nematodes have remained largely uninvestigated as invasive species, despite their influence on ecosystem services (Bongers, 1990; Eisenhauer et al., 2011; Ferris, 2010; Neher, 2010; Wilson and Kakouli-Duarte, 2009; Yeates, 2003).

Of all the nematodes recorded, most of the information available is on parasites because of their importance as agricultural pests (Neher, 2010), as pathogens of vertebrates including humans (Hoberg, 2010; Taraschewski, 2006) or as biocontrol agents (Dillon et al., 2008), even though these groups represent less than half of the known nematode species (Hodda et al., 2009). Vertebrate nematode parasites are better studied as invasive species than plant parasites (Hoberg, 2010; Taraschewski, 2006). Knowledge of plant-parasitic nematodes is lagging even for species which are known to cause economically significant yield losses to important food, forest and fibre crops. Studying the species characteristics and processes which lead to invasions by PPN may provide insights into the how and why of biological invasions and contribute towards strengthening measures to prevent future invasions.
The aims of the paper are: to review what is known about PPN as invasive species, to identify gaps, and to identify the characteristics contributing to invasiveness. We review PPN as invasive species, mainly in agro-ecosystems and consider their potential as invasive species in relation to their characteristics and biological adaptations as plant parasites. The series of steps in biological invasions can be broken down using the PAB framework: where P = Propagule pressure, based on size and frequency of introductions; A= Abiotic factors, incorporating ecosystem invasibility based on physical conditions and B= Biotic factors, including the characteristics of invading species, recipient community and their interactions (Catford et al., 2009). Using this framework, we assess the characteristics of thirty-eight suspected invasive PPN species from fourteen genera listed on the CABI invasive species compendium (CABI, 2011) (Table 2.1). We examine potential invasion pathways and mechanisms of spread and discuss the biotic and abiotic characteristics that could contribute to the survival and persistence of PPN. The uncertainty in studying invasive PPN and biosecurity implications are discussed. We conclude by summarising the most important characteristics of invasive PPN.

2.3 Biogeography of plant-parasitic nematodes

There are two competing hypotheses on the biogeography of microorganisms, which include many of the PPN: Baas Becking’s hypothesis ‘everything is everywhere but the environment selects’ and Foissner’s ‘moderate endemcity model’ which suggests that many microorganisms have restricted distributions (Fontaneto and Brodie, 2011). Microbial species have been shown to have geographic patterns in their distributions with many species having clearly restricted ranges similar to macroorganisms (Litchman, 2010; Martin et al., 2006; Telford et al., 2006; Whitaker et al., 2003). Similarly in the case of nematodes, most evidence supports restricted distributions of species (Ferris and Ferris, 1985; Ferris et al., 1976; Hominick et al., 1996; Monroy et al., 2012; Powers et al., 2009; Procter, 1984).

On a local scale, PPN distributions are influenced by soil edaphic factors (such as soil pH, organic matter composition, soil type and soil moisture), and host availability (Norton, 1978; Norton and Hoffmann, 1974; Robertson and Freckman, 1995). On larger scales, climatic conditions have some effect, but an understanding of the biogeographic patterns of nematode distributions on these scales is still fragmentary (Coomans, 2002; Neher, 2010). Some effort has been made to understand the biogeography of economically important quarantine PPN species.
<table>
<thead>
<tr>
<th>Species</th>
<th>Host range *</th>
<th>Main hosts</th>
<th>Distribution</th>
<th>Feeding habit</th>
<th>Mode of reproduction</th>
<th>Duration of Life cycle</th>
<th>Survival adaptations</th>
<th>Dispersal</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aphelenchoides arachidis</em></td>
<td>Omniphagous</td>
<td>Groundnut</td>
<td>Africa</td>
<td>Migratory endoparasite or ectoparasite</td>
<td>—</td>
<td>—</td>
<td>Anhydrobiosis</td>
<td>Seeds</td>
</tr>
<tr>
<td><em>Aphelenchoides besseyi</em></td>
<td>Polyphagous</td>
<td>Rice, strawberry</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Migratory ectoparasite or endoparasite</td>
<td>Amphilictic and Parthenogenetic</td>
<td>10-12 days at 30ºC</td>
<td>Anhydrobiosis</td>
<td>Seeds</td>
</tr>
<tr>
<td><em>Aphelenchoides fragariae</em></td>
<td>Polyphagous</td>
<td>Strawberry</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Migratory ectoparasite or endoparasite</td>
<td>Bisexual and Amphilictic</td>
<td>Life cycle 10-11 days at 18ºC</td>
<td>Anhydrobiosis</td>
<td>Bulbs, cut flowers and propagative materials</td>
</tr>
<tr>
<td><em>Aphelenchoides ritzemabosi</em></td>
<td>Polyphagous</td>
<td>Strawberry, chrysanthenum</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Migratory ectoparasite or endoparasite</td>
<td>Bisexual</td>
<td>10-13 days</td>
<td>Anhydrobiosis</td>
<td>Bulbs, cut flowers and propagative material</td>
</tr>
<tr>
<td><em>Belonolaimus longicaudatus</em></td>
<td>Polyphagous</td>
<td>Cotton, turf, carrots, maize</td>
<td>Asia, North America, Central America, Caribbean</td>
<td>Migratory ectoparasite</td>
<td>Amphilictic</td>
<td>28 days at 30ºC</td>
<td>—</td>
<td>Propagative material, turf, soil contaminant</td>
</tr>
<tr>
<td><em>Bursaphelenchus xylophilus</em></td>
<td>Omniphagous</td>
<td>Pinus spp.</td>
<td>Asia, Africa, North America, Europe</td>
<td>Migratory endoparasite</td>
<td>Amphilictic</td>
<td>12 days at 15 ºC</td>
<td>Dauer stage juveniles adapted for dispersal</td>
<td>Beetle vector, wood</td>
</tr>
<tr>
<td><em>Bursaphelenchus cocophilus</em></td>
<td>Omniphagous</td>
<td>Coconut, palm trees</td>
<td>North America, Central America, South America</td>
<td>Migratory endoparasite</td>
<td>—</td>
<td>9-10 days</td>
<td>Dauer stage juveniles adapted for dispersal</td>
<td>Beetle vector, wood</td>
</tr>
<tr>
<td><em>Ditylenchus africanus</em></td>
<td>Polyphagous</td>
<td>Groundnut</td>
<td>Africa</td>
<td>Migratory ectoparasite and endoparasite</td>
<td>—</td>
<td>6-7 days at 28ºC</td>
<td>Anhydrobiosis</td>
<td>Groundnut seeds</td>
</tr>
<tr>
<td><em>Ditylenchus angustus</em></td>
<td>Omniphagous</td>
<td>Rice</td>
<td>Asia</td>
<td>Migratory ectoparasite and endoparasite</td>
<td>—</td>
<td>10-20 days at 30ºC</td>
<td>Anhydrobiosis</td>
<td>Seeds and or infected planting materials</td>
</tr>
<tr>
<td><em>Ditylenchus destructor</em></td>
<td>Polyphagous</td>
<td>Potato, bulbs, ornamentals</td>
<td>Asia, Africa, North America, South America, Europe, Oceania</td>
<td>Migratory endoparasite</td>
<td>—</td>
<td>6-7 days at 28ºC</td>
<td>Coiling and clumping.</td>
<td>Seeds and propagative materials</td>
</tr>
<tr>
<td>Genus</td>
<td>Type of Nematode</td>
<td>Hosts</td>
<td>Geographic Range</td>
<td>Life Cycle</td>
<td>Propagation Method</td>
<td>Notes</td>
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<tr>
<td><em>Ditylenchus dipsaci</em></td>
<td>Polyphagous</td>
<td>Onions, garlic, cereals, legumes, ornamentals</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Migratory endoparasite</td>
<td>20 days at 15°C</td>
<td>Anhydrobiosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Globodera pallida</em></td>
<td>Omniphagous</td>
<td>Potato, tomato, aubergine</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Sedentary endoparasite</td>
<td>45 days</td>
<td>Cysts</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Globodera rostochiensis</em></td>
<td>Omniphagous</td>
<td>Potato, tomato, aubergine</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Sedentary endoparasite</td>
<td>45 days</td>
<td>Cysts</td>
<td></td>
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<tr>
<td><em>Globodera tabacum</em></td>
<td>Omniphagous</td>
<td>Tobacco</td>
<td>Asia, Africa, North America, South America, Europe</td>
<td>Sedentary endoparasite</td>
<td>32 days at 30 ºC, 36 days at 26ºC, 72 days at 20ºC</td>
<td>Cysts</td>
<td></td>
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</tr>
<tr>
<td><em>Helicotylenchus multicinctus</em></td>
<td>Polyphagous</td>
<td>Banana</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Migratory ectoparasite</td>
<td>39 days (Karakaş, 2007)</td>
<td>—</td>
<td></td>
<td></td>
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<tr>
<td><em>Heterodera cajani</em></td>
<td>Polyphagous</td>
<td>Peas, beans</td>
<td>Asia, Africa</td>
<td>Sedentary endoparasite</td>
<td>16 days at 29ºC, 45-80 days at 10-25 ºC</td>
<td>Cysts</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Heterodera ciceri</em></td>
<td>Omniphagous</td>
<td>Chickpeas, lentil</td>
<td>Asia, Europe</td>
<td>Sedentary endoparasite</td>
<td>38 days from Juvenile to cyst (Kaloshian et al., 1986)</td>
<td>Cysts</td>
<td></td>
<td></td>
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<tr>
<td><em>Heterodera glycines</em></td>
<td>Polyphagous</td>
<td>Soybean</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe</td>
<td>Sedentary endoparasite</td>
<td>22-35 days (Wang et al., 2009)</td>
<td>Cysts</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Heterodera goettingiana</em></td>
<td>Omniphagous</td>
<td>Pea, broad bean</td>
<td>Asia, Africa, North America, Europe</td>
<td>Sedentary endoparasite</td>
<td>40-55 days from juvenile to cyst (Greco et al., 1986)</td>
<td>Cysts</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hirschmanniella miticausa</em></td>
<td>—</td>
<td>Taro</td>
<td>Oceania</td>
<td>Migratory endoparasite</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Meloidogyne acronea</em></td>
<td>Polyphagous</td>
<td>Cotton</td>
<td>Africa</td>
<td>Sedentary endoparasite</td>
<td>19 days at 33ºC</td>
<td>Egg mass gelatinous matrix protects eggs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: See Table 2.3 for propagation material.
<table>
<thead>
<tr>
<th><strong>Meloidogyne arenaria</strong></th>
<th>Polyphagous</th>
<th>Many hosts</th>
<th>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</th>
<th>Sedentary endoparasite</th>
<th>Mitotic parthenogenesis</th>
<th>21 days</th>
<th>Egg mass gelatinous matrix protects eggs</th>
<th>Planting material, soil contaminant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meloidogyne chitwoodi</strong></td>
<td>Polyphagous</td>
<td>Potato, carrots, lucerne</td>
<td>Asia, Africa, North America, South America, Europe</td>
<td>Sedentary endoparasite</td>
<td>Meiotic parthenogenesis (Chitwood and Perry, 2009)</td>
<td>21-28 days (Charchar and Santo, 2008)</td>
<td>Egg mass gelatinous matrix protects eggs</td>
<td>Planting material (seed potatoes), soil contaminant</td>
</tr>
<tr>
<td><strong>Meloidogyne decalineata</strong></td>
<td>—</td>
<td>Coffee</td>
<td>Africa</td>
<td>Sedentary endoparasite</td>
<td>—</td>
<td>—</td>
<td>Egg mass gelatinous matrix protects eggs</td>
<td>Planting material, soil contaminant</td>
</tr>
<tr>
<td><strong>Meloidogyne enteroblobii</strong></td>
<td>Polyphagous</td>
<td>Bean, coffee, cucumber, guava, watermelon, bell pepper, tomato, aubergine</td>
<td>Asia, Africa, Central America, Caribbean, North America, South America, Europe</td>
<td>Sedentary endoparasite</td>
<td>Mitotic parthenogenesis (Chitwood and Perry, 2009)</td>
<td>24 days (Westerich et al., 2011)</td>
<td>Egg mass gelatinous matrix protects eggs</td>
<td>Planting material, soil contaminant</td>
</tr>
<tr>
<td><strong>Meloidogyne fallax</strong></td>
<td>Polyphagous</td>
<td>Carrots, potato, black salsify</td>
<td>Europe, Oceania</td>
<td>Sedentary endoparasite</td>
<td>Meiotic parthenogenesis (Chitwood and Perry, 2009)</td>
<td>—</td>
<td>Egg mass gelatinous matrix protects eggs</td>
<td>Planting material, soil contaminant</td>
</tr>
<tr>
<td><strong>Meloidogyne graminicola</strong></td>
<td>Polyphagous</td>
<td>Rice</td>
<td>Asia, Africa, North America, South America</td>
<td>Sedentary endoparasite</td>
<td>Meiotic parthenogenesis (Chitwood and Perry, 2009)</td>
<td>19 days at 22-29°C, 23-27 days at 26 ºC</td>
<td>Egg mass gelatinous matrix protects eggs</td>
<td>Planting material, soil contaminant</td>
</tr>
<tr>
<td><strong>Meloidogyne hapla</strong></td>
<td>Polyphagous</td>
<td>Carrots, peanut, sugarbeet, chicory, strawberry, soybean, lucerne, lettuce, tomato, potato</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Sedentary endoparasite</td>
<td>Race A - Meiotic parthenogenesis, Race B - Mitotic parthenogenesis (Chitwood and Perry, 2009)</td>
<td>35 days (Charchar and Santo, 2009)</td>
<td>Survives 0 ºC, Egg mass gelatinous matrix protects eggs</td>
<td>Planting material, soil contaminant</td>
</tr>
<tr>
<td><strong>Meloidogyne incognita</strong></td>
<td>Polyphagous</td>
<td>More than 1000 plant hosts</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Sedentary endoparasite</td>
<td>Mitotic parthenogenesis (Chitwood and Perry, 2009)</td>
<td>20-25 days</td>
<td>Egg mass gelatinous matrix protects eggs</td>
<td>Planting material, soil contaminant</td>
</tr>
<tr>
<td>Species</td>
<td>Life History</td>
<td>Hosts</td>
<td>Geographical Distribution</td>
<td>Reproductive System</td>
<td>Developmental Period(s)</td>
<td>Life Stage Characteristics</td>
<td>Planting Material, Soil Contaminant</td>
<td></td>
</tr>
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<tr>
<td><em>Meloidogyne javanica</em></td>
<td>Sedentary endoparasite</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Mitotic parthenogenesis 27 days at 28-35°C (Khan et al., 2009) Egg mass gelatinous matrix protects eggs</td>
<td></td>
<td>Planting material, soil contaminant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pratylenchus coffeae</em></td>
<td>Migratory endoparasite</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Amphimictic (Roman and Triantaphyllou, 1969) 27 days at 25-30°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pratylenchus goodeyi</em></td>
<td>Migratory endoparasite</td>
<td>Africa, Europe, Oceania</td>
<td>— 24-35 days (Prasad et al., 1999)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Radopholus citri</em></td>
<td>Migratory endoparasite</td>
<td>Asia</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td>Planting material, soil contaminant</td>
<td></td>
</tr>
<tr>
<td><em>Radopholus similis</em></td>
<td>Migratory endoparasite</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Hermaphroditic or Amphigony (Kaplan and Opperman, 2000) 20-25 days at 24-32°C</td>
<td></td>
<td>Males survive longer than females under unfavourable conditions (Chabrier et al., 2010)</td>
<td></td>
<td>Planting material (Banana suckers), soil contaminant</td>
<td></td>
</tr>
<tr>
<td><em>Scutellonema bradys</em></td>
<td>Migratory endoparasite</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America</td>
<td>—</td>
<td></td>
<td>— damage symptoms appear 8 weeks after inoculation (Coyne et al., 2011)</td>
<td></td>
<td>Planting material, yams tubers, soil contaminant</td>
<td></td>
</tr>
<tr>
<td><em>Tylenchulus semipenetrans</em></td>
<td>Semi endoparasite</td>
<td>Asia, Africa, North America, Central America, Caribbean, South America, Europe, Oceania</td>
<td>Amphimictic or meiotic parthenogenesis</td>
<td></td>
<td>— females start laying eggs 5 weeks after hatching</td>
<td></td>
<td>Planting material, soil contaminant</td>
<td></td>
</tr>
<tr>
<td><em>Xiphinema diversicaudatum</em></td>
<td>Migratory ectoparasite</td>
<td>Asia, Africa, North America, Europe, Oceania</td>
<td>—</td>
<td></td>
<td>2 years to develop from egg to adult</td>
<td></td>
<td>Planting material, soil contaminant</td>
<td></td>
</tr>
<tr>
<td><em>Xiphinema index</em></td>
<td>Migratory ectoparasite</td>
<td>Asia, Africa, North America, South America, Europe, Oceania</td>
<td>—</td>
<td></td>
<td>22-27 days at 24°C, 7-9 months at 20-23°C</td>
<td></td>
<td>Planting material, soil contaminant</td>
<td></td>
</tr>
</tbody>
</table>

* polyphagous = able to reproduce on host plants from two or more families; omniphagous = able to reproduce on hosts from one family (usually with few main hosts and alternative hosts from the same plant family); — = uncertain/information not available
such as *Globodera pallida* and *Globodera rostochiensis* (commonly known as Potato Cyst Nematodes, hereafter PCN) and *Bursaphelenchus xylophilus* (commonly known as Pine Wood Nematode, hereafter PWN). However, the origins of most PPN species are still controversial (Trudgill, 1995; Trudgill *et al.*, 1994) and determining the native range remains a major challenge. Deciphering the origins and evolutionary history of PPN is particularly difficult because there are very few fossil records. Often, detailed studies using modern phylogenetic methods and analysis of distributions and co-evolution of PPN with their host plants are required (Subbotin *et al.*, 2010).

Not knowing the native range of PPN species presents a major problem in determining their status as native or alien. Unless invading PPN species are causing noticeable economic damage, they may go undetected, as do invasions by other microorganisms (Bent and Forney, 2008). Knowledge of prior and current community composition is required to determine native or invasive status of species (Litchman, 2010). Information on nematode communities is rarely available because most of the world has not been thoroughly surveyed. Even where there are surveys, many nematodes are not identified to species level, because of dwindling taxonomic expertise (Coomans, 2002).

Even for recognised PPN, biogeographic data are limited. A large majority of PPN species are cryptogenic (obscure or unknown origin) and their status as native or introduced is not easily determined (also see (Carlton, 1996)). Studies have been completed for only two nematodes. PCN, native to the Andes in South America, were introduced to Europe with tubers imported as resistant to other diseases in the late eighteenth century (Turner and Evans, 1998) while PWN, native to North America, were introduced to Asia through trade (Nickle *et al.*, 1981). More recently, genetic studies have been used to reconstruct the invasion routes for known invasive species (Blanchet, 2012; Estoup and Guillemaud, 2010; Handley *et al.*, 2011), thus enabling better understanding of biological invasions by cryptogenic species, and these confirmed the routes of invasion for PCN into Europe (Plantard *et al.*, 2008) and PWN into Asia (Zhang *et al.*, 2008). Therefore, the occurrences of PCN in North America, Europe, Africa, Asia, Caribbean and Oceania and PWN in parts of Asia and Europe are both outside their native ranges and so represent genuine biological invasions. Based on these examples (also see Table 2.1), PPN clearly have the potential to invade other regions; however, which species become invasive and
where, are important questions which need to be addressed to better understand the invasion processes and the biosecurity risks.

2.4 Processes involved in plant-parasitic nematode invasions

Invasion is a multi-stage process (Richardson et al., 2011) with distinct stages including transport, introduction, establishment (colonization, naturalization), spread and impact (Blackburn et al., 2011; Catford et al., 2009). The success of an invasive organism depends on a combination of factors such as: the propagule pressure (e.g. introduction pathways, introduction frequency, ability to survive transit); existing biological and ecological adaptations to new ranges; ability to adapt; means to establish and spread (i.e. presence of hosts, vectors and means of dispersal); suitability of climatic conditions and habitats (Bucharova and van Kleunen, 2009; Handley et al., 2011; Lambdon et al., 2008; Lee, 2002; Lockwood et al., 2005; Meimberg et al., 2010; Miller and Ruiz, 2009; Pysek et al., 2011; Wonham et al., 2000). The PAB framework (see Introduction) is a good way to organise these factors, so in the following sections we assess the characteristics of invasive PPN using this framework.

2.5 Propagule pressure

2.5.1 Pathways

Pathways link donor and recipient regions. The pathway for introduction is important for estimating the chances of a species arriving, establishing and becoming invasive, especially in conjunction with biological and ecological characteristics. The duration and conditions during transit also affect how invasion proceeds (e.g. alien plants introduced by different pathways differed in invasion success (Pysek et al., 2011; Wilson et al., 2009)).

Many of the pathways proposed for invasive organisms generally have been reported for international movement and introduction of PPN over long distances (Table 2.2). Human assisted pathways seem most important for nematodes to spread both locally (short distance within and between farms) and internationally (with trade of produce, seeds, movement of people and machinery, see Table 2.3).

PPN cannot move long distances on their own (Timper and Davies, 2004; Wallace, 1968a). However agricultural crops are moved frequently and in large quantities, and so provide many opportunities for movement of PPN. Infected planting material, seeds or ornamental garden and pot plants are the most common means of entry reported for PPN from interception records and
Table 2.2 Potential pathways associated with the international movement and introduction of exotic PPN

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Some examples of associated nematode genus and species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil, including soil clods as contaminant with seeds, from land vehicles (e.g. agricultural machinery, construction vehicles, military vehicles, cars), associated with shipping containers, with passenger baggage, on footwear, sand, gravel, compost and organic manure and growing medium accompanying plants</td>
<td>Apelenchoides sp., Globodera sp., Globodera pallida, G. rostochiensis, G. tabacum, Helicotylenchus dihystera, Heteroderma sp., H. avenae, H. glycines, H. humuli, H. cruciferae, H. goettingiana, H. trifoli, H. zeae, Meloidogyne sp., Pratylenchus coffeae, Pratylenchus sp., Quinisaicus capitatus, Rotylenchulus reniformis, Tylencorrhynchus sp., Tylencorrhynchus crassicaudatus, T. nudus</td>
<td>(Dawabah and Al-Hazmi, 2007; Esser, 1984; Gadgil et al., 2000; Hughes et al., 2010; Lal and Lal, 2006; MAF-NewZealand, 2007; Mani, 1998; Mathur et al., 1981; McNeill et al., 2011; Mulvey, 1960; Plumas et al., 2002; Queneherve et al., 1998; Sewell, 1967; Wheeler, 1949)</td>
</tr>
<tr>
<td>Packaging wood</td>
<td>Apelenchoides spp., Bursaphelenchus spp. B. aberrans, B. arthuri, B. chenii, B. conicaudatus, B. curvicaudatus, B. douii, B. fungivorus, B. hofmanni, B. hylobianum, B. lini, B. mucronatus, B. pinasteri, B. rainulfi, B. singaporenatis, B. thailandae, B. vallesianus, B. xylophilus.</td>
<td>(Gu et al., 2006; Hongmei et al., 2008; Sousa et al., 2011; Wang et al., 2005)</td>
</tr>
<tr>
<td>Wood chips</td>
<td>Bursaphelenchus eggersi, B. fungivorus, B. leoni, B. mucronatus, B. poligraphi, B. sexdentati, B. vallesianus, B. willibaldi, B. xylophilus.</td>
<td>(Braasch, 1996; Halik and Bergdahl, 1992; Hisai et al., 2007; Panesar et al., 1994; Schonfeld et al., 2006)</td>
</tr>
<tr>
<td>Sawn timber</td>
<td>Bursaphelenchus borealis, B. fraudulentus, B. fungivorus, B. helenicus, B. hylobianum, B. leoni, B. mucronatus, B. paracorneolus, B. rainulfi, B. xylophilus.</td>
<td>(Akamovitch and Ryss, 2009; Ambrogioni et al., 2004; Braasch et al., 2001; Braasch and Enzian, 2004; Robinet et al., 2009; Sousa et al., 2011)</td>
</tr>
<tr>
<td>Bonsais, flower plants, ornamental plants</td>
<td>Meloidogyne arenaria, M. hapla, M. incognita, M. javanica, Pratylenchus sp. P. penetrans, P. coffeae, Rotylenchulus reniformis, Tylencorrhynchus crassicaudatus, T. nudus</td>
<td>(Mulvey, 1960; Queneherve et al., 1998; Roques and Auger-Rozenberg, 2006; Xiang et al., 2006)</td>
</tr>
<tr>
<td>Bulbs</td>
<td>Apelenchoides sabtenis, Ditylenchus destructor, D. dipsaci, Xiphinema insigne</td>
<td>(Hastings, 1935; Karnkowski, 1999; Lal and Lal, 2006; Plumas et al., 2002; Saigusa and Yamamoto, 1971)</td>
</tr>
<tr>
<td>Corms, rhizomes, tubers</td>
<td><em>Aphelenchoides fragariae</em>, <em>Ditylenchus destructor</em>, <em>Pratylenchus brzeskii</em>, <em>P. coffeae</em>, <em>P. danensis</em>, <em>P. penetrans</em>, <em>Scutellonema bradyi</em></td>
<td>(de la Peña et al., 2011; Lal and Rajan, 2005; Plumas et al., 2002)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------</td>
</tr>
<tr>
<td>Roots or rootstocks</td>
<td><em>Meloidogyne hapla</em>, <em>M. incognita</em>, <em>M. thailandica</em>&lt;sup&gt;a&lt;/sup&gt; <em>Heterodera humuli</em>, <em>Pratylenchus coffeae</em>, <em>P. crenatus</em>, <em>P. penetrans</em>, <em>P. vulnus</em>, <em>Tylenchorhynchus</em> sp.</td>
<td>(Handoo et al., 2005; Lal and Rajan, 2005; Mulvey, 1960)</td>
</tr>
<tr>
<td>Seedlings or nursery stock, micropropagated plants</td>
<td><em>Aphelenchoides</em> sp., <em>A. fragariae</em>, <em>Boleodorus</em> sp., <em>Criconemella curvata</em>, <em>Ditylenchus dipsaci</em>, <em>Heterodera schachtii</em>, <em>H. trifolii</em>, <em>Pratylenchus brachyurus</em>, <em>P. coffeae</em>, <em>Punctodera punctata</em>, <em>Radopholus similis</em>, <em>Scutellonema brachyurus</em>, <em>Tylenchus</em> sp.</td>
<td>(Gao and Zhang, 1994; Lal and Rajan, 2005; Mathur et al., 1986; Mulvey, 1960; Plumas et al., 2002; Silva et al., 2005; Tenente et al., 1996)</td>
</tr>
<tr>
<td>Seeds and nuts including seeds with shell</td>
<td><em>Anguina</em> sp., <em>A. tritici</em>, <em>Aphelenchoides</em> sp., <em>A. arachidis</em>, <em>A. besseyi</em>, <em>A. subtenuis</em>, <em>Aphelenchus</em> sp., <em>Bursaphelenchus</em> sp., <em>Cercomermella</em> sp., <em>Criconema</em> sp., <em>Criconemella</em> sp., <em>Ditylenchus</em> sp., <em>D. angustus</em>, <em>D. destructor</em>, <em>D. dipsaci</em>, <em>Globodera pallida</em>, <em>G. rostochiensis</em>, <em>Hemicriconemoides</em> sp., <em>Hoplolaimus</em> sp., <em>Meloidogyne</em> sp., <em>M. arenaria</em>, <em>M. chitwoodi</em>, <em>M. fallax</em>, <em>M. incognita</em>, <em>M. javanica</em>, <em>Nacobbus aberrans</em>, <em>Tylenchorhynchus</em> sp.</td>
<td>(Lal and Lal, 2006; Mendes et al., 1996; Plumas et al., 2002)</td>
</tr>
<tr>
<td>Sea swept plant debris floating long distances</td>
<td><em>Pratylenchus brzeskii</em>, <em>P. danensis</em>, <em>P. penetrans</em></td>
<td>(de la Peña et al., 2011)</td>
</tr>
<tr>
<td>Aquarium plants</td>
<td><em>Hirschmanniella caudacrena</em>, <em>Radopholus bridgei</em></td>
<td>(Ryss and Karnkowski, 2010a; Ryss and Karnkowski, 2010b)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Species names in bold are considered as invasive on the CABI invasive species compendium. Also note that many interception records are identified only to genus level because of difficulty in identifying many nematode species. The interception records presented are from many different countries. <sup>b</sup> Denotes new nematode species described from intercepted infested material.
### Table 2.3 Methods for local dispersal of PPN

<table>
<thead>
<tr>
<th>Method</th>
<th>Some examples of associated nematode genus or species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water dispersal e.g. irrigation water runoff</td>
<td><em>Aphelenchoides besseyi, A. fragariae, A. ritzemaabosi, Ditylenchus dipsaci, Heterodera, Globodera, Pratylenchus, Radopholus</em></td>
<td>(Cadet et al., 2002; Chabrier et al., 2009; Esser, 1979; Faulkner and Bolander, 1966; Hugo and Malan, 2010; Roccuzzo and Ciancio, 1991)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Baujard and Martiny, 1994; Carroll and Viglierchio, 1981; de Rooij-van der Goes et al., 1997; Gaur, 1988; Nkem et al., 2006; Orr and Newton, 1971; White, 1953)</td>
</tr>
<tr>
<td>Spread of nematodes by birds</td>
<td><em>Globodera rostochiensis</em> cyst, <em>Heterodera glycines</em> cyst, <em>Punctodera punctata</em></td>
<td>(Brodie, 1976; Epps, 1971; Green et al., 2008; Radice et al., 1984)</td>
</tr>
<tr>
<td>Spread of cysts in seed bags</td>
<td><em>Heterodera glycines</em> cyst</td>
<td>(Epps, 1968)</td>
</tr>
<tr>
<td>Nematode infested seeds, planting material, ornament and flower plants, nursery stock, or plant debris</td>
<td><em>Aphelenchoides besseyi, Ditylenchus dipsaci, Globodera pallida, G. rostochiensis, Heterodera schachtii, Helicotylenchus spp., Pratylenchus spp., Pratylenchus brzeskii, P. dunensis, P. penetrans, Trichodorus spp., Xiphinema spp.</em></td>
<td>(Atkinson and Sykes, 1981; Brown, 1957; Chamberlain, 1952; de la Peña et al., 2011; Green and Sime, 1979; McNeill et al., 2006; Triffitt, 1935; Uebayashi and Imamura, 1972)</td>
</tr>
<tr>
<td>Soil attached to tools, machinery and footwear and animals</td>
<td><em>Globodera pallida, G. rostochiensis, G. tabacum, Helicotylenchus dihystera, Heterodera sp., H. avenae, H. glycines, H. humuli, H. cruciferae, H. goettingiana, H. trifolii, H. zeae, Longidorus elongatus, Meloidogyne sp., Pratylenchus brachyurus, Pratylenchus coffeae, Quinusalcius capitatus, Rotylenchulus reniformis, Rotylenchulus robustus, Trichodorus primitivus, Tylencythynchus sp.</em></td>
<td>(Boag, 1985; Chamberlain, 1952; Esser, 1984; Esser, 1980; Mai, 1977; Mai et al., 1968; McNeill et al., 2011)</td>
</tr>
<tr>
<td>Human assisted dispersal</td>
<td><em>Bursaphelenchus xylophilus, Globodera pallida, G. rostochiensis, Ditylenchus destructor, D. dipsaci</em></td>
<td>(Banks et al., 2012; Dawabah and Al-Hazmi, 2007; Robinet et al., 2009)</td>
</tr>
<tr>
<td>Insect vector</td>
<td><em>Bursaphelenchus xylophilus, Fergusobia spp., Bursaphelenchus cocophilus</em></td>
<td>(Davies and Giblin-Davis, 2004; Robinet et al., 2009)</td>
</tr>
</tbody>
</table>
historical evidence (Bingefors, 1967; Lal and Rajan, 2005; Lehman, 1994; Mendes et al., 1996; Southey and Aitkenhead, 1972; Tenente et al., 1996) and a PPN would be extremely difficult to detect, particularly if the host plant is asymptomatic. Another common pathway for spread of PPN is as contaminants with a wide range of animate and inanimate objects (plants, parts of plants, or animals, and soiled clothing, footwear, soil with machinery, building or construction materials, respectively (Table 2.3)).

PPN have different feeding habits as described in Table 2.1 which could affect their association with the hosts and dispersal pathways. Ectoparasites remain on the surface of the plant tissues and feed by inserting their stylet into cells within their reach. Foliar ectoparasites feed on epidermal cells of young leaves, stems and flower primordial often enclosed by other foliage. Root ectoparasites such as have short stylet and feed mainly on outer root cells and root hairs. Meanwhile root ectoparasites with long stylets insert their stylet deep into the root tissues normally at the growing tip and some can become relatively immobile. Migratory endoparasites can completely penetrate the plant tissues remaining mobile and vermiform and feeding as they move through tissues, they can often migrate between soil and roots. The foliar or stem endoparasites feed in stems, leaves, flower primordial or seeds. The below ground endoparasites are found throughout different tissues in roots, corms, bulbs, tubers and seeds. Sedentary endoparasites become immobile after entering the plant tissues and develop a permanent feeding site which may appear as galls on the roots. Semi endoparasites partially penetrate the roots and become immobile in a fixed feeding site leaving the posterior half or two thirds of their body, projecting into the soil (Bridge and Starr, 2007).

PPN have many and varied adaptations for introduction and dispersal as contaminants. Some PPN form cysts, which are dead females forming a protective coat around hundreds of viable but quiescent eggs. Cysts can be transported as soil contaminants with plants which might not be their hosts (Tovar-Soto et al., 2006). Propagative materials such as seed potato tubers can have soil attached carrying PPN both parasitic on potato and not parasitic on potato. For example, seed potatoes produced in a field in rotation with wheat have been shown to act as means of dispersal for cysts of cereal cyst nematode Heterodera avenae (Dawabah and Al-Hazmi, 2007). In addition, PPN with wide host ranges have been intercepted on plants which were not reported hosts (Karnkowski and Ignatowicz, 1998). Ornamental or pot plants have
been reported as frequently spreading PPN unknowingly because live plants and potting materials conducive to PPN are involved (Table 2.3). A wide range of other pathogens including important fungal and bacterial pathogens have also been introduced with infected planting materials or seeds (Baker and Smith, 1966; Elmer, 2001; Grousset et al., 2012; Smith et al., 2007). This indicates that introduction with planting material is excellent for movement of invasive microorganisms generally.

Establishment of PPN is also favoured by being frequently transported with plant propagative material because the hosts are transported with the nematodes and so will be present to feed on in the recipient region. Furthermore, upon arrival, conditions suitable for host plants will be also suitable for the nematodes (Baker and Smith, 1966). Examples of invasive nematode species introduced and spread worldwide with planting materials include the burrowing nematode (*Radopholus similis*), seed gall nematode (*Anguina tritici*) and PCN (Table 2.2).

Once PPN have established in an area they can be dispersed locally by many methods (Table 2.3). The international pathways listed in Table 2.2 can also contribute to local spread of nematodes between farms. Estimates of natural dispersal rates range from a relatively constant average rate of 5.3 km per year for PCN (Banks et al., 2012) to about 7.5 km per year for PWN in China, mediated by the long-horn beetle (Robinet et al., 2009). Human assisted dispersal can increase the rate of spread greatly (e.g. 111-339 km for PWN in China (Robinet et al., 2009)). Many other species of PPN have adaptations allowing human transport, and therefore are likely to disperse locally by this method after introduction.

Of all the pathways listed in Table 2.2, many invasive PPN species are associated with seeds, plants, soil and bonsai and seedling nursery stock or micro-propagative materials (Figure 2.1). Similarly the most PPN genera are associated with plants, seedling nursery stock or micro-propagative materials, seeds and soil (Figure 2.1) indicating these pathways are important for spreading potentially invasive species. Multiple pathways exist for the well known invasive PPN genera and species with more pathways also tend to be more widespread (Table 2.4).
Although pathways remain important in assessing the risks from invasive species, if pathways are not known, invasive species can be easily overlooked. The lack of published interception records and skewing of the interception records due to sampling effort (sample size and sampling procedure) are major limitations of using interception data to determine the risks from potential invasive species.

### 2.5.2 Quantity and quality of propagules

In addition to pathways, frequent introductions of many and viable propagules are important in invasion (Lockwood et al., 2005; Simberloff, 2009; Wonham et al., 2000) and are also characteristics of many PPN. Even small quantities of soil, plant material or seeds can easily carry hundreds or even thousands of PPN to act as propagules because they are generally small. For example, a single gall the size of a wheat grain can contain thousands of *Anguina tritici* (Krall, 1991). Other species can be transported in similar numbers as either endoparasites, ectoparasites or as soil particle contaminants (Southey and Aitkenhead, 1972). Also, many PPN eggs or aggregates of individuals (e.g. galls of *Anguina, Ditylenchus*) may be carried by the
different pathways including with seeds, propagative materials and in soil, hence single introduction events may provide many viable propagules for establishment.

Genetic variability is often required for successful invasion and is generally available for PPN because large numbers of propagules are transported often (see above). For example in the PCN invasion of Western Europe, as much genetic variability occurred in the PCN on a single plant as in a field or even a whole region (Plantard et al., 2008). Therefore the introduction infected potato plants from outside of Europe could increase the genetic variability of European PCN populations (Hockland et al., 2012).

The number of individuals arriving also influences the chances of establishment (Drake, 2004; Drake and Lodge, 2006). Small initial populations are more likely to go extinct locally through random fluctuations in population size and difficulties in finding mates. This is known as the Allee effect (Allee, 1927; Stephens et al., 1999). The critical population density threshold required for successful invasion has yet to be quantified for PPN, but the critical population may be quite small. Parthenogenetic PPN — for example the root-knot nematode Meloidogyne incognita (Triantaphyllou and Hirschmann, 1964) — may require fewer propagules to establish successfully than sexually reproducing species — for example the PWN Bursaphelechus xylophilus (Xie et al., 2009) — which need enough individuals from both sexes to establish viable populations. Even for amphimictic species, the number of PPN required for successful invasion may be quite small. There were enough propagules in a single introduction of PWN into Portugal for successful invasion (Vieira et al., 2007).

After arrival, populations of PPN may take some time to build to sufficient numbers to make detection possible. As a result time may elapse before they reach high enough abundance to damage local plants sufficiently to be noticed (Crooks, 2005; Lewis and Kareiva, 1993). Further time may elapse before a correct identification of the causal organism is completed, which can be extended further by delays in reporting. The net result can be a considerable lag-time between arrival of a pest and its recognition as invasive (Figure 2.2a & b).
Figure 2.2a Lag times for recognition of invasive species

A theoretical representation of lag times for recognition of invasive species: $a =$ lag time between arrival and earliest possible detection, $b =$ lag time while population adapts and becomes persistent after introduction, $c =$ lag time for populations to reach damaging levels and be noticed, $d =$ lag time taken for damage to be recognised, causal organism to be identified and reported - sooner if crop is of major importance or later if crop is of minor importance, $a + b + c + d =$ total time lag between arrival and recognition as invasive.

Figure 2.2b Schematic representation of the scenarios for recognition of invasive species not yet major pests

Lag times of zero to twenty years have been estimated for PCN (Banks et al., 2012), but few other PPN have been examined. Lag times may be very different if generation times are shorter and dispersal is by different mechanisms. *Bursaphelenchus hunanensis* invasion into Australia
was apparently detected very soon after introduction because symptoms were severe and rapid (Hodda et al., 2008). This is uncommon for most nematodes because their symptoms are generally not distinctive and take some time to develop. There may be many unsuccessful introductions or successful invasions which are never reported, which may skew analysis of lag times and invasions. Lag times are therefore important in the invasion processes, so the lack of information on lag times is a major information gap.

2.6 Biotic factors

2.6.1 Characteristics influencing success of invasive plant-parasitic nematodes

The general biotic characteristics of organisms contributing to their invasion success have been widely studied (Hendrix, 2006; Labrie et al., 2006; Marchetti et al., 2004; Pysek et al., 2012; Catford et al., 2012). Whether invasive species adapt and proliferate in a new environment depends on attributes such as resource use efficiency, ability to compete with native species, flexibility in behaviour, mutual interactions and genetic interactions (e.g. hybridization) (Funk and Vitousek, 2007; Mooney and Cleland, 2001; Stratton and Goldstein, 2001). PPN have not been studied specifically, but have several of the characteristics and adaptations which may aid their survival and persistence in a new environment. Adaptations contributing to success as plant parasites can also contribute to success as invaders. In the following section we discuss the characteristics of PPN, and compare them with other known invasive species.

2.6.2 Host range or specificity

Many successful invasive species are able to survive on many hosts or have a wide range of food sources (e.g. freshwater mussel Anodonta woodiana in Europe (Douda et al., 2012), flatworm Platydemus manokwari in Pacific (Sugiura, 2010), Asian earthworm Amynthas agrestis in North America (Zhang, 2010)). Twenty-six of the thirty-eight species (68%) of PPN listed in Table 2.1 are polyphagous and able to reproduce on plant hosts from two or more families. A quarter are omniphagous (24% or nine species) and are able to reproduce on hosts from one family or have only a few main hosts. The remaining 8% (three species) have only one main host recorded or lack information. Examples of polyphagous, widespread invasive PPN include the cosmopolitan Meloidogyne arenaria, M. incognita and M. javanica. All are known to parasitise and reproduce on alternative hosts such as weeds, enabling them to survive in the absence of a main crop host (Kutywayo and Been, 2006; Shah et al., 2010; Singh et al., 2010). This may favour their establishment and spread.
Being polyphagous may assist invasion of PPN, but not be absolutely necessary. Omniphagous species, such as PWN and PCN, may be extremely successful invaders if their hosts are cosmopolitan (pine trees and potato, respectively). Thus, there is still a gap in understanding how host range influences invasive success.

2.6.3 Duration of life cycle

A short life cycle and multiple generations per year are common traits in many invasive species (Litchman, 2010; Pockl, 2009) and most PPN have these characteristics. Of the thirty-eight nematode species listed in Table 2.3, thirty (79%) can complete their life cycles in less than two months. Only one species (*Xiphinema diversicaudatum*) requires longer than a year to complete its life cycle (information on the duration of life cycles was not available for the remaining species).

Although the duration of PPN life cycles can be relatively short compared to macroorganisms, the number of generations actually occurring in a year can depend on local conditions such as the temperature and host availability. In PPN, temperature partly determines the number of days required to complete the life cycle and consequently the number of generations possible per year (Trudgill and Perry, 1994). For example *Meloidogyne incognita* and other tropical species are able to complete more generations per year under ideal conditions than in cooler areas.

Some PPN have short life cycles when active, but complete only one generation per year because they are dormant during periods of adverse conditions. For example, the life cycle of *Heterodera avenae* is about four months, but it completes only one generation per year because it is dormant when hosts are absent or conditions are too hot, cold or dry (Valdeolivas and Romero, 1985). For PPN like *Heterodera avenae*, the availability of a suitable host within 18 months since the time of introduction may be important for establishment success. Other PPN, such as *Meloidogyne incognita*, are active at a wide range of temperatures, and thus timing of introduction is not as important. These PPN may pose greater risks for invading new areas.

2.6.4 Adaptations for survival

In addition to having multiple hosts and short life cycles, PPN have several adaptations that enable them to survive adverse conditions, thus assisting their chances of establishment and persistence in new areas.
i) Cryptobiosis
Cryptobiotic mechanisms are physiological changes which enable PPN to survive for long periods under unfavourable conditions (e.g. in the absence of a host, unfavourable temperatures, and during storage or transportation) by entering a quiescent or dormant state. *Ditylenchus dipsaci* has been recorded surviving without water in anhydrobiosis for twenty-three years, *Globodera rostochiensis* for twenty-five years and *Heterodera glycines* for six years (Norton, 1978). Species with such adaptations to survive adverse conditions may have an advantage during and after introduction over those without.

ii) Eggs, cysts and egg mass
The chances of PPN surviving transit may be dependent also on the life-stage associated with a particular pathway because adaptations to survive adverse conditions in new areas may be specific to particular life stages. Live, non-quiescent adult nematodes can survive in plant materials and soil contaminants for relatively short periods, but eggs can survive longer. The eggs of PPN are enclosed in a multilayered eggshell typically with three layers: an outer vitelline layer; a middle chitinous layer; and an inner lipid layer (Decraemer and Hunt, 2006). The eggshell protects the contents from desiccation, extreme temperatures and chemicals such as nematicides (see Perry and Moens, 2011b and references therein). The eggshell in PCN enables enclosed juveniles to supercool to temperatures below – 38 ºC (Devine, 2010; Wharton and Ramløv, 1995), enabling them to survive extreme winter temperatures. In addition to the protection of the eggshell, many hundreds of eggs may be contained within another protective package formed by the tanning of the cuticle of the dead fertilized female. This is known as the “cyst” and occurs in *Heterodera*, *Globodera* and related PPN genera. Cysts have been implicated in the widespread dispersal of these PPN as they can survive harsh conditions and be spread by many different pathways including being ingested by birds and released with their droppings, as well as being blown in dust storms (see Table 2.3). In root-knot nematode *Meloidogyne* spp., hundreds of eggs are laid in a gelatinous matrix which protects the eggs from environmental stresses (Wallace, 1968b) and also from parasitic soil microorganisms (Orion et al., 2001).

iii) Host location and invasion by juveniles
For successful establishment, PPN need to find a susceptible host. Adaptations for host finding are well developed in the best-known PPN. For example, in *Globodera*, *Heterodera* and *Meloidogyne*, root exudates from host plants stimulate egg hatching thereby synchronising the presence of juveniles and hosts, which increases juvenile survival. Second stage juveniles are
also specifically adapted for host location and are the infective stage in the life cycle. In *Meloidogyne* it has been shown that secretions from juveniles can influence root development and responsiveness to nematodes, similar to mechanisms used by *Rhizobia* bacteria while forming root nodules (Weerasinghe *et al.*, 2005). What mechanisms are used by other PPN to locate and invade their hosts are not fully understood (Mitchum *et al.*, 2012). Hence, knowledge of the efficiency of most PPN finding and successfully invading hosts is an important gap in predicting invasion success in PPN, with the exception of the genera mentioned above.

**iv) Specially adapted juveniles**

Other strategies frequently found in PPN are lipid reserves in the intestine of juveniles and specially adapted stages for dispersal. Lipid reserves act as a food source while the nematodes are trying to locate host plants (Rocha *et al.*, 2010; Storey, 1983; Vangundy *et al.*, 1967). Phoretic juveniles with greater lipid reserves are formed in response to adverse conditions in PWN (Aikawa, 2008). These juveniles are adapted for dispersal by beetle vectors from the genus *Monochamus* and are able to survive in their beetle vector for six to twelve months (Aikawa, 2008; Kondo and Ishibashi, 1978). Similarly in *Ditylenchus dipsaci*, special fourth stage juveniles are formed in response to adverse conditions which are larger, have more lipid reserves, tend to aggregate by coiling and clumping, and can survive up to twenty-three years longer than juveniles formed under ideal conditions (Perry and Moens, 2011a).

**v) Changing sex ratios**

Adult PPN are adapted for feeding and reproduction rather than dispersal and finding hosts like the eggs and juveniles. Female nematodes require greater amounts of nutrients than males. As a survival mechanism, the sex of developing juveniles in species of *Globodera*, *Heterodera* and *Meloidogyne* can change with food availability (Betka *et al.*, 1991; Grundler *et al.*, 1991; Grundler and Hofmann, 2011; Triantaphyllou, 1973; Trudgill, 1967). More adult females are produced than males when food is abundant, whereas more adult males develop when food is scarce. Such a strategy is thought to reduce competition for food because males can survive longer without a host: for example, *Radopholus similis* males survive longer than females (Chabrier *et al.*, 2010). This mechanism may affect the establishment and persistence of invasive PPN by altering resource use efficiency, but further studies are required to determine if there is a correlation with invasion success. Observations in animal parasitic nematodes of variation in sex ratios relative to level of infection, host size and temperature have also been linked to reduced competition for food and hence success in invasion of the host (D'Ávila *et al.*, 2011; Golestaninasab *et al.*, 2012).
2.6.5 Persistence of introduced plant-parasitic nematodes

The persistence of an introduced PPN will be influenced by its ability to adapt to the new environment. Unless there are vacant niches (Figure 2.3a), introduced PPN must either outcompete or coexist and persist with native nematodes, bacteria, fungi and other fauna in the soil. The roles of competition, coexistence and persistence are poorly understood for most PPN in many situations, so how they affect whether introduced PPN become invasive remain uncertain. Most evidence is indirect, but suggests that competitive ability or adaptability is a key factor in PPN becoming invasive.

![Vacant niche](image)

Vacant niche: existing species have different seasonal requirements (e.g. species A has different seasonal requirements to species B) and there is a period when neither is favoured; over the unfavourable period abundance of native PPN is low and there is no dominant species, thus creating opportunities for exotic PPN which can exploit the conditions. When exotic species are introduced to a suitable vacant niche, they are likely to establish, persist and may become invasive.

i) Competition

Competition has been investigated for few PPN, but of those studied; most seemed able to outcompete native PPN when introduced to new areas. For example, the PWN species *B. xylophilus* had better boarding of beetle vectors and dispersal than the very closely-related and ecologically very similar *B. mucronatus*. These abilities allowed *B. xylophilus* to competitively displace *B. mucronatus* and successfully invade Japanese and other Asian pine forests (Cheng et al., 2009; Jikumaru and Togashi, 2003; Vincent et al., 2008; Xie et al., 2009). In addition, recent studies demonstrated that the invasive gall forming PPN *Meloidogyne incognita* successfully competed with, and displaced, aboveground herbivores such as phloem-feeding aphids (Kaplan et al., 2011). Hence, when introduced to new areas, many PPN may become
superior competitors to native PPN and non-nematode parasites or pathogens, which will favour colonization success (Figure 2.3b).

**Figure 2.3b Competitive displacement**

![Competitive displacement graph](image)

Competitive displacement: two species occupy the same niche and one (e.g. species A) is a superior competitor over the other (e.g. species B); the inferior competitor must either adapt to persist in the same niche or find a new niche; if it fails to adapt then the inferior competitor is likely to go extinct. When introduced species are better competitors than native species they are likely to become invasive.

**ii) Coexistence and persistence**

If introduced PPN are not immediately superior competitors then they must persist and coexist until conditions change and abundance can then increase above the damage threshold, thus becoming invasive (Figure 2.3c). There is little data on this aspect of nematode ecology, but more than one species of PPN have often been found co-habiting and infecting the same host in the field (Brinkman *et al.*, 2005).

To become invasive, coexisting and persisting introduced PPN may outcompete the indigenous fauna when conditions change (Figure 2.3d). Nematodes appear to divide niches very finely so it is likely that any alien PPN will dominate at some stage (Sohlenius, 1985; Yeates, 1999). There are several observations of trophically similar nematode species altering their competitive ability when climatic conditions change (Boag and Alphey, 1988; Khan *et al.*, 1997; Kraus-Schmidt and Lewis, 1979; Esser *et al.*, 1993; O'Bannon and Stokes, 1978). Different PPN may thus become dominant as climatic conditions or seasons change. However, there are no direct observations of changing competitive dominance in invasive PPN. How the outcomes of
competition among species change under different conditions is a major gap in understanding how PPN become invasive.

**Figure 2.3c Persistence**

![Persistence Graph](image)

Persistence: an existing species dominates in an area (e.g. species A), introduced species must maintain a sufficient population to persist (e.g. species B); an introduced species may not persist if unable to maintain a population above the persistence threshold (e.g. species C). During the persisting period, the introduced species—depending on their genetic and phenotypic plasticity—may be able to adapt to the local conditions, so when conditions (e.g. temperature, resource availability) become favourable for the persisting PPN, they may become invasive.

**Figure 2.3d Coexistence by niche partitioning**

![Coexistence Graph](image)

Niche partitioning: species are active during different seasons in a year due to different temperature and host requirements enabling two or more species to coexist in the same habitat. The native species A (solid line) is most abundant and active when the introduced species B (broken line) is least abundant and inactive, and vice versa. The introduced species thus finds a suitable niche then they may become dominant, damaging and hence invasive.
The presence of nematode predators may also influence invasion success. Naturally occurring predators may control PPN including introduced species, but very little is known about whether this affects persistence, coexistence, competition and ability of the introduced species to reach damaging thresholds.

2.6.6 Host plant resistance to plant-parasitic nematodes
The host plant species and their characteristics can be important in determining herbivore or plant-parasite invasion success (Atsatt and Odowd, 1976; Le Guigo et al., 2012; Price et al., 1980). Based on historical evidence, hosts seem generally less resistant to invasive PPN than to local PPN. Hence, in new areas, introduced PPN may face less host resistance than in their native range, where native host species have co-evolved to have better resistance (Carpentier et al., 2012; Grenier et al., 2010). For example, in their native range the hosts of PCN and PWN are more resistant than in their introduced range. The solanaceous hosts of PCN in the native Andean region are less susceptible than solanaceous hosts in Europe (Canto Saenz and Mayer de Scurrah, 1977; Hockland et al., 2012). Similarly, the native North American pine hosts (e.g. *Pinus taeda*), which co-evolved with PWN, have better resistance to this nematode compared to the highly susceptible Asian pine tree species (e.g. *Pinus densiflora*) (Dwinell and Nickle, 1989).

Some PPN seem able to overcome host resistance, which may increase their invasive ability. For example, *Meloidogyne enterolobii* can overcome resistance in tomato and pepper cultivars resistant to other PPN (Castagnone-Sereno, 2012; Kiewnick et al., 2009) and hence this species has emerged as an invasive species. How many PPN can overcome resistance is subject to continuing investigation and remains a gap in understanding invasions by PPN.

2.6.7 Genetic variability and evolutionary potential
Genetic variability and evolutionary potential may affect invasion success (Lee, 2002; Novak, 2007; Whitney and Gabler, 2008). Introduced species must interact, adapt and respond to a new environment to invade successfully, and this is dependent, at least in part, on genetic variability and evolutionary potential. Introduced species also exert a selection pressure on the native species, which may evolve in response: the introduced species must have enough variability to respond in turn (Strauss et al., 2006). If the introduced species cannot respond, they may not persist.
Studying the mode of reproduction is one way of understanding genetic variability. Many PPN reproduce by amphimixis, which increases genetic variability within species through cross fertilization and recombination of genetic materials (Table 2.1). Many other species of PPN are parthenogenetic or hermaphroditic (Table 2.1). These means of reproduction may help colonization, particularly for sedentary PPN, because fewer individuals are needed in a breeding population (above) and advantageous genotypes are retained (Castagnone-Sereno, 2002; Castagnone-Sereno, 2006). Genetic variability seems retained in parthenogenetic PPN by structural rearrangements of the chromosome (deletions, duplications and translocations), aneuploidy and polyploidy (Castagnone-Sereno, 2002; Semblat et al., 2000). Thus most PPN seem to have mechanisms for maintaining genetic variability, which may assist invasion, but specific studies of genetic variability in PPN invasions are lacking. Also, the reproductive modes of about one third of PPN are unknown, which is another major gap in understanding invasion by PPN.

Introduction of species from elsewhere can lead to greater genetic variation through hybridisation with native genotypes. This increased genetic variation may prime an otherwise benign species to cause increased damage and thus become invasive. Many PPN vary considerably between different geographic areas (above). There is very little data on how introduction of exotic PPN increases genetic variation, leading to a major gap in understanding of how this may affect invasion.

### 2.6.8 Phenotypic plasticity

Phenotypic plasticity refers to an organism’s ability to produce different phenotypes when exposed to different environments (Sommer and Ogawa, 2011; Pigliucci, 2005). Phenotypic plasticity allows adaptation in novel or stressful environments thus increasing chances of colonisation (Bossdorf et al., 2008; Rapp and Wendel, 2005). In nematodes, phenotypic plasticity is manifested by formation of dauer larvae, evolution of parasitism, generation of novel feeding and reproductive traits (Sommer and Ogawa, 2011; Niu et al., 2012). For example, the invasive PPN *B. xylophilus* produced more eggs which hatched earlier under conditions of high food availability; which aided in competing with ecologically similar *B. mucronatus* (Niu et al., 2012). While formation of dauer larvae and plasticity in reproductive traits have been demonstrated to aid survival under adverse conditions in *B. xylophilus* (Aikawa,
Phenotypic plasticity is also exhibited by root-knot nematodes (e.g. *Meloidogyne hapla*) and cyst nematodes (e.g. *Globodera pallida* and *G. rostochiensis*), which produce life stages specifically adapted to survive unfavourable conditions (Devine, 2010; Wharton and Ramlov, 1995; Wang et al., 2010). How common phenotypic plasticity is in other PPN species and its effect on colonization success of PPN in novel environments needs further investigation.

### 2.7 Abiotic factors

Abiotic conditions such as level of disturbance, resource availability and climatic conditions have been related to invasion success in many organisms (Abella *et al.*, 2012; Davis *et al.*, 2000; Kneitel and Perrault, 2006; Olden *et al.*, 2006; Sharma *et al.*, 2011). Many widespread invasive species are present in less diverse and highly disturbed habitats which are more susceptible to invasion than more diverse pristine habitats (Davis and Landis, 2011; Hendrix *et al.*, 2008; Taraschewski, 2006). Many PPN species are associated with agriculture and their habitat often has low diversity and high disturbance which may favour colonisation by introduced species. The biotic adaptations of PPN to cope with low resource availability, tolerance of drought and or low temperatures (see above) are further aids to colonisation. Hence, resource availability and climatic conditions may exert less influence on invasion by PPN than on other organisms.

Although the influence may be less, climatic and edaphic conditions may still partly affect invasion success. Temperature and edaphic conditions affect population growth and pathogenicity of many PPN (Norton and Hoffmann, 1974; Trudgill and Perry, 1994), and hence may affect their invasion success. In a semiarid grassland field experiment, temperature and soil moisture both affected the species diversity and abundance of the nematode fauna (Bakonyi and Nagy, 2000). However, the relative contribution of these abiotic factors to invasive success of PPN species is yet to be studied elsewhere or in detail.

In many organisms, changes in abiotic conditions can facilitate biological invasions (Mainka and Howard, 2010; Smith *et al.*, 2012). In PPN, higher temperatures may increase the geographic range and probability of establishment of some temperature-dependent species (Table 2.1). Also, higher temperatures reduce generation times in many PPN see (Bakonyi and Nagy, 2000; Carter *et al.*, 1996; Ghini *et al.*, 2008; Jacobs *et al.*, 2011; Nethi and Prasad, 2012;
Nethi et al., 2010), and hence populations may increase to damaging levels more rapidly. Range expansion and variations in pathogenicity as a result of changes in climate have not been observed directly in PPN, so this is an important area for investigation.

Range expansion with increased temperature has been observed for nematode vectors, but not for PPN directly. For Monochamus, the vector of PWN, higher temperatures resulted in spread into new areas where the climate was previously unfavourable (Jikumaru and Togashi, 2000). With even warmer temperatures under further climate change, this vector may survive in more areas and invade places previously too cold (Jikumaru and Togashi, 2000; Jones et al., 2008). The severity of pine wilt disease caused by PWN may also increase because nematode transmission is increased by increased longevity of the principal vector of PWN (Monochamus alternatus) at higher temperatures (Rebetez and Dobbertin, 2004).

2.8 Summary on plant-parasitic nematodes as invasive species
PPN, probably due to their cryptic nature, have received relatively little attention as invasive species. However their potential to become invasive is clearly demonstrated by species such as Bursaphelenchus xylophilus, Globodera pallida, G. rostochiensis, Heterodera glycines and Meloidogyne incognita. All these species have spread in the past, and continue to spread, causing economically important damage. Most of these species have most of the characteristics likely to aid species invasions, including adaptations allowing human mediated transport, wide host range or cosmopolitan hosts, multiple pathways, microscopic size (lowering probability of detection during quarantine inspections), transport of numerous propagules with a single introduction event, high fecundity, short life cycle, cryptobiotic abilities to survive harsh environmental conditions by having cysts, egg masses and dauer stage juveniles with specific adaptations for long term survival, variation of male to female sex ratio to survive abiotic or biotic stresses, and ability to break down host plant resistance.

Many other PPN have many of these characteristics as well (Tables 2.1, 2.2 and 2.3), but they have not been recognised as potentially invasive. Absence of information on some of their biological characteristics and unquantified impacts seem to be the major reasons these species have not been recognised as invasive, even when most of the known characteristics and data suggests they should be. The lack of information and uncertainty presents major challenges to assessing invasiveness in PPN particularly in the areas of biogeographical patterns, species
distributions and biology. PPN species identification is also a major challenge as specialist expertise is required.

### 2.9 Uncertainty

For many PPN, uncertainty about many of the features related to invasiveness discussed above, (such as native range, host range, duration of life cycle, mode of reproduction and correct species identification) makes it difficult to properly assess or predict their invasiveness. The impact of many PPN is also uncertain. This is significant to assessments of invasiveness because if information is insufficient, then non-invasiveness is assumed under many of the codes governing biosecurity (FAO-ISPM-2, 2007; FAO-ISPM-11, 2013). Hence PPN may be wrongly classified as non-invasive purely on absence of information rather than evidence or characteristics suggesting non-invasiveness. Many PPN are not considered invasive mainly because they are poorly known.

Further, the uncertainty about biological characteristics and impacts are linked, and this can seriously bias which PPN are considered invasive. The most widespread and economically important PPN are best studied and therefore have highest likelihood of being considered invasive (e.g. PCN, PWN, *Meloidogyne incognita*, *M. arenaria*, *M. javanica*, *M. hapla*, *Heterodera avenae*, *H. glycines*, *Ditylenchus dipsaci*). Other PPN, which may be economically important pests but have more restricted distributions, have lower likelihood of being considered invasive (e.g. *Meloidogyne chitwoodi*, *M. fallax*, *Heterodera ciceri*). PPN which have not been well studied, particularly those from countries where nematological expertise is scarce, have even lower likelihood of being considered invasive (e.g. *Aphelenchoides arachidis*, *Ditylenchus africanus*, *Hirschmanniella miticausa*, *Scutellonema bradys*). PPN which have little recorded impact and currently have a narrow distribution, have lowest likelihood of being considered invasive (e.g. *Ibipora lolii*, *Meloidogyne citri*, *M. donghaiensis*, *Subanguina chilensis* and *Subanguina hyparrheniae*).

It is important to note that **BEFORE** they became invasive both of the well recognised invasive nematodes, PCN and PWN, had limited and local impacts only. It was only **AFTER** these species were recorded as successfully invading somewhere that their biological characteristics and impacts were studied in sufficient detail to become considered invasive.
Uncertain impacts of PPN are common because assessing and quantifying the damage caused by each species is challenging. PPN may affect plants directly or indirectly through other soil organisms or effects on the ecosystem as a whole. Hence, the full direct and indirect effects of PPN are seldom quantified, and underestimation of impacts may mean that invasive PPN are not recognised, but how frequently this occurs is not known. Better estimation of more of the complex impacts would clarify this in both natural and agricultural landscapes.

The direct interactions between PPN, their hosts and other soil fauna are complex. PPN can damage plants directly through their feeding on roots. They can damage plants by increasing the effects of drought or nutrient deficiency (Evans et al., 1993; Luc et al., 2005). They can act as vectors of damaging viruses or bacteria (Riley and McKay, 1991; Taylor and Brown, 1997) and there are several other ways that PPN damage plants (Hewitt et al., 1958; Powell, 1971; Sidhu and Webster, 1974).

Indirect and ecosystem effects of PPN include several mechanisms as well. PPN form disease complexes predisposing plants to attack by soil fungi and bacteria through entry wounds or by compromising plants defence (Hewitt et al., 1958; Powell, 1971; Sidhu and Webster, 1974). PPN may also affect the wider soil biota and the ecosystem by modifying interactions among components of the ecosystem. For example, the soil biota can influence competition between plants by affecting one species more than another. When new elements are introduced to the soil biota then their relative influence on the different plant species may change, and favour undesirable or invasive plants (Callaway et al., 2004; Klironomos, 2002; Turnbull et al., 2010; Wolfe and Klironomos, 2005). Effects such as those mentioned above have never been studied for PPN, but could be substantial (Barker et al., 1994; Freckman and Ettema, 1993; Yeates et al., 1993). The soil biota may also influence soil properties, so changes resulting from introduced species may adversely affect soils as well. Introduced PPN may also affect the vulnerability of native ecosystems such as grasslands or forests to invasions by undesirable plants (Huston, 2004; Prober and Wiehl, 2012; Stohlgren et al., 2002); another impact of invasion by PPN never quantified.

2.10 Biosecurity implications
Biological invasions highlight the difficulty for biosecurity systems to prevent entry and establishment of invasive species. The effectiveness of biosecurity systems to prevent biological
invasions depends on identification of potential risks, so that resources can be best allocated and particular vulnerabilities can be addressed. Two of the most challenging characteristics of PPN for biosecurity systems are difficulty of detection and potential introduction via multiple pathways. Similar challenges have been noted for several other groups of potentially invasive organisms (McKirdy et al., 2012; Bacon et al., 2012), so designing a system effective for PPN can strengthen biosecurity systems in general.

PPN can be overlooked easily during quarantine inspections and hence escape detection if they are not specifically sampled (Ward and Hockland, 1996). For many species, symptoms of infestation are not visible to the naked eye, so specific extraction procedures and microscopic examination are required to detect their presence. Some PPN are endoparasites of roots. Thus apparently symptomless plant material may still harbour numerous nematodes in or on them (Lehman, 1994; Lehman, 1996). PPN can also be present with soil particle contaminants on plant parts or bare root cuttings and so travel with non-host plants, machinery or footwear (Table 2.3). There are two potentially effective strategies to cope with this and to avoid introduction of PPN.

One is certification at source of freedom from nematodes (Inserra et al., 2005; Lehman et al., 1996). This strategy may be effective for high risk pathways such as seeds or planting material. An example of successful application of certification in reducing spread of PPN is Anguina tritici. Seeds are a known major pathway for spread in this species and the use of clean, certified seed has been successful in eliminating the species from farms in countries using clean certified seeds, but remains a problem where seed is not certified (CABI, 2010). By comparison, in the absence of certification, nematode infested planting material may be the main factor contributing to the widespread occurrence of the burrowing nematode Radopholus similis and its spread into areas of Asia, Africa, Caribbean and Latin America (Khan, 1999; Marin et al., 1998; Price, 2006).

The other potentially effective strategy is identification of target species and pathways to know where and how to sample. This has been the basis of implementing quarantine measures against PPN which are already well known, but has proven problematic for the many PPN where considerable uncertainty remains about characteristics and impacts. Investigations on improved
methods for estimating and prioritising risks from poorly-known PPN are reported in subsequent chapters.

Correct species diagnosis is another component of detection that may be a critical vulnerability in biosecurity systems for PPN. Correct diagnosis is necessary for accurately identifying biogeographic patterns (Price, 2006; Coomans, 2002) which can have important implications for predicting invasions and species distributions. It also is important in guiding efforts at detection and deciding what to do after detection (Boykin et al., 2011; Maynard and Nowell, 2009). It is important for correctly estimating the risks of invasion and avoiding loopholes in biosecurity systems designed to prevent spread of invasive species (Thum et al., 2012). The taxonomy of many genera of PPN, such as *Aphelenchoides, Ditylenchus, Heterodera, Meloidogyne* and *Xiphinema*, is difficult, hence misidentifications of species are common. For example, *Meloidogyne enterolobii* was considered until recently only a minor pathogen and not widely investigated. Then the more widely studied and economically important *M. mayaguensis* was determined as the same species and synonymized with *M. enterolobii* (Karssen et al., 2012; Castagnone-Sereno, 2012). *M. enterolobii* had also been misidentified as *M. incognita* because it is very similar morphologically (Fargette et al., 1994). These misidentifications make a difference: *M. enterolobii* is quarantined, but *M. incognita* is widespread and not quarantined.

Development and use of molecular identification techniques in addition to morphological characteristics may enable more accurate PPN species identifications (for example reference diagnostic protocols are provided by EPPO and sequence information from the DNA databank – see Qbank; [http://www.q-bank.eu/](http://www.q-bank.eu/)) and thus address some of the biosecurity challenges due to species misidentification.

Improved detection and surveillance are required to decrease the time taken between arrival and discovery of exotic species. A delay in detection allows invasive species to spread while undetected and unregulated and can affect the invasion success of a species. Early detection of PPN may allow minimisation of spread and better success in eradication while distributions are restricted to small areas (Hodda et al., 2008; Pluess et al., 2012). Early detection was important in two successful eradication programmes for invasive PPN: *Buraphelenchus hunanaensis* from Victoria, Australia (Hodda et al., 2008) and *Globodera rostochiensis* from Western Australia following a twenty-four year eradication campaign (IPPC, 2010). By comparison, detection may have been delayed for the PWN invasion in Portugal, resulting in the eradication
campaigns being unsuccessful thus far (Abelleira et al., 2003; Rodrigues, 2008). PWN has continued to spread and damage Portuguese and Spanish pine forests. Simulation models now predict a low probability of eradication (Okland et al., 2010).

2.11 Conclusions
Most PPN seem to have many of the characteristics associated with invasiveness; however only a few have been recognised as invasive. Many characteristics seem to contribute to the general propensity of PPN to have invasion potential. However, the relative contributions of the different characteristics towards the invasion success of PPN are still unknown and require further research. Uncertainty about biological characteristics and impacts seem to be the major causes of PPN not being recognised as invasive.

Biosecurity systems for PPN may be improved through two main strategies: by improving identification of which characteristics of PPN are most important in invasiveness; and by improving detection (including reducing lag times between arrival and recognition), targeting and diagnosis.

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Plant-parasitic nematodes of potential phytosanitary importance, their main hosts and reported yield losses

Sunil K. Singh, Mike Hodda and Gavin J. Ash

3.1 Abstract

**Keywords:** biosecurity; quarantine, nematodes, pest risk analysis

3.2 Introduction
Annual crop losses caused by plant-parasitic nematodes are estimated at 8.8 to 14.6 % of total crop production and 100–157 billion USD worldwide (Abad et al., 2008; Koenning et al., 1999; Nicol et al., 2011; Sasser & Freckman, 1987). Actual losses may be even higher because there is no data from many countries where nematological expertise is lacking. Yield loss data are also difficult to obtain because of the complex interactions of plants, nematodes, other soil organisms and soils (Coyne & Plowright, 2002; Koenning et al., 1999; Noling, 1986; Seinhorst, 1982). Often the most noticeable localised damage is investigated and reported while less obvious but more widespread damage is overlooked (Nicol et al., 2011).
Substantial crop losses from plant-parasitic nematodes (hereafter PPN), could be much greater if species currently causing localised damage became more widespread. Many PPN have low impact in their native range, but much greater impact when introduced to new areas. Examples include: *Bursaphelenchus xylophilus* (Pine Wood Nematode) in Japan, China, Portugal and most recently in Spain (Abelleira et al., 2011; Braasch & Enzian, 2004; Cheng et al., 2007; Mota et al., 1999; Robertson et al., 2011; Togashi & Jikumaru, 2007), *Heterodera glycines* (Soybean Cyst Nematode) in USA (Riggs, 1977; Winstead et al., 1955); and the Potato Cyst Nematodes in Europe, the USA, Canada and Australia (Hafez & Sundararaj, 2007; Stanton, 1987; Stone et al., 1977; Sun et al., 2007; Turner & Evans, 1998). Cumulative losses from potato cyst nematodes introduction to Australia were estimated at 370 million AUD over 20 years (Hodda & Cook, 2009) and Pine Wood Nematode introduction to Europe estimated at 22 billion EUR over 22 years (Soliman et al., 2012).

Quarantine measures against known damaging PPN are effective in preventing their spread, thus effectively and economically preventing crop losses (Inserra et al., 2005; Lehman et al., 1996; Sikora et al., 2005). The presence of PPN during quarantine inspections may also act as a bio-indicator for consignments that do not meet the phytosanitary requirements of plants being grown in sterile environments and could be carrying other plant pathogens and microorganisms (Hockland & Anderson, 2012). Quarantine measures can also prevent spread of non-target species which are potentially invasive (Schrader & Unger, 2003). Quarantine and other phytosanitary measures are particularly important for PPN because other management methods such as chemical control or crop resistance can be far more costly and difficult to implement without other adverse effects (Hockland et al., 2013; Nicol et al., 2011; Zasada et al., 2010a). Furthermore, targeting PPN is important because of the wide range of survival adaptations and dispersal routes available (Singh et al., 2013).

An important first step in implementing quarantine measures is to determine which species should be regulated under international trade rules. The International Plant Protection Convention (IPPC) defines a quarantine pest as ‘a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled’ (FAO, 2011a). Organisms which meet this definition can be
regulated. Countries determine their lists of regulated pests according to guidelines set by the IPPC and International Standards for Phytosanitary Measures No. 19 (FAO, 2011b).

Countries may also establish lists of regulated non-quarantine pests. The IPPC defines these as follows: ‘a non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party.’ All lists of regulated pests are dynamic, with species added or removed from lists as phytosanitary risks change or as previously benign species emerge as pests (EPPO, 2007; FAO, 2011b).

Species other than those on official lists of quarantine pests may also pose a biosecurity risk. Only a small proportion of nematode species have been described (Blaxter, 2011; Hodda, 2011). New species are being described regularly, and species new to science have been intercepted during phytosanitary inspections (e.g. *Radopholus bridgei* and *Meloidogyne thailandica*), both described from material intercepted in quarantine (Handoo *et al.*, 2005; Siddiqi & Hahn, 1995)). Records of non-compliance for PPN show species outside of official lists of regulated pests being frequently detected (Gao & Zhang, 1994; Lal & Rajan, 2005; Lal & Lal, 2006; Mani, 1998; McNeill *et al.*, 2011; Plumas *et al.*, 2002; Queneherve *et al.*, 1998; Roques & Auger-Rozenberg, 2006). Information allowing assessment of the risks of the many PPN not on official lists of regulated pests is often not available (Singh, S.K., Hodda, M., Ash, G.J, Banks, N.C. unpublished data). To partially fill this gap, this paper presents a list of the 250 PPN likely to be most important for quarantine at a global scale, selected from all named PPN. The authors present notes on their main hosts, information on yield losses where available and their quarantine status globally if known, together with an extensive bibliography. This paper also discusses how such a list could be used in the pest risk analysis process.

### 3.3 Methods

Data on hosts, geographic distribution, yield loss and quarantine status were compiled from published records, the majority from online databases (Table 3.1). Species names of all known PPN were obtained from: Hunt, (1993; 2008) for Aphelenchoidea and Longidoridae; Decraemer, (1995) for Trichodoridae; Coomans *et al.*, (2001) for *Xiphinema*; Ebsary, (1991) and Siddiqi (2000) for Tylenchida; and Esser, (1991) and Fortuner, (1984) for general checklists of phytoparasitic nematodes. Synonyms were sourced from the database of nematode names
created for the classification of phylum Nematoda (Hodda, 2011). CABI abstracts, Web of Knowledge and Google Scholar were searched using these genus and species names. For species with over 200 hits, the search was refined using the advanced search option using the terms “distribution”, “hosts”, “yield”, “damage”, “disease”, “pathotype” and “virus-vector”. Full texts were acquired where available, either online or through inter-library loans. Records up to 31st May 2012 were included.

To be considered of phytosanitary importance PPN species had to meet at least one of the following criteria:

1. recorded in the peer reviewed scientific literature as pathogenic (causing disease) or parasitic (infecting or associated as ectoparasites) of an economically-important crop host;
2. recorded in the peer reviewed scientific literature as acting as vectors of, or forming disease complexes with, other pathogens such as bacteria, fungi and viruses;
3. currently on an official list of regulated pests for at least one country.

**Table 3.1 Online sources on organisms of phytosanitary importance**

<table>
<thead>
<tr>
<th>Resource</th>
<th>Notes on resource and their coverage of plant parasitic nematodes</th>
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</thead>
<tbody>
<tr>
<td>CABI Invasive Species Compendium</td>
<td>The Invasive Species Compendium (ISC) includes known invasive species of all taxonomic groups affecting natural and managed ecosystems, except human pathogens, concentrating on those species that have the greatest impacts on livelihoods and environment. It also includes some species which currently have a low impact where they currently occur but may be a threat to other regions if introduced (L Charles, pers. comm.). There were 38 species of PPN with detailed datasheets listed on the ISC database. The database is open access. <a href="http://www.cabi.org/ISC/">http://www.cabi.org/ISC/</a></td>
</tr>
<tr>
<td>CABI Crop Protection Compendium</td>
<td>The Crop Protection Compendium (CPC) is an encyclopaedic resource that brings together a wide range of different types of science-based information on all aspects of crop protection. The crop protection compendium covers pests of agricultural and horticultural crops and, since 2004, forest trees. The species that have been selected for inclusion as full datasheets are those of major global or regional economic or phytosanitary importance. In addition to crops; weeds, invasive plants of rangelands and woody invasive plants are also included on the CPC. There were 97 species of PPN with detailed datasheets listed on the CPC database. Access to the detailed datasheets is restricted to users with a subscription. <a href="http://www.cABI.org/CPC/">http://www.cABI.org/CPC/</a></td>
</tr>
<tr>
<td>CABI Distribution Maps of Plant Diseases</td>
<td><em>The Distribution Maps of Plant Diseases (DMPD)</em> “cover important diseases affecting agriculture, horticulture and forestry. Two sets of maps are produced each year, consisting mostly of new maps with a number of map revisions (where significant changes have merited a revision). There are 18 diseases per map set covering fungi, bacteria, viruses and, from 1999 onwards, nematodes” (CABI, DMPD). There were 91 species distribution maps of PPN. Access to detailed maps is restricted to users with a subscription. <a href="http://www.cABI.org/DMPD/">http://www.cABI.org/DMPD/</a></td>
</tr>
<tr>
<td><strong>International Plant Protection Convention</strong></td>
<td>The International Plant Protection Convention (IPPC) is an international agreement on plant health with 177 current signatories. It aims to protect cultivated and wild plants by preventing the introduction and spread of pests. Countries communicate their list of quarantine organisms, new pest records and phytosanitary alerts via the IPPC website. <a href="http://www.ippc.int/">http://www.ippc.int/</a></td>
</tr>
<tr>
<td><strong>EPPO PQR database</strong></td>
<td>The EPPO PQR database provides data on all the pests of the EPPO A1 and A2 lists, pests on the EPPO alert list, and quarantine pests and invasive plants of interest to other regions of the world. For each pest, lists of host plants, commodities able to act as pathways in international trade, nomenclatural and taxonomic details, and geographical distribution are presented (EPPO PQR). PQR lists 77 species of PPN with quarantine categorization for 47 species. The database is open access and can be downloaded from the EPPO website. <a href="http://www.ippc.int/DATABASES/pqr/pqr.htm">http://www.ippc.int/DATABASES/pqr/pqr.htm</a></td>
</tr>
<tr>
<td><strong>National Agricultural Pest Information System</strong></td>
<td>The US National Agricultural Pest Information System (NAPIS) stores and manages pest survey data collected by Cooperative Agricultural Pest Surveys and other Plant Protection Quarantine survey programs. Information is provided on the distribution of plant pests of regulatory significance to the USA and the risks associated with entry, establishment and spread of animal, plant pests and noxious weeds to the USA. There are 63 species of PPN listed on the database with survey maps and host maps. The risk maps are specific to the USA and its states. <a href="http://pest.ceris.purdue.edu/index.php">http://pest.ceris.purdue.edu/index.php</a></td>
</tr>
<tr>
<td><strong>Invasive and Exotic Species of North America</strong></td>
<td>The University of Georgia (USA) Center for Invasive Species and Ecosystem Health website provides information on invasive species whose introduction causes or is likely to cause economic or environmental harm or harm to human health. There are 29 species of PPN listed as potentially invasive to North America. <a href="http://www.invasive.org/species/nematodes.cfm">http://www.invasive.org/species/nematodes.cfm</a></td>
</tr>
<tr>
<td><strong>California Department of Food and Agriculture</strong></td>
<td>The California Department of Agriculture website provides a rating system for pests based on the potential and actual harm to California’s agriculture and environment. Pest ratings for 109 species of PPN for the state of California, USA are given. <a href="http://www.cdfa.ca.gov/plant/ppd/nematology/nema_by_rating.html">http://www.cdfa.ca.gov/plant/ppd/nematology/nema_by_rating.html</a></td>
</tr>
<tr>
<td><strong>Nematodes of quarantine concern</strong></td>
<td>The University of Nebraska website provides information on the top 15 species of PPN causing severe damage to crops which are the most widely regulated species worldwide. <a href="http://nematode.unl.edu/quaranem.htm">http://nematode.unl.edu/quaranem.htm</a></td>
</tr>
<tr>
<td><strong>Society of Nematologists</strong></td>
<td>Professional association web site presenting results of a working group evaluation of phytosanitary risks to the USA from 68 species of PPN. Distribution and hosts are also provided. (Last updated September 2003) <a href="http://nematode.unl.edu/projectpest.htm">http://nematode.unl.edu/projectpest.htm</a></td>
</tr>
<tr>
<td><strong>Q-bank and QBOL</strong></td>
<td>Dutch Ministry of Economic Affairs sponsored database (Q-bank) providing descriptions of well-characterized regulated plants pests. It comprises ecological, morphological, physiological, and sequence data of materials that are available in physical collections of plant-pathogenic bacteria, fungi, insects, nematodes, phytoplasmas, viruses and viroids, and invasive plants. <a href="http://www.q-bank.eu/">http://www.q-bank.eu/</a> The EU financed QBOL project aimed to develop rapid identification for prioritized quarantine organisms relevant for the EU, using DNA barcode sequences. There are 76 species of PPN on the nematode priority list. <a href="http://www.qbol.wur.nl/UK">http://www.qbol.wur.nl/UK</a></td>
</tr>
<tr>
<td><strong>Pacific Island Pest List database</strong></td>
<td>Website produced by South Pacific Commission. The Pacific Island Pest List database (PLD) provides records of pests that are currently known to affect agriculture, forestry and the environment in Pacific Island countries and territories (PLD). There are 31 species of PPN listed with information on their distribution and hosts. <a href="http://www.spc.int/pld/">http://www.spc.int/pld/</a></td>
</tr>
</tbody>
</table>
3.4 Results

Almost all of the 250 PPN species selected, satisfied criterion 1 (i.e. directly pathogenic or associated with economically important plants including crop, forest or pasture species (Table 3.2)). The only exception, *Ditylenchus weischeri* which parasitises a weed; is included due to its recent taxonomic separation from the economically important *D. dipsaci* species complex (Chizhov et al., 2010). Half of these species (52%, N=129) also satisfied criterion 2 (could form disease complexes with other organisms and or acted as vectors for viruses). A total of 126 species from 33 PPN genera were currently recognised as regulated pest species in one or more countries, worldwide (Figure 3.1). All PPN species reported as a regulated pest satisfied more than 2 criteria and were often associated with economically important plants. Over a third (35%, N= 88) species satisfied all 3 criteria.

The information from the sources in Table 3.1 varied in quality and consistency. The reasons for selecting particular species were not always explicitly stated. The CABI databases, EPPO PQR database, Society of Nematologists, University of Nebraska nematodes of quarantinable concern, were especially comprehensive information sources. Information was gathered from several databases and cross-checked with primary sources. Except for a few PPN species and countries worldwide (UK, EPPO countries, USA and Australia), detailed risk assessments leading to the quarantine listing of a species were not publicly available. In most other instances, the broad definition of a pest species under IPPC and ISPM were used to justify the quarantine status of a species.

The quarantine listing of PPN species varied between countries. PPN species well known for their damaging impacts (including for example: *Anguina tritici, Aphelenchoides besseyi, A. fragariae, Bursaphelenchus xylophilus, B. cocophilus, Ditylenchus destructor, D. dipsaci, Globodera pallida, G. rostochiensis, Heterodera glycines, H. schachtii, Nacobbus abberans, Radopholus similis, Xiphinema americanum* and *X. index* (Source: Nematodes of quarantinable concern, Table 3.1)) were the most widely regulated. Other species, although damaging, are not regulated in many countries often because they are usually widespread. However, some countries regulate specific races e.g. of *Meloidogyne incognita, M. javanica, M. arenaria* which are not present in their territory. Distinguishing races is a major challenge for inclusion of races on list of regulated pests and further research is needed to develop rapid and specific methods for their distinction (see discussion).
International standards (ISPM) on pest risk analysis have been developed in the IPPC framework to determine the phytosanitary risks and biosecurity importance of species. The scarcity of publicly available documentation on pest risk analysis from countries worldwide and lack of transparency in the process leads to differences in how the phytosanitary importance of species are rated. For instance, some countries consider *Bursaphelenchus mucronatus* as of phytosanitary importance, but others do not. This is partially due to uncertainty on the whether the species can cause disease. Recent studies have demonstrated that the species can carry pathogenic bacteria (Zhao *et al.*, 2009) hence it was included on our list under criterion 2.

**Figure 3.1** Number of regulated species per plant parasitic nematode genus

![Graph showing number of regulated species per plant parasitic nematode genus](image)

**Table 3.2** List of plant parasitic nematode species of phytosanitary importance

<table>
<thead>
<tr>
<th>Species</th>
<th>Notes on main hosts, vector relationships and yield losses</th>
<th>Regulated pest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Achlysiella williamsi</em></td>
<td>Parasitises sugarcane (Blair <em>et al.</em>, 1999; Hunt <em>et al.</em>, 1989).</td>
<td>-</td>
</tr>
<tr>
<td>(Siddiqi, 1964) Hunt, Bridge &amp; Machon, 1989</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. <em>Anguina agropyri</em></td>
<td>Acts as vector of bacterium <em>Rathayibacter</em> (Evtushenko <em>et al.</em>, 1994). The potential of the bacteria-nematode complex to cause toxicity in livestock is yet to be determined.</td>
<td>-</td>
</tr>
<tr>
<td>Kirjanova, 1955</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Steinbuch, 1799) Filipjev, 1936</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. <em>Anguina australis</em> Steiner,</td>
<td>Acts as vector of bacterium <em>Rathayibacter toxicus</em> (syn.</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>Species</td>
<td>Description</td>
</tr>
<tr>
<td>------</td>
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<td>-------------</td>
</tr>
<tr>
<td>1940</td>
<td><em>Clavibacter toxicus</em></td>
<td>on annual veldtgrass under experimental conditions only (Riley et al., 2001). The association of <em>A. australis</em> with <em>R. toxicus</em> has not been recorded in the field under natural conditions.</td>
</tr>
<tr>
<td>6.</td>
<td><em>Anguina graminis</em> (Hardy, 1850) Filipjev, 1936</td>
<td>Acts as vector of bacterium <em>Rathayibacter festucae</em> on red fescue (Dorofeeva et al., 2002). The potential of the bacteria-nematode complex to cause toxicity in livestock is yet to be determined.</td>
</tr>
<tr>
<td>9.</td>
<td><em>Anguina tritici</em> (Steinbuch, 1799) Filipjev, 1936</td>
<td>Important parasite of wheat in countries which do not have access to clean/certified nematode free seeds. Reduced wheat yields by 43-100% in India (Khan &amp; Athar, 1996; Paruthi et al., 1987), 69-93% in Pakistan (Anwar &amp; Inam-ul-Haq, 1992) and 32% in Turkey (Ozberk et al., 2011). Is of limited importance in countries using clean/certified nematode free seeds for planting. Under experimental conditions, <em>A. tritici</em> can also act as a potential vector of bacterium <em>R. toxicus</em> (Riley et al., 2001).</td>
</tr>
<tr>
<td>10.</td>
<td><em>Aphasmatylenchus straturatus</em> Germani, 1970</td>
<td>Pathogenic to peanut and cowpeas (Baujard &amp; Martiny, 1995b). This species is only known from the south western region of Burkina Faso and yield loss of peanut can be as great as 70% in heavily infested fields (Dickson &amp; De Waele, 2005).</td>
</tr>
<tr>
<td>12.</td>
<td><em>Aphelenchoides arachidis</em> Bos, 1977</td>
<td>Pathogenic to peanut. Species is seed borne hence there is high risk of spread, recently reported from Egypt from peanut (Montasser et al., 2008) and also intercepted in seeds imported to India (Lal &amp; Lal, 2006).</td>
</tr>
<tr>
<td>13.</td>
<td><em>Aphelenchoides besseyi</em> Christie, 1942</td>
<td>Pathogenic to rice and causes rice white tip disease. Species is seed borne and has been disseminated in most rice growing areas of Africa, North, Central and South America, Asia, Eastern Europe and Pacific Islands. Importance and severity can vary with country locality and rice environment. Rice yield loss varies from 10-50% (Muthukrishnan et al., 1974; Silva &amp; Da Silva, 1992).</td>
</tr>
<tr>
<td>14.</td>
<td><em>Aphelenchoides bicaudatus</em> (Imamura, 1931) Filipjev &amp; Schuurmans Stekhoven, 1941</td>
<td>Parasitises cultivated mushrooms (Grewal &amp; Siddiqi, 1993), yield loss depends on nematode population density and can be up to 45% (McLeod, 1967).</td>
</tr>
<tr>
<td>15.</td>
<td><em>Aphelenchoides blastophthorus</em> Franklin, 1952</td>
<td>Pathogen of ornamental plant <em>Scabiosa caucasica</em>, causes destruction of inflorescence and distortion of laminae (Franklin, 1952). Little information is available on the species.</td>
</tr>
</tbody>
</table>
16. *Aphelenchoides composticaola* Franklin, 1957  
Parasitises edible mushrooms *Agaricus bisporus* (Gitanjali & Nandal, 2005; Goodey, 1960; Grewal & Siddiqi, 1993; McLeod, 1967).

17. *Aphelenchoides fragariae* (Ritzema Bos, 1890)  
Parasitic to strawberry and reduced yields by up to 60% (Bohmer, 1981; Duggan, 1969).

18. *Aphelenchoides ritzemabosi* (Schwartz, 1911) Steiner & Buhrer, 1932  
Parasitises edible mushrooms (Gitanjali & Nandal, 2005; Goodey, 1960; Grewal & Siddiqi, 1993; McLeod, 1967).  
Pathogenic to strawberry and reduced yields by 65% (Bohmer, 1981) and reduced chrysanthemum yields by 45-92% (Baranowski, 1976).  
Yes

19. *Aphelenchoides sacchari* Hooper, 1958  
Caused yield losses of up to 100% (Aman et al., 2002).

20. *Aphelenchoides saprophilus* Franklin, 1957  
Parasitises garlic (Balasubramanian et al., 2002) and edible mushroom (Grewal & Siddiqi, 1993; McLeod, 1968).  
Parasitises edible mushrooms Agaricus bisporus (Gitanjali & Nandal, 2005; Goodey, 1960; Grewal & Siddiqi, 1993; McLeod, 1967).

21. *Aphelenchoides subtenuis* (Cobb, 1926) Steiner & Buhrer, 1932  
Parasitises bulbous crops such as *Crocus*, *Allium* and some species of *Tulipa* and *Narcissus* (van Leeuwen & Trompert, 2011).

22. *Aphelenchus avenae* Bastian, 1865  
Parasitises edible mushroom *Calocybe indica* and reduced yield by 11% (Kumar et al., 2010).  
The species is also associated with a wide range of crop plants but is not pathogenic.

23. *Belonolaimus gracilis* Steiner, 1949  
Parasitises a wide range of cultivated crops (Christie et al., 1952; Esser, 1976), turf (Christie et al., 1954). Also forms disease complex with fungal pathogens (Holdeman & Graham, 1952).

Pathogenic to potato (Crow et al., 2000b), turf (Crow, 2005), soybean (McSorley & Dickson, 1989). Can reduce the yield of cotton at low population densities or result in crop failure when population density is high (Crow et al., 2000a; Koenning et al., 2004). Species is listed as of quarantine concern (Karnkowski, 2007).

Pathogenic to peanut (Dickson & De Waele, 2005; Reddy et al., 1984) and is capable of parasitizing other crops (Thakar et al., 1986).

26. *Bitylenchus dubius* (Bütschli, 1873) Filipjev, 1934  
Parasitises sugarbeet (Kalatur, 2008), wheat (Jones, 1979), turf (Blackburn et al., 1997) and flax (Skarbilovich, 1971).

Parasitises small millet (Jain, 2009), wheat (Patel & Thakar, 1989) and maize (Jain, 1982).

Pathogenic to palm trees and causes red ring disease (Inserra et al., 2005).

29. *Bursaphelenchus hunanensis* Yin Fang & Tarjan, 1988  
Pathogenic to pine trees; causes wilt disease however very little information is available on biology and ecology of this species (Smith et al., 2008).

30. *Bursaphelenchus mucronatus* Mamiya & Enda, 1979  
Pathogenic to *Pinus thunbergii* and *P. taiwanensis* seedlings (Chen et al., 2010). Is able to carry bacteria responsible for the pine wilt disease complex (Zhao et al., 2009). The quarantine status of this species is not clear (See Society of Nematologists expert comments and
| Page | Discussion on This Species | Pathogenic to Pine Trees; Causes Pine Wilt Disease (Kiyohara & Tokushige, 1971). Widely Quarantined Species (Dwinell & Nickle, 1989; Mota & Vieira, 2008; Zhao et al., 2008). | Parthenophytoidea Cacti (Filipjev & Schuermans-Stekhoven, 1941) Krall & Krall, 1978 Parasitises Cactus (Zygocactus truncatus and Hyllocereus trigonus) and Commonly Grown Ornamental Cacti (Cho et al., 1995; Narbaev & Mirsalimova, 1989; Paneque & Sampedro, 1986). | Parasitises Barley and is able to reproduce on weed hosts (Tovar-Soto et al., 2007; Tovar-Soto et al., 2003). | Parasitises Barley (Cid del Prado & Miranda, 2008). | Parasitises Groundnut Seeds and Pods. Downgrades Groundnut Kernel Quality by 32-64% (McDonald et al., 2005). | Important Pest of Rice. Caused Yield Losses of 10-100% (Cox & Rahman, 1980; Hashioka, 1963; Latif et al., 2011; Rahman et al., 1994). | Important Pest of a Wide Range of Root and Tuber Crops Including Potato, Sweet Potato, Garlic, Onions, Peanut. Caused Potato Yield Loss of up to 94% in Sweden (Andersson, 1971), 5-10% in Iran (Barooti, 1997). Controlling D. destructor in Sweet Potato Resulted in Yield Increase (Jin et al., 2008). | Important Pest of a Wide Range of Crops. Yield Loss can be as High as 60-80% of Onions and Garlic (Sturhan & Brzeski, 1991), Damages Potato (Goodey, 1956), Canola (Taylor & Szot, 2000). There are about 30 Biological Races which Have Different Host Ranges (Sturhan et al., 2008). Forms Disease Complexes with Fungal Pathogens (Hillnhutter et al., 2011). | Pathogenic to Beans. Recently-Described Species from the D. dipsaci Species Complex Previously Referred to as the Giant Race of D. dipsaci (Vovlas et al., 2011). | Found on the Leaves, Stems and Roots of Medicago Sativa (Commonly Grown as Pasture) (Wasilewska, 1965) and Has Been Found Associated with Other Crops Such as Wheat (Erum & Shahina, 2010; Kheiri et al., 2002). | Parasitises Edible Mushroom (Goodey, 1960; Grewal & Siddiqi, 1993). Caused Yield Loss of up to 70% (Aman et al., 2002). | Recently-Described Species Parasitising Cirsium Arvense (Weed) Previously Reported as a Race of D. dipsaci (Chizhov et al., 2010). Is of Phytosanitary Importance Because of Potential Confusion with D. dipsaci. | Pathogenic to Celery, Tomato and Maize (Paracer et al., 1968; Perry, 1953). | Important Pest of Potato with Reported Yield Loss of up to 80% (Talavera et al., 1998). Yield Loss Varies Depending on |
46. *Globodera rostochiensis*<sup>+</sup>  
(Wollenweber, 1923) Skarbilovich, 1959  
Important pest of potato with reported yield loss of up to 85% (Greco *et al.*, 1984). Yield loss varies depending on potato cultivar, population density and species pathotypes (Evans & Franco, 1979).  
Yes

47. *Globodera tabacum*  
(Lownsbery & Lownsbery, 1954) Skarbilovich, 1959  
Important pest of tobacco with reported yield losses of 40-60% (LaMondia, 1995; LaMondia, 2002). Forms disease complex with *Fusarium oxysporum* (LaMondia & Taylor, 1987).  
Yes

48. *Helicotylenchus dihystera*  
(Cobb, 1893) Sher, 1961  
Pathogenic to eggplant, tomato, wheat (Firoza & Maqbool, 1995), peanut, millet (Baujard & Martiny, 1995d) and fruit trees (Rossi & Camargo Barbosa Ferraz, 2005). Parasitizes a wide range of crop plants.  
-

49. *Helicotylenchus microcephalus* Sher, 1966  
Pathogenic to banana; causes root necrosis and stunted growth of banana (Bridge & Page, 1984). Also associated with fruit trees (Rossi & Camargo Barbosa Ferraz, 2005).  
-

50. *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956  
Important pest of banana; caused yield loss of up to 29% (Brentu *et al.*, 2004; Speijer *et al.*, 1999).  
Yes

51. *Helicotylenchus oleae*  
(Inserra, Vovlas & Golden, 1979)  
Parasitises olive trees (Inserra *et al.*, 1979; Vovlas & Larizza, 1994). Yield loss estimates are not available.  
Yes

52. *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956  
-

53. *Helicotylenchus varicaudatus* Yuen, 1964  
Parasitises wheat (Jones, 1978), carnations (Khanna & Jyot, 2002) and marram grass (Reis *et al.*, 2010).  
-

54. *Helicotylenchus vulgaris* Yuen, 1964  
Parasitises olives (Abrantes *et al.*, 1987) and sugarbeet (Ebrahimi *et al.*, 2004; Spaull, 1982). Data on yield loss not available.  
-

55. *Hemicriconemoides cocophilus*  
(Loos, 1949) Chitwood & Birchfield, 1957  
Parasitises small millets (Jain, 2009), rice (Sharma *et al.*, 1992a), sugarcane (Cadet & Albrecht, 1992), ornamental plants, medicinal plants (Rathour *et al.*, 2003) and root crops (Ray *et al.*, 1992).  
-

56. *Hemicriconemoides litchi*  
(Edward & Misra, 1964)  
Parasitises litchi (Nath *et al.*, 2008) and mango (Liu & Feng, 1995; Ni *et al.*, 2004).  
-

57. *Hemicriconemoides mangiferae* Siddiqi, 1961  
-

58. *Hemicyclophiophora arenaria*  
(Raski, 1958)  
-

59. *Hemicyclophiophora poranga*  
(Monteiro & Lordello, 1978)  
Pathogenic to tomato (Chitambar, 1993; Chitambar, 1994) and celery (Emilse *et al.*, 2011). Associated with maize (Jamali *et al.*, 2004).  
-

60. *Hemicyclophiophora similis*  
(Thorne, 1955)  
Pathogenic to carrots in glasshouse experiment (McKewen, 1979) and cranberry (Bird, 1963).  
-

61. *Heteroderda avenae*<sup>†</sup>  
(Wollenweber, 1924)  
Important pest of wheat with reported yield loss of 20-50% (Meagher, 1982; Meagher & Brown, 1974).  
Yes
<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Description</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.</td>
<td><em>Heterodera cajani</em> Koshy, 1967</td>
<td>Pathogenic to pigeon pea, reduced yield by 20-67 % and mungbean yield by 86% (Saxena &amp; Reddy, 1987; Sharma et al., 1993a).</td>
<td>Yes</td>
</tr>
<tr>
<td>63.</td>
<td><em>Heterodera carotae</em> Jones, 1950</td>
<td>Pathogenic to carrots, reduced yield by 50% (Bossis &amp; Mugniéry, 1989; Greco, 1987).</td>
<td>Yes</td>
</tr>
<tr>
<td>64.</td>
<td><em>Heterodera ciceri</em> Vovlas, Greco &amp; Di Vito, 1985</td>
<td>Pathogenic to chickpeas and lentils, yields reduced by 20-100% depending on population density of nematode (Greco et al., 1988; Greco et al., 1993).</td>
<td>Yes</td>
</tr>
<tr>
<td>65.</td>
<td><em>Heterodera cruciferae</em> Franklin, 1945</td>
<td>Pathogenic to cabbage, rape, radish, rutabaga (Chizhov et al., 2009; Evans &amp; Webb, 1989; Krnjaic´ &amp; Krnjaic´, 1987).</td>
<td>Yes</td>
</tr>
<tr>
<td>66.</td>
<td><em>Heterodera daverti</em> Wouts &amp; Sturhan, 1978</td>
<td>Pathogenic to carnations (Ambrogioni &amp; D'Errico, 1994) and Egyptian clover (Massoud et al., 1988).</td>
<td>-</td>
</tr>
<tr>
<td>67.</td>
<td><em>Heterodera delvii</em> Jairajpuri, Khan, Setty &amp; Govindu, 1979</td>
<td>Parasitises cereals (Jairajpuri et al., 1979; Krishna Prasad et al., 1980).</td>
<td>-</td>
</tr>
<tr>
<td>68.</td>
<td><em>Heterodera elachista</em> Ohshima, 1974</td>
<td>Parasitises upland rice with reported yield loss of 16% (Ohshima, 1974; Shimizu, 1976).</td>
<td>-</td>
</tr>
<tr>
<td>69.</td>
<td><em>Heterodera fici</em> Kirjanova, 1954</td>
<td>Pathogenic to figs (Braasch, 1973). Can also occur in glasshouses (Krnjaic´ et al., 1994; Monteiro et al., 1977; Subbotin et al., 1989).</td>
<td>Yes</td>
</tr>
<tr>
<td>70.</td>
<td><em>Heterodera filipjevi</em> (Madzhidov, 1981) Stelter, 1984</td>
<td>Pathogenic to wheat with reported yield loss of up to 48% (Hajihasani et al., 2010a).</td>
<td>Yes</td>
</tr>
<tr>
<td>71.</td>
<td><em>Heterodera glycines</em> Ichinohe, 1952</td>
<td>Important pest of soybean with reported yield loss of up to 70% (Ichinohe 1955). Also see (Wrather et al., 1997).</td>
<td>Yes</td>
</tr>
<tr>
<td>72.</td>
<td><em>Heterodera goettingiana</em> Liebscher, 1892</td>
<td>Pathogenic to peas and broad beans; pea yield reduced by 68-85% (Greco &amp; Di Vito, 1994; Greco et al., 1991).</td>
<td>Yes</td>
</tr>
<tr>
<td>73.</td>
<td><em>Heterodera graminis</em> Stynes, 1971</td>
<td>Parasitises turf grass (<em>Cynodon dactylon</em>) on bowling greens (Stynes, 1971). Data on economic importance is not available.</td>
<td>Yes</td>
</tr>
<tr>
<td>74.</td>
<td><em>Heterodera hordecalis</em> Andersson, 1975</td>
<td>Parasitises cereals, commonly associated with barley, rye, grasses (Andersson, 1975) and wheat (Lombardo et al., 2009).</td>
<td>-</td>
</tr>
<tr>
<td>75.</td>
<td><em>Heterodera humuli</em> Filipjev, 1934</td>
<td>Pathogenic to hops (Hafez et al., 1999b; Hay &amp; Pethybridge, 2003).</td>
<td>-</td>
</tr>
<tr>
<td>76.</td>
<td><em>Heterodera latipons</em> Franklin, 1969</td>
<td>Pathogenic to wheat, reduced yields by 55% (Hajihasani et al., 2010b).</td>
<td>Yes</td>
</tr>
<tr>
<td>77.</td>
<td><em>Heterodera medicaginis</em> Kirjanova, 1971</td>
<td>Pathogenic to lucerne (<em>Medicago sativa</em>, <em>M. falcata</em> and <em>M. lupulina</em>); reduced yields by 46% at high population densities (Alpašev et al., 1980; Artokhina, 1984; Terent'eva, 1982).</td>
<td>-</td>
</tr>
<tr>
<td>78.</td>
<td><em>Heterodera mediterranea</em> Vovlas, Inserra &amp; Stone, 1981</td>
<td>Pathogenic to olive and pistachio (Castillo &amp; Vovlas, 2002; Castillo et al., 1999; Vovlas &amp; Inserra, 1983; Vovlas et al., 1981).</td>
<td>-</td>
</tr>
<tr>
<td>81.</td>
<td><em>Heterodera sacchari</em> Luc &amp; Merny, 1963</td>
<td>Pathogenic to upland rice, reduced yields by 57% (Babatola, 1983; Coyne &amp; Plowright, 2000; Lamberti et al., 1991). Damage to rice is severe under drought</td>
<td>Yes</td>
</tr>
<tr>
<td>Number</td>
<td>Species</td>
<td>Conditions</td>
<td>Economic Importance</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------</td>
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</tr>
<tr>
<td>82</td>
<td><em>Heterodera schachtii</em> Schmidt, 1871</td>
<td>Important pest of sugarbeet (Muller, 1999) and pathogenic on a number of crops. Reduced yield of spinach by 49%, table beet by 30%, rutabagas by 35% and cabbage by 24% (Olthof et al., 1974).</td>
<td>Yes</td>
</tr>
<tr>
<td>83</td>
<td><em>Heterodera skohensis</em> Kaushal, Sharma &amp; Singh, 2000</td>
<td>Parasitises rice (Kaushal et al., 2000). Data on economic importance is not available.</td>
<td>-</td>
</tr>
<tr>
<td>84</td>
<td><em>Heterodera trifolii</em> Goffart, 1932</td>
<td>Important pest of clover (Clements &amp; Cook, 1998; Mowat, 1974; Sarathchandra et al., 1995; Sikora, 1977; Yeates, 1978). Also damages sugarbeet (Heijbroek &amp; Maas, 1982) and carnations (Lamberti et al., 1987b).</td>
<td>Yes</td>
</tr>
<tr>
<td>85</td>
<td><em>Heterodera zeae</em> Koshy, Swarup &amp; Sethi, 1971</td>
<td>Pathogenic to maize, reduced yield by 13-73% (Hashmi et al., 1993; Ismail et al., 1996; Krusberg et al., 1997).</td>
<td>Yes</td>
</tr>
<tr>
<td>86</td>
<td><em>Hirschmanniella gracilis</em> (de Man, 1880) Luc &amp; Goodey, 1964</td>
<td>Parasitises rice (Dash et al., 2008; Mei et al., 2009), sunflower (Khan et al., 2011), tall fescue (Prior et al., 2010b) and pondweed potamogeton (Prejs, 1986).</td>
<td>Yes</td>
</tr>
<tr>
<td>87</td>
<td><em>Hirschmanniella imamuri</em> Sher, 1968</td>
<td>Pathogenic to rice, reduced yields by 31-37% under experimental conditions (Babatola, 1979; Babatola &amp; Bridge, 1979; Ichinohe, 1988; Kuwahara &amp; Iyotami, 1970).</td>
<td>Yes</td>
</tr>
<tr>
<td>88</td>
<td><em>Hirschmanniella miticausa</em> Bridge, Mortimer &amp; Jackson, 1983</td>
<td>Pathogenic to taro (Colocasia esculenta) and causes corm rot or ‘mitimiti’ disease (Bridge &amp; Page, 1984). Yield loss estimates not available.</td>
<td>Yes</td>
</tr>
<tr>
<td>89</td>
<td><em>Hirschmanniella orzyae</em> (van Breda de Hann, 1902) Luc &amp; Goodey, 1964</td>
<td>Important pest of rice, yield reduced by 25-39% (Babatola &amp; Bridge, 1979; Cho et al., 1994; Jonathan &amp; Velayutham, 1987; Ying et al., 1996; Youssef &amp; El-Hamawi, 1996).</td>
<td>Yes</td>
</tr>
<tr>
<td>90</td>
<td><em>Hirschmanniella spinicaudata</em> (Schuurmans Stekhoven, 1944) Luc &amp; Goodey, 1962</td>
<td>Parasitises rice (Babatola &amp; Bridge, 1979; Babatola &amp; Bridge, 1980; Rinaudo &amp; Germani, 1981).</td>
<td>Yes</td>
</tr>
<tr>
<td>91</td>
<td><em>Hoplolaimus</em> (Basirolaimus) columbus Sher, 1963</td>
<td>Important pest of cotton (Bond &amp; Mueller, 2007; Koenning &amp; Bowman, 2005; Koenning et al., 2004; Noe &amp; Imbriani, 1986) and soybean (Kinloch, 1980; Perez et al., 2003).</td>
<td>Yes</td>
</tr>
<tr>
<td>92</td>
<td><em>Hoplolaimus</em> (Hoplolaimus) galeatus (Cobb, 1913) Thorne, 1935</td>
<td>Pathogenic to soybean (Rodriguez-Kabana &amp; Thurlow, 1980; Weaver &amp; Rodriguez-Kabana, 1987), pine (Stokes, 1982) and turf (Giblin-Davis et al., 1995; Nambiar et al., 2008). Also parasitises lucerne (Ng &amp; Chen, 1985).</td>
<td>Yes</td>
</tr>
<tr>
<td>94</td>
<td><em>Hoplolaimus</em> (Ethiolaimus) pararobustus* (Schuurmans Stekhoven &amp; Teuniessen, 1938) Sher, 1963</td>
<td>Parasitises coffee (Vovlas &amp; Lamberti, 1985), banana (Mateille, 1992; Speijer et al., 2001) and cowpea (Baujard &amp; Martiny, 1995c).</td>
<td>-</td>
</tr>
<tr>
<td>95</td>
<td><em>Hoplolaimus</em> (Basirolaimus) seinhorsti Luc, 1958</td>
<td>Parasitises rice, cowpea, pepper, eggplant, tomato, black gram, soybean, pineapple, cocoa (Lamberti et al., 1993), pigeon pea (Sharma &amp; Nene, 1988), mulberry (Toida &amp; Keereewan, 1991) and sandalwood (Sivaprakash et al., 1995c).</td>
<td>-</td>
</tr>
</tbody>
</table>
96. *Ibipora jara* Monteiro & Lordello, 1977  
Parasitises sugarcane (Monteiro & Lordello, 1977). Not much information available on the species.

Parasitises sugarcane (Roman, 1968). Not much information available on the species.

98. *Ibipora lolii* (Siviour, 1978) Siviour & McLeod, 1979  
Pathogenic to turf and pasture grasses (Siviour, 1978; Siviour & McLeod, 1979).

Parasitises celery and *Chenopodium quinoa* (Bleve-Zacheo et al., 1977) and *Chenopodium quinoa* (Bleve-Zacheo et al., 1984). Also acts as vector of Artichoke Italian Latent Virus (Lamberti & Bleve-Zacheo, 1977).

100. *Longidorus arthensis* Brown, Grunder, Hooper, Klingler & Kunz, 1994  
Acts as vector of Cherry Rosette Disease caused by Nepovirus (Brown et al., 1994a; Kunz, 2003).

101. *Longidorus attenuatus* Hooper, 1961  
Parasitises sugarbeet and associated with docking disorder (Whitehead & Hooper, 1970). Also acts as vector of Tomato Black Ring Virus (Gibbs & Harrison, 1964b; Harrison, 1964) and Artichoke Italian Latent Virus (Taylor et al., 1976).

102. *Longidorus diaeductus* Eveleigh & Allen, 1982  
Vector of Peach Rosette Mosaic Virus (Allen et al., 1982; Eveleigh & Allen, 1982). Is subject to compulsory control in European Union (Karnkowski, 2004).

103. *Longidorus elongatus* (de Mannon, 1876) Micoletzky, 1922  

Vector of Artichoke Italian Latent Virus (Brown et al., 1997; Roca et al., 1982).

105. *Longidorus leptocephalus* Hooper, 1961  
Vector of Cherry Leaf Roll Virus (Jones et al., 1981) and feeds directly on the roots of *Lolium perenne* (Robertson et al., 1984).

106. *Longidorus macrosoma* Hooper, 1961  
Vector of Raspberry Ringspot Virus (Bercks, 1968; Buser, 1999; Trudgill & Brown, 1978), Carnation Ringspot Virus (Fritzche & Schmelzer, 1967) and Cherry Leaf Roll Virus (Jones et al., 1981). Also causes direct damage to rose (Winfield, 1974).

Vector of Mulberry Ringspot Virus (Bellizzi, 2004; Yagita & Komuro, 1972).

Associated with peas (Edward et al., 1964), soybean (Fourie et al., 2001), maize (De Waele & Jordaan, 1988a) and sugarcane (Spaull & Heyns, 1991).

Parasitises black pepper (Freire & Monteiro, 1978), Associated with sugarcane (Rossi et al., 1996), cocoa (Sharma & Loof, 1974) and fig (Campos, 1997).

Associated with peach tree short life syndrome (Lowsbrey et al., 1973; Nesmith et al., 1981; Nyczepir et al., 1983). Parasitises clover, carnation, tomato (Hussey et al., 1992) and other herbaceous plants (Zehr et al., 1986).
<table>
<thead>
<tr>
<th>No.</th>
<th>Species Name</th>
<th>Host/Pathogenicity</th>
<th>Quarantine Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>111</td>
<td><em>Meloidogyne acronea</em></td>
<td>Coetzee, 1956: Pathogenic to cotton and sorghum, reduced yields by 90% and 56% respectively (Page 1983 cited in CABI ISC).</td>
<td>Yes</td>
</tr>
<tr>
<td>114</td>
<td><em>Meloidogyne arenaria</em></td>
<td>(Neal, 1889) Chitwood, 1949: Polyphagous and pathogenic to a wide range of crops. Reduced yield of groundnuts by 13-50% (Patel et al., 1996) and oriental melon by 45% (Kim &amp; Ferris, 2002). Species is not included on list of regulated pests in many countries because of its widespread occurrence.</td>
<td></td>
</tr>
<tr>
<td>115</td>
<td><em>Meloidogyne artiellia</em></td>
<td>Franklin, 1961: Pathogenic to chickpeas (Greco et al., 1994; Greco et al., 1992). Forms disease complex by interacting with <em>Fusarium oxysporum</em> (Castillo et al., 2003).</td>
<td>Yes</td>
</tr>
<tr>
<td>116</td>
<td><em>Meloidogyne brasiliensis</em></td>
<td>Charchar &amp; Eisenback, 2002: Pathogenic to tomato (Charchar &amp; Eisenback, 2002) and is able to parasitise pea and tomato with the root knot resistance <em>Mi</em> gene (Charchar et al., 2010).</td>
<td></td>
</tr>
<tr>
<td>117</td>
<td><em>Meloidogyne brevicauda</em></td>
<td>Loos, 1953: Pathogenic to tea (Loos, 1953; Mehta &amp; Somasekhar, 1998).</td>
<td>Yes</td>
</tr>
<tr>
<td>118</td>
<td><em>Meloidogyne citri</em></td>
<td>Zhang, Gao, &amp; Weng, 1990: Parasitises citrus (Shaosheng et al., 1990; Zhang &amp; Xu, 1994), limited information is available on this species.</td>
<td>Yes</td>
</tr>
<tr>
<td>120</td>
<td><em>Meloidogyne donghaiensis</em></td>
<td>Zheng, Lin, &amp; Zheng, 1990: Parasitises citrus (Zheng et al., 1994), limited information is available on this species.</td>
<td></td>
</tr>
<tr>
<td>121</td>
<td><em>Meloidogyne enterolobii</em></td>
<td>Yang &amp; Eisenback, 1983: Polyphagous species; caused yield loss in tomato by 65% (Cetintas et al., 2007) and damage on guava (Iwahori et al., 2009; Tigano et al., 2010). Can reproduce on cultivars with the <em>Mi</em> resistance gene (Brito et al., 2007; Cetintas et al., 2008; Cetintas et al., 2007; Kiewnick et al., 2009). Also see (Castagnone-Sereno, 2012) on the quarantine significance of this species.</td>
<td>Yes</td>
</tr>
<tr>
<td>122</td>
<td><em>Meloidogyne ethiopica</em></td>
<td>Whitehead, 1968: Pathogenic to kiwi fruit (Carneiro et al., 2003), grape (Di Vito et al., 2009) and tomato (Strajnar et al., 2012). Potential quarantine species in Europe.</td>
<td>Yes</td>
</tr>
<tr>
<td>123</td>
<td><em>Meloidogyne exigua</em></td>
<td>Goeldi, 1892: Pathogenic to coffee; caused yield loss of 45% (Barbosa et al., 2010; Barbosa et al., 2004). Parasitises rubber trees (Correa &amp; Rodella, 2002; Schwob et al., 1999).</td>
<td>Yes</td>
</tr>
<tr>
<td>124</td>
<td><em>Meloidogyne fallax</em></td>
<td>Karssen, 1996: Pathogenic to potato and carrots; reduces produce market value (Karssen et al., 2009).</td>
<td>Yes</td>
</tr>
<tr>
<td>125</td>
<td><em>Meloidogyne floridensis</em></td>
<td>Handoo, Nyczepir, Esmenjaud, van der Beek, Castagnone-Sereno, Carta, Skantar &amp; Higgins, 2004: Parasitises peach (Handoo et al., 2004) and ornamental plants (Brito et al., 2010). Is able to overcome <em>Mi</em> gene resistance in tomato (Stanley et al., 2009).</td>
<td></td>
</tr>
<tr>
<td>126</td>
<td><em>Meloidogyne fujianensis</em></td>
<td>Pan, 1985: Parasitises citrus (Pan et al., 1999; Pan, 1985). Limited information is available on this species.</td>
<td>Yes</td>
</tr>
<tr>
<td>127</td>
<td><em>Meloidogyne graminicola</em></td>
<td>Important pest of rice, caused yield losses of 11-73% in rice.</td>
<td>Yes</td>
</tr>
<tr>
<td>Page</td>
<td>Reference</td>
<td>Description</td>
<td>Status</td>
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</tr>
<tr>
<td>129.</td>
<td>Meloidogyne hapla</td>
<td>Polyphagous, pathogenic to many crops. Tomato yield reduced by up to 50% (Barker et al., 1976) and lettuce yield reduced by up to 64% (Olthof &amp; Potter, 1972; Viaene &amp; Abawi, 1996).</td>
<td>Yes</td>
</tr>
<tr>
<td>130.</td>
<td>Meloidogyne incognita</td>
<td>Polyphagous, pathogenic to many crops. Vegetable yield reduced by up to 90% (Bhatti &amp; Jain, 1979; Lamberti et al., 1988), tomato yield reduced by up to 85% (Barker et al., 1976) and cotton yield reduced by up to 47% (Davis &amp; May, 2005). Species is not included on list of regulated pests because of its widespread occurrence.</td>
<td>-</td>
</tr>
<tr>
<td>131.</td>
<td>Meloidogyne indica</td>
<td>Parasitises Amaranthus, watermelon, cowpea (Patel et al., 2003) and medicinal plants (Lin et al., 2004).</td>
<td>-</td>
</tr>
<tr>
<td>132.</td>
<td>Meloidogyne javanica</td>
<td>Parasitises peanut and banana yield reduced by up to 30% (Brentu et al., 2004). Species and is not included on list of regulated pests because of its widespread occurrence.</td>
<td>-</td>
</tr>
<tr>
<td>133.</td>
<td>Meloidogyne jianyangensis</td>
<td>Pathogenic to mandarin orange (Yang et al., 1990; Zhu et al., 1991), limited information is available on this species.</td>
<td>-</td>
</tr>
<tr>
<td>134.</td>
<td>Meloidogyne kongi</td>
<td>Pathogenic to turf grass, causes yellow patch disease on golf courses (Karssen et al., 2004). Parasitises potato (De Weerdt et al., 2011; Thoden et al., 2012). For a discussion on the phytosanitary importance of this species, see Turner &amp; Fleming, (2005) and Morris et al. (2011).</td>
<td>Yes</td>
</tr>
<tr>
<td>135.</td>
<td>Meloidogyne naasi</td>
<td>Polyphagous species, pathogenic to cereals and reduced barley yields by up to 50% (York, 1980). Parasitises peas and beans (Bélaire et al., 2006; Caubel et al., 1971; Ediz, 1972; Gooris &amp; D'Herde, 1969; Gooris &amp; D'Herde, 1972).</td>
<td>Yes</td>
</tr>
<tr>
<td>136.</td>
<td>Meloidogyne oryzae</td>
<td>Polyphagous species, pathogenic to rice and reduced yields by 10-15% in greenhouse experiments (Segeren &amp; Sanchit, 1984). Additional hosts include grasses, wheat, tomato and potato (Maas et al., 1978).</td>
<td>Yes</td>
</tr>
<tr>
<td>137.</td>
<td>Meloidogyne paranaensis</td>
<td>Pathogenic to turf grass, causes yellow patch disease on golf courses (Karssen et al., 2004). Parasitises potato (De Weerdt et al., 2011; Thoden et al., 2012). For a discussion on the phytosanitary importance of this species, see Turner &amp; Fleming, (2005) and Morris et al. (2011).</td>
<td>Yes</td>
</tr>
<tr>
<td>138.</td>
<td>Meloidogyne pisi</td>
<td>Parasitises peas, bean (Phaseolus), tobacco, tomato and potato (Charchar et al., 2008a).</td>
<td>-</td>
</tr>
<tr>
<td>139.</td>
<td>Meloidogyne phaseoli</td>
<td>Parasitises peas, bean (Phaseolus), tobacco, tomato and potato (Charchar et al., 2008a).</td>
<td>-</td>
</tr>
</tbody>
</table>
Eisenback, Charchar & Boiteau, 2008

144. Meloidogyne polycephannulata
Parasitises tomato and carrots (Charchar et al., 2009). -

145. Meloidogyne salasi
Lopez, 1984
Pathogenic to rice (Lopez, 1984; Sancho et al., 1987). Parasitises black rice and sedge (Medina et al., 2009). Yes

146. Meloidogyne thailandica
Handoo, Skantar, Carta & Erbe, 2005
Parasitises ginger (Handoo et al., 2005) species described from intercepted material however information on this species in its native range is not available. -

147. Meloidogyne thamesi
Chitwood in Chitwood, Specht & Havis, 1952
Parasitises tomato (Amin, 1993), cowpeas, lettuce (Ponte, 1987; Ponte & Santos, 1981), Bohemeria nivea (Mishra & Mandal, 1988), tea (Huan, 1983) and Paullinia cupana var. sorbilis (Ferraz & Campelo, 1988). Yes

148. Meloidogyne trifoliophila
Bernard & Eisenback, 1997
Pathogenic to clover (Bell et al., 2006; Bernard & Eisenback, 1997; Mercer, 2005; Zahid et al., 2001). Parasitises soybean, broad bean, garden pea, Korean lespedea, sweet clover and common vetch (Bernard & Jennings, 1997). -

149. Merlinius brevidens
(Allen, 1955) Siddiqi, 1970
Pathogenic to wheat and associated with reduced yields (Jordaan et al., 1992; Smiley et al., 2006). Yes

150. Merlinius microdorus
(Geraert, 1966) Siddiqi, 1970
Pathogenic to lettuce and strawberry (Szczypielska, 1981). Associated with Rumex sp., Acer sp. (Ivanova, 1978) and barley (Andersen, 1979). -

151. Merlinius nanus
(Allen, 1955) Siddiqi, 1970
Parasitises watermelon (Tan & Okten, 2011). Associated with fruit trees (Lišková et al., 2007), cereals, pulses, vegetables (Erdal et al., 2001; Kepenekci & Okten, 1996) and bermuda grass (Ibrahim et al., 2000). -

152. Nacobbus aberrans²
(Thorne, 1935) Thorne & Allen, 1944
Pathogenic to tomato, potato, beans and sugarbeet. Reduced yields of tomato by up to 83%, potato by 65%, beans by 36% and sugarbeet by 10-20% (Cristobal-Alejo et al., 2006; Manzanilla-Lopez et al., 2002). Yes

153. Neodolichodorus australis
Hodda & Nambiar, 2005
Pathogenic to carrots (Hodda & Nambiar, 2005). -

154. Neodolichodorus citri
s’Jacob & Loof, 1996
Associated with plum, peach, corn and Chenopodium (s’Jacob & Loof, 1996; Vovlas et al., 2003). -

155. Paralongidorus australis
Stirling & McCulloch, 1984
Pathogenic to rice (Lehman & Stirling, 1988; Stirling & McCulloch, 1984; Stirling et al., 1989). -

156. Paralongidorus maximus
(Bütschli, 1874) Siddiqi, 1964
Acts as vector of Cherry Leaf Roll Virus, Raspberry Ringspot Virus and Tomato Black Ring Virus (Hübschen et al., 2004; Jones et al., 1994; Jones et al., 1981). Pathogenic to Scots pine and European larch (Boag et al., 1977). -

157. Paratrichodorus allius
(Jensen, 1963) Siddiqi, 1974
Acts as vector of Tobacco Rattle Virus which causes corky ringspot disease in potatoes (Charlton et al., 2010; Gieck et al., 2007; Ingham et al., 2007b; Mojtahedi & Santo, 1999). Associated with corn and wheat (Mojtahedi et al., 2002). Yes

158. Paratrichodorus anemones
(Loof, 1965) Siddiqi, 1974
Pathogenic to barley and wheat (Spaull, 1980; Spaull & Newton, 1984; Spaull & Murphy, 1983). Acts as vector of Pea Early Browning and Tobacco Rattle Tobraviruses -
159. *Paratrichodorus hispanus*  
Roca & Arias, 1986


160. *Paratrichodorus minor*  
(Colbran, 1956) Siddiqi, 1974

Pathogenic to sorghum, cowpea and eggplant (Baujard & Martiny, 1995a). Parasitises wheat (Jordaan et al., 1992), rice (Coyne et al., 2001), sugarcane (Blair & Stirling, 2007), pasture grasses *Dactylis glomerata*, * Lolium multiflorum*, *L. perenne*, * Festuca arundinacea* (Bell & Watson, 2001) and turf grass (Crow, 2005). Yes

161. *Paratrichodorus mirzai*  
(Siddiqi, 1960) Siddiqi, 1974

Associated with cotton (Bajaj & Bhatti, 1982), sugarcane (Maqbool & Hashmi, 1987) and litchi (Saha et al., 2006).

162. *Paratrichodorus nanus*  
(Allen, 1957) Siddiqi, 1974


163. *Paratrichodorus pachydermus*  
(Seinhorst, 1954) Siddiqi, 1974

Acts as vector of Tomato Black Ring Virus, Tobacco Rattle Virus and Pea Early Browning Virus (Brown et al., 1989; Gibbs & Harrison, 1964b; Ploeg et al., 1992). Parasitises pine (Choleva & Samuleyan, 1996). Associated with potato (De Pelsmaeker et al., 1985; Leshcheva, 1982) and fruit trees (Kumari, 2010; Lišková et al., 2007). Yes

164. *Paratrichodorus porosus*  
(Allen, 1957) Siddiqi, 1974

Associated with barley (Sheedy et al., 2010), sugarcane (Carneiro et al., 1982), grapevine (Aballay & Eriksson, 2006; Wang et al., 1996) and citrus (Park et al., 2008).

165. *Paratrichodorus renifer*  
Siddiqi, 1974

Parasitises blueberry (Braasch, 1976; Forge et al., 2009; Zasada et al., 2010b). Associated with ornamentals, eucalyptus, pine tree nurseries (Braasch & Sturhan, 1991; Ferraz et al., 1984) and azaleas (Brinkman, 1977; Cotten & Hooper, 1991).

166. *Paratrichodorus teres*  
(Hooper, 1962) Siddiqi, 1974

Acts as vector of Tobacco Rattle Virus which causes corky ringspot disease of potato (Bos & Krikke, 1991; De Pelsmaeker, 1987; Riga & Neilson, 2005). Parasitises artichoke (Karanastasi et al., 2005). Yes

167. *Paratrichodorus tunisiensis*  
(Siddiqi, 1963) Siddiqi, 1974


168. *Paratylenchus bukowinensis*  
Micoletzky, 1922

Pathogenic to parsley (Brzeski & Radzikowska, 1980) and celery (Brzeski, 1975). Parasitises peppermint (Monteiro, 1978).

169. *Paratylenchus hamatus*  
Thorne & Allen, 1950

Pathogenic to dry land peas and lentils, associated with reduced yields (Riga et al., 2008), parsley (Sprau, 1969), mint (Lisetskaya, 1971) and roses (Macdonald, 1976).

170. *Paratylenchus minutus*  
Linford in Linford, Oliveira & Ishii, 1949

Associated with wheat (Jordaan et al., 1992), sugarcane (Decker et al., 1970), pineapples (Linford et al., 1949) anthuriums and other tropical ornamentals (Bala & Hosein, 1996).

171. *Pratylenchus alleni*  
Ferris, 1961

Parasitises soybean (Acosta, 1982; Ferris & Bernard, 1962), tomato (Dickerson, 1979) and raspberry (Doucet et al., 2005).
<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>173.</td>
<td><em>Pratylenchus brachyurus</em> (Godfrey, 1929) Filippiev &amp; Schuurmans Stekhoven, 1941</td>
<td>Pathogenic to soybean (McSorley &amp; Dickson, 1989), maize (Inomoto, 2011), citrus (Inserra &amp; Vovlas, 1977) and pineapple (Dias-Arieira et al., 2010). Associated with <em>Miscanthus giganteus</em> and <em>Panicum virgatum</em> used for biofuels (Mekete et al., 2011). Yes</td>
</tr>
<tr>
<td>174.</td>
<td><em>Pratylenchus coffeae</em> (Zimmermann, 1898) Filipjev &amp; Schuurmans Stekhoven, 1941</td>
<td>Pathogenic to coffee (Inomoto et al., 2007; Trinh et al., 2011), citrus (O’Brien &amp; Tomerlin, 1973), banana (Brentu et al., 2004), reduced yield of banana cv. Grand Naine by 34% (van den Bergh et al., 2006). Yes</td>
</tr>
<tr>
<td>175.</td>
<td><em>Pratylenchus convallariae</em> Seinhorst, 1959</td>
<td>Pathogenic to <em>Convallaria</em> spp. (Cayrol &amp; Ritter, 1962). Parasitises sweet potato (Huan &amp; Xu, 1985), corn (Urek et al., 2003) and peach (Wu et al., 1993). Yes</td>
</tr>
<tr>
<td>176.</td>
<td><em>Pratylenchus crenatus</em> Loof, 1960</td>
<td>Parasitises potato, corn, cereals, grasses, olives, roses, raspberry and other hosts (Castillo &amp; Vovlas, 2007). Yes</td>
</tr>
<tr>
<td>177.</td>
<td><em>Pratylenchus delattrei</em> (Luc, 1958) Handoo &amp; Golden, 1989</td>
<td>Parasitises a wide range of crops including cotton, sugarcane, maize, oats, pearl millet, wheat, pigeon pea and peanut (Castillo &amp; Vovlas, 2007; Sharma et al., 1992b; van Biljon &amp; Meyer, 2000). -</td>
</tr>
<tr>
<td>178.</td>
<td><em>Pratylenchus fallax</em> Seinhorst, 1968</td>
<td>Parasitises a wide range of crops including cereals (Corbett, 1972), fruit trees and ornamental plants (Castillo &amp; Vovlas, 2007). Yes</td>
</tr>
<tr>
<td>179.</td>
<td><em>Pratylenchus flakkensis</em> Seinhorst, 1968</td>
<td>Parasitises a wide range of crops including corn, sweet potato, fruit trees and grasses (Castillo &amp; Vovlas, 2007). -</td>
</tr>
<tr>
<td>180.</td>
<td><em>Pratylenchus goodeyi</em> Sher &amp; Allen, 1953</td>
<td>Pathogenic to banana and forms disease complex with bacterial wilt pathogens (Pattison et al., 2002; Peregrine &amp; Bridge, 1992; Talwana et al., 2003). Associated with grapevine, strawberry and other crops (Castillo &amp; Vovlas, 2007). Yes</td>
</tr>
<tr>
<td>181.</td>
<td><em>Pratylenchus hexincisus</em> Taylor &amp; Jenkins, 1957</td>
<td>Parasitises corn, soybean, citrus, peach, apricot and <em>Pennisetum purpureum</em> (Castillo &amp; Vovlas, 2007). -</td>
</tr>
<tr>
<td>182.</td>
<td><em>Pratylenchus loosi</em> Loof, 1960</td>
<td>Pathogenic to tea (Gnanapragasam et al., 1987) pasture grasses (Inserra et al., 1996) and oranges (Castillo &amp; Vovlas, 2007; Ushiyama &amp; Ogaki, 1970). Yes</td>
</tr>
<tr>
<td>183.</td>
<td><em>Pratylenchus mediterraneus</em> Corbett, 1983</td>
<td>Parasitises chickpea, lentils, potato, carrots, wheat and chrysanthemum (Choi et al., 2006; Greco &amp; Di Vito, 1994; Orion &amp; Glazer, 1987; Orion et al., 1995; Orion et al., 1988). -</td>
</tr>
<tr>
<td>184.</td>
<td><em>Pratylenchus neglectus</em> (Rensch, 1924) Filipjev &amp; Schuurmans Stekhoven, 1941</td>
<td>Important pest of cereals, reduced wheat yields by up to 36%: (Smiley &amp; Machado, 2009; Taylor et al., 1999). Pathogenic to potato (Hafez et al., 1999a; Olthof, 1990), canola (Fatemy et al., 2006), oilseed rape (Kumari, 2012) and parasitises a wide range of other crops (Castillo &amp; Vovlas, 2007). Yes</td>
</tr>
<tr>
<td>185.</td>
<td><em>Pratylenchus penetrans</em> (Cobb, 1917) Filipjev &amp; Schuurmans Stekhoven, 1941</td>
<td>Pathogenic to a wide range of crops including cereals, corn potato, sweet potato, fruit trees and ornamental plants (Castillo &amp; Vovlas, 2007). Forms disease complex with fungal pathogen <em>Verticillium dahlia</em> to cause early dying complex in potato (Martin et al., 1982b). Yes</td>
</tr>
<tr>
<td>186.</td>
<td><em>Pratylenchus</em></td>
<td>Parasitises wheat and other cereals (Corbett, 1969; Corbett, -</td>
</tr>
<tr>
<td><strong>Pratylenchus pratensis</strong> (de Man, 1880) Filipjev, 1936</td>
<td>Pathogenic to cereals, winter wheat and rye (Goffart, 1942; Stepanchuk, 1978). Parasitises a wide range of crops (Castillo &amp; Vovlas, 2007). Forms disease complex with <em>Fusarium moniliforme</em> var. <em>subglutinans</em> (Revelo Moran et al., 1993).</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Pratylenchus pseudopratensis</strong> Seinhorst, 1968</td>
<td>Parasitises a wide range of crops including corn, millet, soybean, tea, fruit trees, strawberry and date palms (Castillo &amp; Vovlas, 2007). Associated with potato (Akgul et al., 2010).</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pratylenchus scribneri</strong> Steiner in Sherbakoff &amp; Stanley, 1943</td>
<td>Pathogenic to potato (Martin et al., 1982a) and reduced maize yield by 26% under experimental conditions (Olowe, 2011). Parasitises a wide range of other crops (Castillo &amp; Vovlas, 2007).</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Pratylenchus sudanensis</strong> Loof &amp; Yassin, 1971</td>
<td>Pathogenic to cotton and forms disease complex with <em>Fusarium oxysporum</em> f.sp. <em>vasinfectum</em> (Saadabi &amp; Yassin, 2007). Parasitises pigeon pea and lubia bean (Yassin &amp; Mohamed, 1980), potato, sweet potato, yams and sugarcane (Castillo &amp; Vovlas, 2007).</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pratylenchus teres</strong> Khan &amp; Singh, 1975</td>
<td>Parasitises cotton, millet and tobacco (Carta et al., 2002), soybean (Fourie et al., 2001), mango (Liu &amp; Feng, 1995), pine (Savkina, 1989), sugarcane (van den Berg &amp; Queneherve, 2000) and potato (Khan &amp; Singh, 1974).</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pratylenchus thornei</strong> Sher &amp; Allen, 1953</td>
<td>Important pest of cereals and causes wheat yield loss of up to 70% (Smiley et al., 2005; Taylor et al., 1999; Thompson et al., 2008). Parasitises a wide range of other crops (Castillo &amp; Vovlas, 2007).</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Pratylenchus vulnus</strong> Allen &amp; Jensen, 1951</td>
<td>Pathogenic to plum (McKerry, 1989) and other <em>Prunus</em> species (Pinochet et al., 1996), strawberry (yields reduced by 80%: Mohotti et al., 1997), chrysanthemum (Lee et al., 2008). Over 80 species of plants are reported as hosts (Castillo &amp; Vovlas, 2007).</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Pratylenchus zeae</strong> Graham, 1951</td>
<td>Pathogenic to maize causing complete crop failure: (Patel &amp; Patel, 2000), sugarcane (Tarte et al., 1977) and a wide range of other crops (Castillo &amp; Vovlas, 2007).</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Punctodera chalcoensis</strong> Stone, Sosa Moss &amp; Mulvey, 1976</td>
<td>Pathogenic to maize (Mundo et al., 1987; Suarez et al., 1985; Tovar-Soto et al., 2006).</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Punctodera matadorensis</strong> Mulvey &amp; Stone, 1976</td>
<td>Parasitises grasses (Mulvey &amp; Stone, 1976). Associated with potato, recorded during a routine survey for PCN (Handoo et al., 2010). Can be confused with other regulated cyst nematodes.</td>
<td>-</td>
</tr>
<tr>
<td><strong>Punctodera punctata</strong> (Thorne, 1928) Mulvey &amp; Stone, 1976</td>
<td>Parasitises grasses <em>Poa annua</em> (annual bluegrass), <em>Poa pratensis</em> (Merion Kentucky bluegrass), <em>Lolium perenne</em> (perennial ryegrass) and <em>Festuca rubra rubra</em> (Cook et al., 1992; Radice et al., 1984; Vandenbossche et al., 2011). Associated with potato and sugarbeet (Urek &amp; Lapajne, 2001).</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Pathogenic to melon (Khan & Khanzada, 1990) and maize (Khan *et al.*, 1988). Associated with ornamental plants (Hung *et al.*, 2011).


Pathogenic to citrus (Machon & Bridge, 1996). Limited information available on this species.


204. *Radopholus nativus* Sher, 1968
Pathogenic to wheat (Riley & Kelly, 2001). Parasitises canola, triticale, oat, field pea, faba bean, durum wheat, narrow-leaved lupin and chickpea (Vanstone, 2010).

205. *Radopholus similis* (Cobb 1893) Thorne, 1949
Important pest of banana (Gowen *et al.*, 2005) and a wide range of crops, with over 250 plant hosts (O’Bannon, 1977). Has two pathotypes, the banana pathotype is more widespread and parasitises banana plus other crops, while the citrus pathotype parasitises only citrus and is restricted to Florida, USA (Huettel *et al.*, 1984).


Parasitises barley, bean, corn, cowpea and bermuda grass (Dasgupta & Raski, 1968), soybean (Fourie *et al.*, 2001), macadamia, pearl millet, potato, papaya, thyme, tobacco, tomato, sunn hemp, sugarcane and cotton (Robinson *et al.*, 1998).

208. *Rotylenchulus reniformis* Linford & Oliveira, 1940

209. *Rotylenchulus robustus* (de Man, 1876) Filipjev, 1936


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<table>
<thead>
<tr>
<th>Species/Genus</th>
<th>Pathogenicity/Parasitism</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>211. <em>Scutellonema bradys</em> (Steiner, 1937) Andrassy, 1958</td>
<td>Pathogenic to yams (Acosta &amp; Ayala, 1975; Adesiyan et al., 1975), potato (Coyne et al., 2011), Sesamum indicum, Vigna unguiculata, pigeon pea, okra, tomato and melons (Adesiyan, 1976).</td>
<td>Yes</td>
</tr>
<tr>
<td>212. <em>Scutellonema clathricaudatum</em> Whitehead, 1959</td>
<td>Pathogenic to groundnut (Sharma et al., 1992c). Parasitises upland rice (Coyne et al., 2001), sunflower (Eldin &amp; Siddiq, 1995), maize (Talwana et al., 2008), medicinal plant Aloe barbadensis (Kindelan et al., 1991) and grapes (Wang et al., 1991).</td>
<td>-</td>
</tr>
<tr>
<td>214. <em>Scutellonema unum</em> Sher, 1964</td>
<td>Parasitises yams (Park &amp; Khan, 2007), potato, sweet potato (Njuguna &amp; Bridge, 1998), pigeon pea (Sharma et al., 1993b), maize, pineapple, citrus (van den Berg &amp; Heyns, 1973) and grape (Wang et al., 1991). Associated with Prunus amygdalus (Khan &amp; Khan, 1985) and Ficus sp. (Melillo &amp; Troccoli, 1993).</td>
<td>-</td>
</tr>
<tr>
<td>215. <em>Sphaeronema alni</em> Turkina &amp; Chizhov, 1986</td>
<td>Parasitises alder (Subbotin, 1989), downy birch (Prior et al., 2010a) and chestnut (Palomares-Rius et al., 2010).</td>
<td>-</td>
</tr>
<tr>
<td>216. <em>Subanguina chilensis</em> Vovlas Troccoli &amp; Moreno, 2000</td>
<td>Parasitises leaves of tree Nothofagus obliqua (Baldini Urrutia &amp; Aguayo Silva, 2007; Vovlas et al., 2000).</td>
<td>-</td>
</tr>
<tr>
<td>219. <em>Trichodorus cedarus</em> Yokoo, 1964</td>
<td>Parasitises bonsai trees (Hirata &amp; Yuhara, 1986), coniferous trees (Kiyohara, 1970), pear (Zhao et al., 2005), China fir, peach, apricot, persimmon and apple (Xu &amp; Decraemer, 1995).</td>
<td>-</td>
</tr>
<tr>
<td>221. <em>Trichodorus primitivus</em> (de Man, 1880) Micoletzky, 1922</td>
<td>Vector of Pea Early Browning Virus (Harrison, 1966), Spinach Yellow Mottle Virus (Kurppa et al., 1981) and Tobacco Rattle Virus which causes sparing disease of potato (Alphey et al., 1975; Brown &amp; Sykes, 1973; Ploeg et al., 1992).</td>
<td>-</td>
</tr>
<tr>
<td>222. <em>Trichodorus similis</em> Seinhorst, 1963</td>
<td>Vector of Tobacco Rattle Virus (Brown et al., 1996; van Hoof, 1967). Parasitises tobacco (Wyss, 1973; Wyss, 1975), beans (Coosemans, 1993), potato, hops, strawberry (De Pelsmaeker &amp; Coomans, 1985) and gladiolus (Cremer Yes</td>
<td></td>
</tr>
</tbody>
</table>
223. *Trichodorus viruliferus*  
Hooper, 1963  
Vector for Tobacco Rattle Virus (Brown et al., 1989) and Pea Early Browning Virus (Gibbs & Harrison, 1964a). Parasitises a wide range of plants including apple, barley, maize, pea, potato, rye, sugarbeet and wheat (Cooke, 1984; Gibbs & Harrison, 1964a; Hooper, 1963; Pitcher & McNamara, 1971).

224. *Tylenchorhynchus agri*  
Ferris, 1963  
Parasitises rice (Xie et al., 2007), coffee (Mekete et al., 2008), red clover (Amosu & Taylor, 1974; Coates-Beckford, 1982) and citrus (Esser et al., 1993).

225. *Tylenchorhynchus annulatus*  
(Cassidy, 1930)  
Golden, 1971  
Pathogenic to rice (Aly & Shaukat, 2000) and sugarcane (Bond et al., 2004; Hasselrot de Gomez et al., 1980). Parasitises corn (Chen et al., 2006), horseradish (Walters et al., 2004) and potato (Khan & Hussain, 2004).

226. *Tylenchorhynchus brassicae*  
Siddiqi, 1961  
Parasitises to rice (Khan et al., 1990a), tomato (Ahmad & Khan, 1988), cauliflower (Khan et al., 1994), chickpeas (Tiyagi & Alam, 1989) and pigeonpeas (Tiyagi & Parveen, 1990).

227. *Tylenchorhynchus clarus*  
Allen, 1955  

228. *Tylenchorhynchus claytoni*  
Steiner, 1937  

229. *Tylenchorhynchus cylindricus*  
Cobb, 1913  
Parasitises guava (Abivardi, 1973), strawberry (Nesterov & Koev, 1972), maize (Nesterov & Lizogubova, 1972), coconut (Valdez, 1980) and cotton (Tu et al., 1972).

230. *Tylenchorhynchus mashhoodi*  
Siddiqi & Basir, 1959  
Parasitises maize (Mahapatra & Das, 1984), rice (Baqri & Ahmad, 2000), sugarcane (Ray et al., 1994), sweet potato, cassava, yam (Ray et al., 1992), fruit trees (Afshar et al., 2006), chickpeas (Ali, 1993), cabbage (Bilgrami, 1994) and ginger (Luqman Khan & Makhnotra, 1998).

231. *Tylenchorhynchus nudus*  
Allen, 1955  
Parasitises wheat (Smolik, 1972), sorghum (Smolik, 1977), rice (Haider et al., 1996), sugarcane (Haider et al., 1987; Hu & Chu, 1964), bluegrass, bentgrass (Davis et al., 1994; Smolik & Malek, 1973), chilli (Prasad et al., 1991) and banana (Choudhury & Phukan, 1992).

232. *Tylenchulus palustris*  
Inserra, Vovlas, O’Bannon & Esser, 1988  
Parasitises peach, Carolina ash and saltbush (Eisenback et al., 2007; Inserra et al., 1990), swamp plants; *Aster elliottii*, *Liquidambar styraciflua*, *Borrichia arborescens* and *B. frutescens* (Dow et al., 1990).

233. *Tylenchulus semipenetrans*  
(Cobb, 1913)  
Important pest of citrus, responsible for slow decline disease of citrus (Cohn, 1965; Timmer & Davis, 1982). Other hosts include *Calodendrium capensis*, *Citrus volkameriana*, Ruta bracteosa and *R. graveolens* (Cohn, 1966), *Cynodora oblonga*, *Diospyros sp.*, *Olea europaea*, *Philadelphus coronarius*, *Poncirus trifoliata* and *Vitis vinifera* (Kwaye et al., 2008). Hosts differ depending on...
the species pathotype (Kwaye et al., 2008; Murguia et al., 2005; Toktay et al., 2005).

234. **Vittatidera zeaphila**
Bernard, Handoo, Powers, Donald & Heinz, 2010
Parasitises corn and goosegrass (Bernard et al., 2010). Recently described genus and species closely-related to economically important cyst nematodes.

235. **Xiphinema americanum**²
Cobb, 1913 sensu lato
Vector of nepoviruses; Cherry Rasp Leaf, Tobacco Ringspot, Tomato Ringspot (Brown et al., 1993), Soybean Severe Stunt Virus (Evans et al., 2007) and Peach Rosette Mosaic Virus (Allen et al., 1984). Parasitises a wide range of crop and weed hosts (Miller, 1980), including stone fruits, soybean, cotton, sugarcane, rice, tobacco and maize (CABI crop protection compendium). Species complex.

236. **Xiphinema basiri**
Siddiqi, 1959

237. **Xiphinema brasiliense**
Lordello, 1951
Parasitises cocoa (Afolami & Caveness, 1983), ornamental and flowering plants (Costa et al., 2003), citrus (Crozzoli et al., 1998), peach, grape (Maximiniano et al., 1998) and potato (Lordello, 1951).

238. **Xiphinema brevicolle**
Lordello & Da Costa, 1961

239. **Xiphinema bricolensis**
Ebsary, Vrain & Graham, 1989
Vector of Tomato Ringspot Virus (Brown et al., 1994b; Jones et al., 1995). Parasitises grape (Graham et al., 1988) and apple (Ebsary et al., 1989).

240. **Xiphinema californicum**
Lamberti & Bleve-Zacheo, 1979

241. **Xiphinema diversicaudatum**
(Mikoletzky, 1927)
Thorne, 1939
Acts as vector of Arabic Mosaic Virus (Harrison & Winslow, 1961), Raspberry Ringspot Virus (Fritzsche & Kegler, 1968) and Strawberry Latent Ringspot Virus (Trudgill et al., 1981). Parasitises strawberry, raspberry, ryegrass, hops, barley and wheat (Cotten, 1975; Flegg et al., 1970; Griffiths et al., 1982).

242. **Xiphinema ifacolum**
Luc, 1961
Parasitises tomato (Bleve-Zacheo et al., 1987), rice (Lamberti et al., 1987a; Lamberti et al., 1991), soybean (Lamberti et al., 1993), cocoa, citrus (Lamberti et al., 1992a), cowpea, eggplant, tomato, okra (Lamberti et al., 1992b), black pepper (Lamberti et al., 1983) and banana (Adiko, 1988).

243. **Xiphinema index**
Thorne & Allen, 1950

244. **Xiphinema insigne**
Loos, 1949
Pathogenic to grape (Lal et al., 1982) and coconut (Ekanayake & Lamberti, 1987). Parasitises lily bulbs
(Saigusa & Yamamoto, 1971), fruit trees (Chen et al., 2004), *Pennisetum purpureum* (Fang, 1994), bonsai trees (Hirata & Yuhara, 1986), citrus (Zhou et al., 2007) and pomegranate (Hu, 1991).

245. *Xiphinema italicum* Meyl, 1953

Vector of Grapevine Fanleaf Virus (Cohn et al., 1970; Dalmasso et al., 1972). Parasitises grape (Arias et al., 1994; Avgelis & Tzortzakakis, 2001), walnut (Ciancio et al., 1996), citrus (Edongali & El-Majberi, 1988) and fruit trees (Katalan-Gateva, 1980).

Yes

246. *Xiphinema pachtaicum* (Tulaganov, 1938) Kirjanova, 1951

Parasitises grape (Gangl et al., 2009; Kumari, 2004), apricot (Ivanova & Choleva, 1999), walnut (Lišková & Brown, 1998), strawberry (Samaliev & Mohamedova, 2011), cherry, cypress and fig (Koliopanos & Vovlas, 1977).

Yes

247. *Xiphinema rivesi* Dalmasso, 1969

Vector of Tobacco Ringspot, Tomato Ringspot, Cherry Leaf Rasp and Peach Rosette Mosaic Virus (Brown et al., 1994b; Forer et al., 1981; Sirca et al., 2007; Stobbs & Schagen, 1996). Parasitises fruit trees (Georgi, 1988; Gutiérrez-Gutiérrez et al., 2011; Islam et al., 1996; van Driel et al., 1990).

Yes


Parasitises citrus (1994). Associated with oak (Lamberti & Bleve-Zacheo, 1979). Belongs to the economically important *Xiphinema americanum* group (Gozel et al., 2006). Limited information is available on the biology and ecology of this species.

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Parasitises grape (Coiro et al., 2000; Samota et al., 1994), apricot (Ivanova & Choleva, 1999), walnut (Lišková & Brown, 1998), ornamental plants (Brzeski et al., 1978) and fruit trees (Choleva et al., 1984).

Yes


Pathogenic to celery, carrot, pea (Vovlas et al., 1976) and *Viola odorata* (Ambrogioni & Rapetti, 1992). Parasitises a wide range of plants: faba bean, chickpea, lentils (Di Vito et al., 1994; Troccoli & Di Vito, 2002; Vovlas & Inserna, 1977), sugarbeet (Ebrahimi et al., 2004), lucerne (van den Berg, 1989), ornamental plants (Deimi et al., 2008) and forest trees (Talavera et al., 1999).

Yes

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1 on an official list of regulated pests for at least one country globally.
2 See discussion on phytosanitary importance of PPN with pathotypes/races and species complexes.
3 Hunt & Handoo (2009) consider *M. thamesi* a synonym of *M. arenaria*. However measurements reported in Whitehead (1968) and Amin (1993) indicate differences from those of *M. arenaria*. *M. thamesi* also has different host reactions compared to *M. arenaria*. In the absence of more detailed published information supporting synonymising *M. thamesi* with *M. arenaria*, we retain *M. thamesi*.

3.5 Discussion

3.5.1 Importance of economic impact in assessing the phytosanitary risk of plant-parasitic nematodes

A small percentage of the approximately 3400 known species of PPN (Hodda, 2011), are widespread and cause significant losses to crop production (Koenning et al., 1999; Nicol et al., 2011; Sasser, 1988). Of the remainder, their importance as plant pathogens is unknown; some
species have limited distributions and cause localised damage to plants, and some species are recorded only once from their type host and locality. Hence, based on distribution, phytosanitary measures for PPN seem generally justifiable, depending on whether potential impacts outweigh costs.

Assessment of potential impacts on economically-important crops is crucial for determining the phytosanitary status of a pest species. However, yield loss estimates are available mainly for species whose pathogenicity or disease-causing abilities are already well known (Table 3.2). Data on the economic impacts of the majority of PPN remains sparse (Nicol et al., 2011). The available data can also be difficult to interpret and compare.

Yield loss caused by PPN is often used to determine economic importance, but there are limitations in its calculation. Yield loss calculations from different studies and countries do not necessarily use the same methods, with some reporting damage as percentage yield loss and others reporting as tonnes per hectare or as percentage yield gained after application of nematicides or as correlations of yield gains with declining PPN abundance. Indirect losses (see below) also generally remain unaccounted for in most yield loss figures.

Yield loss estimates based on work done long ago and under different nematode management regimes are also likely to require updating. Many frequently-used nematicides, such as Methyl Bromide, have been removed from the market (Noling & Becker, 1994) and alternative nematode management practices have been developed (Chitwood, 2002; Trudgill, 1991; Zasada et al., 2010a). PPN yield loss estimates from old publications may therefore need re-evaluation. In addition, PPN management practices differ between countries, depending on availability of nematicides, resistant varieties and expertise in PPN management (De Waele & Elsen, 2007; Sasser & Krishnappa, 1980; Sharma, 1997). For example, in developing countries, nematicide use is limited and PPN yield loss estimates could be much higher compared to developed countries, which use nematicides widely to reduce crop losses (Nicol et al., 2011; Sasser & Freckman, 1987).

The list of 250 PPN species in this paper, although representative of the major nematode genera, may not cover all species of phytosanitary importance. Some genera such as *Meloidogyne*, *Heterodera* and *Pratylenchus* are well represented in the list with over 20 species each (Table
3.2. All these genera are regarded as of economic importance (Castillo & Vovlas, 2007; Evans et al., 1993; Luc et al., 2005) and this is reflected in large numbers of publications on species from these genera. Lack of published information on the pathogenicity and economic importance of many less well-known species makes it difficult to predict the potential impacts of these species. Although over 100 valid species are known from genera such as *Xiphinema*, *Tylenchorhynchus* and *Hemicycliophora* (Coomans et al., 2001; Siddiqi, 2000) the economic importance of only a few species from these genera has been investigated.

### 3.5.2 Plant-parasitic nematode disease complexes and indirect impacts

Impacts can also be difficult to estimate or severely underestimated because PPN may injure plants in many different ways. One way is by direct feeding action (Bridge & Starr, 2007; Endo, 1975), but this may take many different forms including direct damage, root galls, root stunting or withdrawal of resources from other parts of the plant (Norton & Niblack, 1991). Another way PPN injure plants is through disease complexes formed with other organisms, including other PPN (Davis & Webster, 2005; Powell, 1971; Sidhu & Webster, 1974). This type of damage may also take many forms. Species from the genera *Longidorus*, *Paralongidorus*, *Paratrichodorus*, *Trichodorus* and *Xiphinema* can act as vectors for several important plant viruses (Decraemer, 1995; Hewitt et al., 1958; Taylor & Brown, 1997). Root damage caused by migratory endoparasites from the genera *Pratylenchus* and *Radopholus* allows ingress of damaging rots. The effects on the plant of both organisms together in all these cases are greater than the effects of the nematode alone. Furthermore, the amount of damage may differ according to the nematode pathotype, population levels, crop species or cultivar, nematode/farm management practices, edaphic factors and climatic conditions (Barker & Olthof, 1976; Green & Dennis, 1981; Lehman et al., 1971; Noe & Barker, 1985; Oteifa, 1997; Schouten & Beniers, 1997; Seinhorst, 1970). Damage symptoms and impacts are not always obviously associated with PPN, being frequently misidentified as due to drought, nutrient deficiency or other causes (Barker et al., 1994).

There is also little data on the impact of PPN species on biodiversity. Even though there are provisions under ISPM and IPPC to assess the effects of a species on biodiversity, it is seldom included in assessment of phytosanitary importance of PPN. Therefore a wide range of potential damage and interactions of PPN with other disease-causing organisms were taken into account while compiling the list of species.
Another reason for taking a broad approach is that apparently benign PPN can emerge as pests with changes in cropping patterns, nematode management, climate or arrival in new regions (Nicol et al., 2011). For instance, *Meloidogyne enterolobii* (syn. *M. mayaguensis*), is able to overcome the resistance of tomato and bell pepper genotypes carrying the *Mi-I, N* and *Tabasco* resistance genes widely used for nematode management (Brito et al., 2007; Castagnone-Sereno, 2012; Kiewnick et al., 2009). It has recently been added to the EPPO A2 list of pests recommended for regulation due to its pathogenicity and potential for spread in the EPPO region (EPPO, 2011). Except the European Union countries, elsewhere few PPN species are currently treated as regulated non-quarantine pests, although regulated non-quarantine status could be an effective way of preventing further spread of recently established exotic PPN species.

### 3.5.3 Assessing risks from plant-parasitic nematode pathotypes and species complexes

Another aspect to consider when assessing phytosanitary status is intra-specific variation. This is often not considered because most phytosanitary regulation is on the taxonomic level of the species. However, species from the economically-important genera *Belonolaimus, Ditylenchus, Globodera, Heterodera, Meloidogyne, Nacobbus, Radopholus, Rotylenchulus* and *Tylenchulus* all have pathotypes or races with distinctive host responses and differences in host range (Abugharbieh & Perry, 1970; Anthoine & Mugniéry, 2006; Gottlieb et al., 1986; Michell et al., 1973; Mojtahedi et al., 1988; Robertson et al., 2009; Sturhan et al., 2008; Toktay et al., 2005). Under the IPCC, absence of pathotypes in a country is justification for implementing quarantine measures against exotic pathotypes (FAO, 2013; EFSA, Panel on Plant Health 2012). For example, only the sugarbeet pathotype of *Nacobbus aberrans* is present in the USA, while the potato pathotype is absent, and hence the potato pathotype is a regulated pest in the USA (Inserra et al., 2004). The known highly damaging species such as *Globodera pallida, G. rostochiensis, Heterodera avenae* and *Ditylenchus dipsaci* which have pathotypes, therefore may require further phytosanitary risk assessment specific to pathotypes to prevent the spread of exotic pathotypes and justify regulatory measures (Hockland et al., 2012; Hockland et al., 2013). It is not known how widespread pathotype variability is in the many less well investigated PPN, but if it is as common in the best-known species, then this is another reason to take the broadest approach for assessing phytosanitary risk from PPN.
The recent description of the new species *Ditylenchus gigas* and *D. weischeri*, previously considered as part of *D. dipsaci* species complex (Vovlas *et al.*, 2011; Chizhov *et al.*, 2010), highlight the importance of research into taxonomy and specific identification methods, especially for species complexes and races. With more specific identification methods, the distributions and phytosanitary importance of closely related or cryptic species and races could be assessed more precisely.

Synonymising species names can also have important implications on the phytosanitary status of a species. For example, until recently *M. enterolobii* and *M. mayaguensis* were considered separate species, but their recent synonymization meant that when distribution, host range and other information published under both names were consolidated, the apparent risk increased substantially (see Castagnone-Sereno, 2012; Karssen *et al.*, 2012). Therefore information published under species synonyms also needs to be considered when assessing phytosanitary importance.

### 3.5.4 Biosecurity implications

Despite these limitations, making the best systematic predictions of impact and risk possible, based on as much data as can be obtained, is preferable to the alternative of empirical measurements of actual damage following real introductions of potentially damaging species. Where real introductions of exotic PPN have occurred and been measured, the impacts have mostly been substantial. Of course, by this stage, eradication is seldom an option (Hodda *et al.*, 2008).

More recently PPN species have been used as bio-indicators during quarantine inspections. In the UK, *Helicotylenchus dihystera* is often intercepted with planting materials supposedly grown in sterile conditions, indicating that phytosanitary standards were not met (Hockland & Anderson, 2012). Other microscopic plant pathogens such as fungi, bacteria and viruses may also use similar pathways to PPN (Grousset *et al.*, 2012), so targeting the generally larger PPN during quarantine inspections has assisted in reducing the risks from other microscopic quarantine organisms generally.

Phytosanitary risks are specific to countries or regions. Regulated organisms differ between countries depending on species distributions and regulatory or biosecurity policy in different countries. Formal pest risk analysis processes include multiple stages (initiation, pest
categorization, risk assessment and risk management) and so require considerable time and resources to scientifically assess and determine the regulatory measures commensurate with the risks posed by each species (Petter et al., 2010). Yet, it is important that this process is followed because the regulatory status of a species has important trade implications: countries can impose trade restrictions if certain species are present in the exporting country (Gebrehiwet et al., 2007; Cook et al. 2011).

3.6 Conclusions
The authors hope that the list provided in this paper will assist assessment of the risks from species (including pathotypes and races) in at least the first, initiation stage of the pest risk analysis process globally. It may assist in deciding on which species to build diagnostic and detection capacity. The systematic list should be useful in providing preliminary information and guidance to nematologists in many countries assessing the risks from PPN. As new information becomes available, databases and such lists will need updating. The authors hope that the criteria presented for assessment also facilitate pest risk analysis and prompt appropriate gathering of additional data, thus enhancing nematode biosecurity globally.

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Prioritising plant-parasitic nematode species biosecurity risks using self organising maps

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4.1 Abstract
The biosecurity risks from many plant-parasitic nematode (PPN) species are poorly known and remain a major challenge for identifying potentially invasive species. A Self Organising Map (SOM) was used to prioritise biosecurity risks from PPN to the whole of continental Australia as well as each of the states and the Northern Territory separately. The SOM used the recorded worldwide distributions of 250 systematically selected species from 43 genera, and identified 18 different countries spanning Asia, Africa, North and Central America, Europe and the Pacific with very similar PPN assemblages to Australia as a whole. Many of the species in these countries are not recorded in Australia, and therefore pose a biosecurity risk. Analysed separately, the states and territories were identified as forming five separate clusters, each with a different region of the world, and with different characteristic PPN. Many of the PPN found in the regions clustered with an Australian state have not been recorded from anywhere in Australia, and others have very restricted distributions within Australia, thus posing different biosecurity risks. The SOM analysis ranked the risks of the different PPN based on likelihoods of establishment. The rankings confirmed the risks from frequently quarantined PPN, but more importantly identified species, which upon further investigation could be new risks. This method can be used to identify previously overlooked species for more detailed risk assessments.

Keywords: phytosanitary, pest risk analysis, pest list, new risks, pest species assemblages

4.2 Introduction
The number and taxonomic range of species moving around the world and the threat from invasive species have increased globally (Keller et al. 2011; Pimentel et al. 2005) with growing economic development (Lin et al. 2007; Nunez and Pauchard 2010), movement of people (Tatem 2009; Tatem et al. 2006) and, trade (Hulme 2009; Westphal et al. 2008), changing climates (Walther et al. 2009) and improved transport networks (Tatem and Hay 2007). Transport networks, in particular have expanded in extent, increased in connectivity and accelerated in speed (Hulme 2009; Tatem and Hay 2007). As a result, exotic species have more opportunity and faster means to move from one part of the world to another along transport networks that effectively bring geographically isolated but climatically similar regions closer (Ascunce et al. 2011; Floerl et al. 2009; Hulme 2009; Vilà and Pujadas 2001).

Although data are available for only a few countries, indications are that most countries have only acquired a few of the plant pests and pathogens which can adversely impact on their
agricultural production and environment (Pimentel et al. 2005; Waage and Mumford 2008). This situation is beneficial economically because in the absence of invasive species, production is higher, losses are lower, management interventions are lower, indigenous ecosystems are protected, incidental environmental effects (e.g. destruction of plant and animals in the case of an incursion) are avoided, and market access and trade are facilitated (Cook et al. 2011b; Heikkila 2011; Hodda 2004; Keller et al. 2008). For any country then, biosecurity measures that effectively continue to prevent the establishment and spread of damaging pests; are the most effective and economical means of avoiding crop losses (Cook et al. 2011b; Hockland et al. 2013; Hodda and Cook 2009; Kahn 1991; Perrings et al. 2005; Pyšek and Richardson 2010; Sikora et al. 2005).

The challenge for any biosecurity agency charged with protecting a nation’s agricultural production, is that not all potential invaders pose the same level of risks (Williamson and Fitter 1996). Some species are ill-suited to the climate, fail to adapt, or arrive with insufficient propagules, while others may not have suitable hosts or vectors to complete their life cycles and are unable to establish viable populations (Hayes and Barry 2008; Kolar and Lodge 2001; Simberloff 2009). If these biosecurity risks can be assessed in an objective, repeatable way, resources can be prioritized to risk pathways and inspection regimes at a country’s border can target those species with the greatest risks. In the context of this paper, the term biosecurity risk refers to the potential of an exotic pest to establish in a new range.

Self Organizing Map (SOM) have been used to analyse species assemblages at the global scale to identify and rank potentially invasive species based on their likelihood of establishing in a particular country (Cereghino et al. 2005; Gevrey et al. 2006; Morin et al. 2013; Paini et al. 2010a; Vanninen et al. 2011; Worner and Gevrey 2006). A SOM is an artificial neural network, which recognises patterns in high dimensional data and is widely used in various research areas such as; molecular biology, medicine, geospatial analysis, mineral exploration, metrology, oceanography, data mining and financial risk analysis (Kohonen 2001). A SOM has two properties that make it especially suitable for identifying and prioritizing biosecurity risks. Firstly, a SOM can analyse large data sets, for example the worldwide distribution of up to 10,000 species on a standard desktop computer (Paini et al. 2011). This means that data from amongst the many pests and pathogens identified worldwide can be used in one analysis. Secondly, a SOM can handle the incomplete and suspect distribution records that often
characterise pest and pathogen datasets. A SOM is resilient to up to a 20% error in the species distribution dataset (Paini et al. 2010b) and has been shown to be highly efficient at ranking those species that can establish in a region above those that cannot establish (Paini et al. 2011).

In this paper we use a SOM to analyse the worldwide distributions of 250 plant-parasitic nematodes (PPN) to identify and prioritise the biosecurity risks from these nematodes to Australia. PPN are a major group of pathogens, which cause losses estimated at between 8.8 and 14.6% of total world crop production or US$100-157 billion annually (Abad et al. 2008; Koenning et al. 1999; Nicol et al. 2011; Sasser and Freckman 1987). Australia lacks many of the PPN that cause major losses elsewhere in the world, and quarantine is a major strategy of the Australian government to minimize any future losses (Hodda 2004; Hodda and Nobbs 2008). This is thus an excellent model system on which to apply the methodology and identify those PPN species most likely to establish in Australia.

From the SOM analysis we derive prioritised lists of the PPN posing the greatest biosecurity risks to Australia as a whole, as well as to each state and the Northern Territory. We also identify the countries or regions most likely to be sources of species with the highest likelihoods of establishment. These rankings are supported by what is otherwise known of the biological and ecological characteristics (i.e. species preferences and adaptations compared to the establishment likelihoods) of the PPN.

4.3 Methods
4.3.1 Dataset
The dataset consisted of the distributions of 250 PPN species of greatest phytosanitary importance, based on a number of characteristics: pathogenicity or association with economically important crops; ability to act as virus vectors; interaction with bacterial and fungal pathogens; and quarantine or invasive status (Singh et al. 2013). These species came from 43 genera. A database for the worldwide distribution of these species was created containing the accepted species name, and presence or absence. The presence or absence of species was obtained by searching for the scientific names and synonyms of the 250 PPN on the Web of Knowledge, CABI abstracts and Scopus databases. Synonyms were sourced from the database of nematode names created for the classification of phylum Nematoda (Hodda 2011). Publications reporting the distribution of these species were sourced and presence or absence recorded in the PPN distribution database. Distribution records for synonyms were then
consolidated with the valid names. Distribution records on the CABI distribution map of plant diseases (CABI 2013), CABI crop protection compendium (CABI 2010) and CABI invasive species compendium (CABI 2011) and EPPO PQR (EPPO 2012) databases were retrieved, crosschecked and added to the PPN distribution database. Species without presence or absence information in a given region were assumed to be absent. This procedure produced 6,693 records of presence and 82,057 absence records from 355 world regions spanning 201 countries (large countries were further divided into formally recognised counties, states or provinces). [Please refer to supplementary information 4.1 for PPN species distribution dataset].

4.3.2 Self organising map
The SOM model was implemented using the SOM toolbox (Vesanto et al. 2000) for MATLAB (MathWorks 2007). Details of the SOM algorithm, equations and the implementation can be found in Kohonen (2001) and Vesanto et al. (2000). Input into SOM was the PPN distribution data matrix [355x250] comprised of 250 neurons (one for each PPN species) connected to all 355 regions, thus forming 355 sample vectors of presence and absence records of the species at each of the sites. The linear initialization and batch SOM algorithms were used to model the PPN distribution data (Vesanto et al. 2000). The optimal SOM output size of 104 neurons was determined using the heuristic rule: $5 \times \sqrt{n}$ where $n$ is the number of samples (Vesanto et al. 2000) and using the two largest eigen values from the dataset as the length and width of the SOM (Paini et al. 2011). A total of 52000 iterations were used in the model, based on the recommended formula of 500 x number of neurons (Kohonen 2001). The SOM output after analysing the PPN distribution was represented on a 13x8, hexagonal lattice of 104 neurons.

The SOM assesses species assemblages and associations to generate an index for every species in every region between 0 and 1 (Worner and Gevrey 2006). The SOM clusters countries and regions based on similarities in species assemblages and countries occupying the same neuron have the greatest similarity in species assemblage. Based on the similarities in species assemblages, the SOM analyses where a species has established and which other species are likely to establish in those same regions. The SOM index for a particular species in a particular neuron represents the strength of association of the particular species with the species assemblages found in the countries or regions grouped in the neuron (Paini et al. 2011; Paini et al. 2010a; Worner and Gevrey 2006). Thus the index can be used as a representation of the
likelihood of the species establishing in that country if it arrives and given that the host plant is present. The indices can then be used to rank all species, identifying those species most likely to establish in a particular country or region. The SOM clustering was used to identify countries or regions occupying the same neuron as Australia and its respective jurisdictions.

SOM indices for each species in each country, state or region were extracted from the SOM model output. Ranked lists for Australia as a whole and for each state; New South Wales (NSW), Queensland (QLD), South Australia (SA), Tasmania (TAS), Victoria (VIC), Western Australia (WA) and the Northern Territory (NT), were then extracted. The Australian Capital Territory was excluded from analysis because there is minimal agriculture and few records of PPN. While it was possible to generate a list for Australia as a whole, we wanted to account for the range of climatic and ecological characteristics found throughout Australia by generating jurisdiction specific lists. As such, we analysed each of the jurisdictions (the states and Northern Territory) for comparison with Australia as a whole. Spearman’s rank correlation test was used to statistically compare the species rankings between Australia and each of its jurisdictions. The test was implemented in R (R Development Core Team 2010) using the “pspearman” package (Savicky 2009).

To prioritise species for national quarantine (i.e. A1 species are quarantined nationally), species recorded from anywhere in Australia (N=104) were removed from the 250 species ranked list. The remaining species not recorded from anywhere in Australia (N = 146), were then ranked based on their SOM indices. Using these rankings, the top 50 species absent from Australia with the highest likelihood of establishment were determined for each of the jurisdictions.

To prioritise species for domestic quarantine (i.e. A2 species are quarantined at jurisdiction level only), we used the list of 104 species present in Australia to compare with the species absent from each jurisdiction. Species not recorded in the given jurisdiction (but found somewhere else in Australia) were then ranked based on their SOM indices and the top 10 species with the highest likelihood of establishment were determined. The scheme used here for prioritisation of species for national and domestic quarantine is presented in Figure 4.1.

The top 50 and 10 species were chosen for prioritisation for national and domestic quarantine, respectively, because they represent a realistic number on which more detailed assessments can
be completed within timeframes that will allow implementation of meaningful biosecurity measures if they are justified.

**Figure 4.1** Scheme for prioritisation of species for national and domestic quarantine

![Scheme for prioritisation of species for national and domestic quarantine](image)

**4.4 Results**

**4.4.1 Distribution of plant-parasitic nematodes**

Of the 201 countries in the dataset, most had 20 or fewer PPN species reported as present (64%) and only a few had more than 40 species reported (15%) (Figure 4.2a). No country had more than half of the 250 species investigated. Many of the countries with few records were developing countries or small islands (Figure 4.2b). Most of the published species records came from countries with good nematological expertise, and most were from major agricultural areas, especially for large countries such as Australia, Brazil, Canada, China, India, Russia and USA.

**Figure 4.2a** Percentage of countries on the database by number of reported PPN species

![Percentage of countries on the database by number of reported PPN species](image)

\[a\] Number on top of each column represents the number of countries
Figure 4.2b Worldwide representation by number of PPN species reported

Country colour coding represents number of PPN species records: Orange = 1-10, Brown = 11-20, Blue = 21-40, Yellow = 41-60 and Green = 61-120.

4.4.2 Self organising map clustering of countries and regions

The 355 world regions were clustered into 88 neurons based on similarities in their PPN species assemblages. Most of the neurons (69%) had four or fewer countries and regions while 16 neurons were empty (Figure 4.3). A maximum of 26 countries and/or regions were clustered in one neuron. Generally the individual counties, states or regions of large countries with diverse climates were clustered into different neurons reflecting the differences in species assemblages between the smaller geographic units across these countries. Australia as a whole and the individual jurisdictions were clustered into five different neurons (Figure 4.3). Similarly USA and its states were clustered into 20 different neurons (Supplementary Table 4.2). Species which were recorded in many countries generally had higher SOM indices than species with more restricted distributions.

Australia as a whole had the most similar PPN to South Africa (sharing the same neuron).

When the jurisdictions were considered independently, 18 countries spanning: Asia (7), Africa (5), Central America (2), Pacific (2), Europe (1) and North America (1), were present in the same neurons, (Figure 4.3). Considering each jurisdiction within Australia separately, SA and VIC were clustered together, as were NSW and WA, which were also clustered in the same neuron as Australia as a whole (Figure 4.3). Regions clustered in the same neuron had the same SOM index for all species. Comparing the rankings of all species in each jurisdiction showed that climatically similar jurisdictions had similar PPN rankings (Table 4.1). All rankings for species in the individual jurisdictions were significantly positively correlated except TAS and
NT, which had a significant negative correlation and TAS and QLD, which had no correlation (Table 4.2).

**Figure 4.3** Self-organising map clustering of regions and countries with similar plant parasitic nematode assemblages to Australia as a whole and individual jurisdiction

Each hexagon represents a neuron on the SOM model output layer. Countries or regions with very similar PPN species assemblages to Australia and individual jurisdictions are clustered into the same neuron. The greater the distance between the neurons the greater the dissimilarity in PPN species assemblage between them. For a detailed list of all neurons and the SOM regional clustering, please refer to supplementary Table 4.2.

### 4.4.3 Plant-parasitic nematodes species not recorded in Australia

Of the species not recorded in Australia, the top 50 PPN with the highest likelihood of establishing in Australia as a whole should they be introduced were from 23 genera (Table 4.1). All of the top 50 PPN for Australia as a whole were also ranked in the top 50 for two or more of the individual jurisdictions within Australia considered separately, and 80% of these species (N = 40) were ranked in the top 50 for at least 5 jurisdictions (Table 4.1; also see supplementary Tables 4.3 a-e for a list of all species and their SOM indices). The highest ranked species was *Hirschmaniella oryzae*, followed by *Ditylenchus destructor* and *Heterodera glycines* (Table 4.1).
Table 4.1 Rank comparison of plant parasitic nematode species in top 50 lists of Australia as a whole and individual jurisdictions within

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<td>Longidorus macrosoma</td>
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<td>Meloidogyne oryzae</td>
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<tr>
<td>Radopholus citri</td>
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<td>125</td>
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<td>138</td>
<td>142</td>
<td>144</td>
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</tr>
<tr>
<td>Hirschmanniella miticausa</td>
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<tr>
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<td>Subanguina hyparrheniae</td>
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<tr>
<td>Meloidogyne acronea</td>
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<td>144</td>
<td>137</td>
<td>38</td>
<td>144</td>
<td>136</td>
</tr>
</tbody>
</table>

Species marked with asterisk (*) are already on high priority pest list for Australia, species indicated in bold are in the top 15 ranked species for one or more jurisdictions in Australia and could represent possible risks previously unrecognised.

Ranks in bold represent species in the top 50 ranks of the respective jurisdiction.

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**Table 4.2** Spearman’s rank correlation of plant parasitic nematode species ranks for Australia as a whole and individual jurisdiction within

<table>
<thead>
<tr>
<th></th>
<th>AUST</th>
<th>NSW &amp; WA</th>
<th>QLD</th>
<th>NT</th>
<th>SA &amp; VIC</th>
<th>TAS</th>
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</thead>
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<td>AUST</td>
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<td>NSW &amp; WA</td>
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<tr>
<td>QLD</td>
<td>0.88054*</td>
<td>0.88054*</td>
<td>1</td>
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<tr>
<td>NT</td>
<td>0.469843*</td>
<td>0.469843*</td>
<td>0.591405*</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>SA &amp; VIC</td>
<td>0.90607*</td>
<td>0.90607*</td>
<td>0.651825*</td>
<td>0.23039*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TAS</td>
<td>0.423374*</td>
<td>0.423374*</td>
<td>0.150473*</td>
<td>-0.22208*</td>
<td>0.643982*</td>
<td>1</td>
</tr>
</tbody>
</table>

* indicates significant correlation
There were five species from each of the genera *Heterodera* (cyst nematodes) and *Meloidogyne* (root-knot nematodes) ranked in the top 50 for Australia as a whole (Table 4.1). Other genera with many species ranked in the top 50 included *Hirschmanniella, Tylenchorhynchus* (stunt nematodes), *Scutellonema* (spiral nematodes) and *Trichodorus* (stubby-root nematodes).

When the jurisdictions within Australia were considered separately, *Heterodera* and *Meloidogyne* each had three or more species ranked in the top 50 for every state (Figure 4.4). The other PPN genera mentioned above also contained species in the top 50 for five or more jurisdictions. In addition, another 14 genera contained species ranked in the top 50 for at least four jurisdictions (*Ditylenchus, Paratrichodorus, Pratylenchus, Bursaphelenchus, Globodera, Xiphinema, Hoplolaimus, Longidorus, Hemicyclophyllus, Helicotylenchus, Quinisulcius, Rotylenchulus, Zygorylenchus and Paratylenchus*). Altogether 12 genera had species ranked in the top 50 for three or less jurisdictions: *Anguina, Nacobbus, Punctodera* (SA, VIC, TAS); *Bitylenchus* (NSW, WA, QLD); *Hemicyclophyllus* (QLD); *Aphelenchoides* (QLD, NT); *Merlinius* and *Tylenchulus* (NSW, WA); *Dolichodorus, Ibipora, Radopholus* and *Subanguina* (NT) (Table 4.1).

**Figure 4.4** Plant parasitic nematode genera in top 50 lists of four or more Australian jurisdictions
4.4.4 Plant-parasitic nematode species present in Australia

Of the 104 PPN recorded from Australia, most (64%) were found in three jurisdictions or less: only about one third of the PPN species present (36%) were widespread (in more than three jurisdictions). Of the species recorded from Australia, the numbers per jurisdiction varied from 20 (TAS) to 68 (NSW) (Figure 4.5). PPN species from 19 genera already present in Australia were ranked in the top 10 for jurisdictions where they are not recorded (Supplementary Table 4.4a-g). These PPN generally had higher SOM index values and thus a stronger association with the Australian jurisdictions than the species totally absent from Australia.

**Figure 4.5** Plant parasitic nematode species recorded in Australia, presence and absence by jurisdictions

PPN species absent from particular jurisdictions, and most frequently ranked in the top 10 for other Australian jurisdictions included *Aphelenchoides besseyi, Cactodera cacti, Globodera rostochiensis, Helicotylenchus pseudorobustus, Hemicriconemoides mangiferae, Heterodera fici, Paratrichodorus porosus, P. renifer, Pratylenchus scribneri, Quinisulcius capitatus, Rotylenchulus parvus, R. reniformis, Tylenchorhynchus annulatus, T. claytoni and Xiphinema index* (Supplementary Table 4.4a-g). Species from the genera *Pratylenchus, Heterodera, Xiphinema, Paratrichodorus* and *Tylenchorhynchus* were commonly ranked in the top 10 for five or more jurisdictions (Supplementary Table 4.4a-g).

4.5 Discussion

4.5.1 Use of self organising map to assess biosecurity risks

The basis of using SOM to assess biosecurity risks is that species assemblages integrate the complex interactions of the biotic, abiotic and anthropogenic environments (Begon et al. 1996; Gevrey et al. 2006; Paini et al. 2010b; Worner and Gevrey 2006). All the species in a particular place will have broadly similar niches and form an assemblage (Wisz et al. 2013; Worner and Gevrey 2006). Geographic units sharing many species of an assemblage will also share similar
niches and ecological characteristics (Ferrier and Guisan 2006; Wisz et al. 2013; Worner and Gevrey 2006). Thus, if a particular species is missing from an assemblage at a particular place, but is found in other regions with otherwise similar assemblages, then it is likely to be able to establish in the place where it is absent and should be regarded as a biosecurity risk.

An advantage of a SOM analysis is that it is based on patterns in species distributions and their resulting associations with each other, rather than simple pair-wise comparisons of species between regions. SOM analyses and the resulting indices can differentiate biosecurity risks from many species from all over the world to any given region (Paini et al. 2011; Paini et al. 2010a; Worner et al. 2013; Worner and Gevrey 2006). In this study PPN and Australia were evaluated but any other country included in the dataset could have been analysed similarly. Another advantage is that the SOM index is based on a consistent mathematical calculation of similarity of species assemblages between regions and is more objective than qualitative observations (Paini et al. 2010b). All the species and regions are evaluated using the same framework in the SOM model; hence the SOM index and species rankings between regions are also comparable.

The SOM approach also has some limitations. The quality of dataset used is an important contributor to the limitations of any predictive modelling approach including SOM (Elith et al. 2006; Morin et al. 2013; Wisz et al. 2008). The species selected or represented in a dataset, the correctness of presence and absence of a species and the thoroughness of surveys are all inherent limitations. Only some species that may pose a biosecurity risk are listed on widely used databases such as the CABI crop protection compendium, invasive species compendium and EPPO databases. In the present study, a very comprehensive list of PPN species of phytosanitary importance was compiled (Singh et al. 2013) and used for the SOM. Of the 250 PPN species analysed, only 97 PPN species were listed in the databases listed above. Indeed, studies such as this are one way to identify other species to add to such databases.

The observation that the majority of countries in the world have 20 PPN species records or less, demonstrates the paucity of nematological expertise and scarcity of nematode surveys for most parts of the world (De Waele and Elsen 2007; Nicol et al. 2011; Powers et al. 2009). The lack of PPN species distribution records can result from low sampling effort rather than true absence of species in data poor regions and countries. This can distort understanding of species
biogeography (Bello et al. 1986; Coomans 2002; Navas et al. 1993) and hence is an inherent limitation to using patterns of species assemblage to determine the risks from invasive pest species. Countries and/or regions with fewer than 10 species present are more difficult for the SOM to generate an accurately ranked list (Paini et al. 2011). Nevertheless, a SOM analysis is able to cope with up to 20% errors in the species distribution dataset without causing large changes in species rankings (Paini et al. 2010b). Even allowing for the incomplete data, it is apparent that the distributions of most damaging PPN are still restricted. This was evident within countries in the present study, and presence of biogeographical patterns in nematode species distributions has also been observed elsewhere (Coomans et al. 2001; Ferris and Ferris 1985; Ferris et al. 1976; Porazinska et al. 2012). The power of SOM to make assessments of similarities in species distribution even with incomplete data (Paini et al. 2011) gives confidence to the lists generated here.

Another limitation is that the SOM approach does not provide a measure of the impacts from a species. Therefore the SOM index and species rankings can only be considered as a preliminary measure of biosecurity risks. For instance a species with medium probability of establishing in a region but likely to cause high economic or environmental impact is likely to be rated as of greater importance by experts than a species which has a high probability of establishing but with low economic or environmental impact (Paini et al. 2010a; Paini et al. 2010b).

Expert opinion is commonly used to prioritize invasive species for quarantine or management actions based on their knowledge of the damage and impact species have. However, the process is susceptible to biases depending on knowledge of the taxonomic group, the time available and other external influences (Burgman et al. 2011; Martin et al. 2012). These biases may result in differences of opinion between experts especially when evaluating large numbers of species (McGeoch et al. 2012; Paini et al. 2010b). Another pitfall of using expert opinion is that biosecurity risks may be underestimated when there is uncertainty and a lack of information on the impacts of a species (McGeoch et al. 2012). The biosecurity risks from many PPN may not have been realised due to lack of information and level of uncertainty (Singh et al. 2013). When using a systematic approach such as a SOM analysis, the abovementioned biases can be avoided and there is considerable potential for identification of species which could establish and later become invasive in a particular region.
There are thousands of potential pest species from highly diverse groups such as nematodes, fungi or arthropods and resources to thoroughly evaluate all are not available. Therefore the SOM analysis can be used to rank species, and those with high likelihoods of establishment (SOM indices) can be prioritised for further, more detailed analysis of potential impacts (Cook et al. 2011a; Cook et al. 2011b; Morin et al. 2013). Targeted investigation on species with good chances of establishment could be used to determine species which could become new risks to a particular region.

For example, in the present analysis *Helicotylenchus microcephalus* ranked third for NT and fifth for NSW, QLD and WA and has been reported from many hosts (including economically important crops such as sugarcane, chickpeas, soybean, citrus, grapevine and ornamental plants) from 20 different countries (PPN distribution database, this study). However, the biology and damage caused by *H. microcephalus* is not well known, and after qualitative evaluation based on expert opinion only, it was classified as low risk to northern Australia (Hodda et al. 2012). The classification of *Helicotylenchus microcephalus* as low risk by experts was based on qualitative assessment of available information and did not take into account likelihood of establishment. However, considering the high likelihood of *H. microcephalus* establishing in northern Australia based on SOM index, classification as low risk could be an underestimation of the biosecurity risks by experts. Thus such species are good candidates for further investigation.

In addition to prioritising species, the clustering of countries and regions with similar PPN species assemblages can be used to identify pathways linking these countries and regions to Australia for further analysis and quarantine targeting. The results can also be used for the development of new phytosanitary controls such as phytosanitary certificates. Regions which have similar pest species assemblages can be sources of invasive species (Paini et al. 2010b; Worner and Gevrey 2006). For instance WA and NSW have very similar PPN species assemblages except *Helicotylenchus pseudorobustus*, *Pratylenchus scribneri* and *Rotylenchulus reniformis* which occur in WA, but not in NSW. These three species have high SOM indices for NSW (0.82 - 0.65) and therefore have a high probability of establishing in that state if they were to arrive. Based on this information, the potential pathways for these species between NSW and WA can be targeted during risk assessments. Similar findings have been reported for insects in the USA, where the greatest risks from exotic insect species came
from other states within the USA with very similar insect species assemblages (Paini et al. 2010a).

Although quarantine is mostly controlled at a national level (as, for example, in Australia and the USA), smaller geographic units may be better for analysis and identifying risks, especially for large countries like Australia. Were they countries rather than states or territories, WA, QLD and NT would be the tenth, eighteenth and twentieth largest in the world. Using smaller units captured more variation in PPN assemblages than using the country as a whole, as shown by the divergent lists of risks for the different states, and the different positions of the neurons.

Using smaller units also meant that fewer species were recorded in each, and there was a greater likelihood of false absences. However, the SOM was able to deal with the fewer records from individual jurisdictions well, and produce results consistent with known climatic and other differences. In addition to climate, other biotic (such as crops, cropping history), abiotic and anthropogenic factors (history of colonization, trade, and even research on particular taxa) which affect the known species distributions are taken into account by SOM when clustering regions. Although NSW and WA differ climatically, their clustering into the same neuron can be explained by their similarity in crops and both have winter and summer dominant rainfall zones.

4.5.2 Particular implications for Australia
All PPN listed on the high priority pest list by Plant Health Australia (Plant Health Australia 2012) that are absent from Australia were ranked in the top 15 species of one or more of the jurisdictions considered. This supports the ranking based on expert opinion from these species. The SOM rankings also identified a further 32 PPN species not yet on the priority lists of Plant Health Australia and ranked them in the top 15 for one or more jurisdictions. Species currently not on the high priority pest list but having similar likelihoods of establishment as currently known high priority pests for Australia could be targeted for more detailed analysis. More detailed analysis of these species could result in identification of some as new risks to Australia.

The majority of the species (76% N =29/38) ranked in the top 15 biosecurity risk for any of the jurisdictions were of more general importance for the country as a whole since they occurred in the top 50 for four or more of the individual jurisdictions. For instance *Hirschmanniella oryzae*,...
ranked first for QLD, NSW, WA and NT is also ranked sixth for SA & VIC (with SOM index range of 0.65-0.31). Such a ranking indicates *H. oryzae* can establish in any of the six jurisdictions. This is further supported by evidence from literature confirming the wide ecological tolerance of *H. oryzae* (i.e. survival in moderate to heavy clay soils including flooded soils; soil pH range 5-9, and soil temperatures ranging 10-34 °C (Babatola 1981; Fortuner 1976; Maung et al. 2012)). The evidence from this species indicates that many of the PPN species posing greatest biosecurity risks may have relatively wide environmental ranges.

PPN species from both temperate and tropical regions posed risks to Australia, with 18 countries having very similar species assemblages to at least one Australian jurisdiction. These countries include major trading partners with frequent travel pathways linking to Australia. Hence, PPN potentially invasive to Australia could come from a wide range of countries via a wide range of pathways. The risks from a wide range of PPN species to Australia overall is not unexpected given the diverse range of climates and crops, together with the 18 different agro-climatic regions (Hutchinson et al. 2005; Hutchinson et al. 1992). That risks from different PPN varied among jurisdictions is also expected. There is scope for different targeting of quarantine species for different jurisdictions in a country the size and climatic variability of Australia. TAS and NT have greatest difference in climate and illustrate the scope for different targeting of species. The destination of imported goods should be considered when targeting species, as goods arriving in one port may be transported to other jurisdictions in Australia. While we do not imply that species likely to establish in other jurisdictions should not be targeted by another jurisdiction (see above for species with wide environmental ranges), it is logical for quarantine inspectors in TAS to spend relatively greater sampling effort on species which are more likely to establish in TAS, SA, VIC, NSW and WA than on species which are more likely to establish in NT. Given the microscopic size and difficulties in detecting of PPN and other similar microscopic pests (Ferris et al. 2003; Rajan 2006), targeted sampling effort can make a difference. For example, consignments of root vegetables, bulbs, nursery stock and other goods with the potential to carry targeted nematode species may be sampled more vigilantly to improve chances of detection.

The species ranks for all states in Australia except TAS and NT were positively correlated; indicating some similarity in risks from PPN species. Tasmania and Northern Territory had the greatest difference in their species assemblages indicated by the distance separating the
respective neurons. TAS and NT have contrasting climates and there are thus strong indications that PPN species posing high phytosanitary risks to TAS do not pose the same levels of risk to NT, and vice versa. For example, \textit{Hirschmaniella oryzae} is the top ranked species for NT, NSW, WA and QLD, but is ranked 80\textsuperscript{th} for TAS. \textit{H. oryzae} is an important pest of rice and is known mainly from countries or regions with climates suitable for growing rice (CABI 2013). It is likely to be of minor importance for TAS, because of unfavourable conditions for rice cultivation. Similarly \textit{Scutellonema clathricaudatum} prefers warm (30 -36 °C) tropical climates (Baujard and Martiny 1995) so was identified by SOM as of greatest risk to NT (ranking 2\textsuperscript{nd}) and low risk to TAS (ranking 115\textsuperscript{th}). By contrast, \textit{Ditylenchus destructor} was ranked by SOM as the most important species for TAS, SA and VIC and second most important for NSW and WA but relatively unimportant (ranking 71) for NT. Elsewhere, \textit{D. destructor} is of known importance in countries/regions with cold-temperate climates, and is adapted for surviving in cold climates (Svilponis et al. 2011). It is unable to withstand excessive desiccation (Sturhan and Brzeski 1991) and the monsoonal conditions in NT would be unfavourable for it, particularly during the dry season. These examples illustrate that the SOM rankings generally accord with PPN habitat preferences where they are known.

The clustering of jurisdictions in Australia based on PPN species assemblages (this study) was similar to the SOM clustering of jurisdictions based on insect (Paini et al. 2010b) and fungal species assemblages (Paini et al. 2011). In all three SOM analyses, the country as a whole and its jurisdictions were clustered into 5 different neurons. It thus seems that PPN, fungi and insect species assemblages differ similarly among jurisdictions. SA and VIC consistently clustered in one neuron in all three analyses, indicating these jurisdictions had very similar species assemblages of all three pest groups. All three taxa assemblages seem to capture the climatic, biotic and abiotic characteristics of each region, and all include pests of agricultural crops.

Other modelling methods have also used species assemblages to predict future invaders but with different datasets and model parameters (Diez et al. 2012; Hui et al. 2013). While the SOM model uses species presence and absence data to determine species assemblages more complex models can integrate additional information on species naturalisation and invasion history to build predictive models which provide a probabilistic score on the likelihood of establishment (Diez et al. 2012). The time invasive species spend as part of an assemblage also affects the characteristics of a species assemblage and can be integrated in models for predicting future
invaders (Hui et al. 2013). Compared to the two recent studies, the SOM model uses a relatively simple species presence and absence dataset which is more readily available for plant pests and pathogens than the datasets on invasion histories (Diez et al. 2012) and the invasion residence time of species (Hui et al. 2013). The Bayesian model (Diez et al. 2013) and the functional modularity model (Hui et al. 2013) both require input from experts in the model development and are more complex than the SOM model used in this study.

4.6 Conclusions
Based on this and previous studies (Morin et al. 2013; Paini et al. 2011; Paini et al. 2010a; Paini et al. 2010b; Worner and Gevrey 2006) we suggest the use of a SOM analysis as complementary to expert opinion especially when analysing biosecurity risks from large numbers of pest species. A SOM analysis is a systematic approach, which is free from bias and can provide a preliminary assessment of risks as well as an independent means of cross-validating lists derived through expert elicitation. Systematic assessments generally increase the consistency and reliability of biosecurity risk assessments across geographies (Gordon et al. 2008; Hayes 2003; Holt et al. 2006). The prioritised PPN species from this study could be used for cross validation of expert opinion and also be used for more detailed risk analysis including information on other important variables such as pathogenicity, host range, pathways and association with disease complexes.

4.7 Acknowledgements
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Keeping one step ahead of invasive species: using an integrated framework to screen and target species, for detailed biosecurity risk assessment

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5.1 Abstract
Predicting which species will become invasive in each country or region before they arrive is necessary to devise and implement measures for minimising the costs of biological invasions. Metaphorically, this is keeping one step ahead of invasive species. A structured and systematic approach for screening large numbers of species and identifying those likely to become invasive is proposed in this paper. The Pest Screening and Targeting (PeST) framework integrates heterogeneous information and data on species biogeography, biotic and abiotic factors to first determine an overall risk index, then uses this index to identify species for a second, more detailed, risk evaluation process to provide a final ranking.

Using the PeST framework, 97 species of Plant-Parasitic Nematodes (PPN) were evaluated for their biosecurity risks to Australia. The species identified as greatest risks included both previously unrecognised and currently-recognised species. The former included *Heterodera zeae*, *Meloidogyne graminicola*, *M. enterolobii*, *M. chitwoodi* and *Scutellonema bradys*, while the latter included *Bursaphelenchus xylophilus*, *Ditylenchus destructor*, *Globodera pallida*, *Heterodera glycines* and *H. filipjevi*.

Of the ten criteria used in the PeST framework, emerging pest status, pathogenicity, host range and the SOM index (based on species biogeography) most strongly influenced overall risk. The PeST framework also identified species where research to fill in critical knowledge gaps will be most beneficial (e.g. *Globodera tabacum*, *Heterodera cajani*, *H. filipjevi*, *Meloidogyne ethiopica*, *Pratylenchus fallax* and *P. sudanensis*). Where data were available, the information and associated metadata gathered for the PeST framework can be used to produce species profiles useful for management of the high-risk pests identified.

5.2 Introduction
Risk assessments are a basic part of biosecurity, which is the “strategic and integrated approach to analysing and managing relevant risks to human, animal and plant life and health and associated risks to the environment” (FAO 2007a). Risk assessments are used to estimate the probability of any pest entering some geographic locality on a particular commodity or by a certain pathway, then establishing, and finally causing damage. They are used for contingency planning and implementing measures to avoid or mitigate the negative impacts of exotic species which threaten the sustainability of agriculture, food safety and the environment, including biodiversity (FAO-ISPM-11 2013; FAO 2007a)
Risk assessments are also used to prioritise biosecurity risks. Resources for implementation of biosecurity measures are limited, and so risk assessments that provide some sort of comparative, quantitative and defendable measure or ranking are desirable. Standardised and objective methods offer the best prospects for this, but practical methods have yet to be developed for plant pathogens because of difficulties including all the important characteristics of all potential invaders, and doing so within realistic resource constraints.

Considerable time and resources are often required for detailed risk analysis because extensive data collection and consolidation are needed. There are many more potential threats to plants than can be assessed using such procedures. Therefore, an assessment procedure allowing many species to be assessed relatively quickly with readily available information will be valuable to prioritise which species and pathways warrant more detailed assessments.

Risk assessments can also be used for deciding which biosecurity measures may be best deployed against particular threats because a wide range of measures are potentially available, including targeted sampling procedures, particular detection methods, incursion management plans and ongoing surveillance (Emery et al. 2003; Schrader & Unger 2003; FAO-ISPM-11 2013; Baker et al. 2005; Schaad et al. 2006; Petter, Roy & Smith 2008; Kumschick et al. 2012). Among the most effective means of averting negative impacts from invasive species are those designed to prevent the entry of potentially harmful exotic species (Wittenberg & Cock 2001; Leung et al. 2002; Cook et al. 2011b). However, such measures have the potential to form an unnecessary barrier to trade, which is mostly seen as undesirable (Gebrehiwet, Ngqangweni & Kirsten 2007; Cook et al. 2011a). Hence, identification of biosecurity risks and minimisation measures now need to be formalised so that they are justifiable, transparent and proportionate to the risks (FAO 2007b).

Despite considerable work improving risk assessment methodology, determining potentially invasive exotic species remains a complex and difficult task (Andersen et al. 2004a; Andersen et al. 2004b; Stohlgren & Schnase 2006; MacLeod et al. 2010; Devorshak 2012). Numerous factors contribute in myriad combinations towards the risks from exotic species, including: the abiotic properties of an environment; the biotic characteristics of species and ecosystems; the propagule pressure; the levels of economic development; and the trade volumes, modes and routes, together with the characteristics of the networks goods travel through (Hayes 2003;

Furthermore, there are often high levels of uncertainty in many of these factors, which affect perception and estimates of risks (Liu et al. 2011a; Liu et al. 2011b). Some of these factors (e.g. propagule pressure, trade and transportation networks) are also dynamic, hence the risks from exotic species can change over time (Venette et al. 2010). Incorporating all or most of these factors so that risks can be fully understood and evaluated requires synthesis of concepts and knowledge from many different sources involving multiple stakeholders such as academia, governments, industry and community (Devorshak 2012).

Using the FAO-ISPM guidelines, national and regional plant protection agencies devote significant time and resources to pest risk assessments and a plethora of methods are available for detailed pest risk assessments (Leung et al. 2012). Most of the current pest risk assessment methods focus on detailed evaluation of one species at a time and systematic methods which evaluate and prioritise the risks from a large number of species are lacking.Because comprehensive biosecurity risk analysis is such a time consuming and resource intensive task, it is not feasible to comprehensively analyse the risks from every exotic species. Therefore, screening tools are necessary to initially systematically evaluate large numbers of exotic species and determine which species should be analysed more comprehensively (Daehler & Strong Jr 1993; Sutherland et al. 2008; Sahlin et al. 2011). Such tools can save both time and resources (Daehler & Strong Jr 1993). The Weed Risk Assessment (Pheloung, Williams & Halloy 1999) and Fish Invasiveness Scoring Kit (Copp, Garthwaite & Gozlan 2005) are two widely used screening tools which have been modified for use in many different countries (Gordon et al. 2008; Vilizzi & Copp 2012). Both these tools use a semi-quantitative approach to score plant or fish species against a set of questions based on their biogeography, biology and ecology. These methods have been used effectively to evaluate plant or fish species imports respectively, and decide whether to accept, reject or further evaluate import of any particular species.

Unlike plants and fish imports, most plant pathogens are not deliberately imported, but arrive ‘uninvited’ as contaminants via numerous potential pathways (Hulme et al. 2008; Hulme 2009; McNeill et al. 2011; Bacon, Bacher & Aebi 2012). These pathways also need to be prioritised
for targeting sampling, choosing methods and requiring pre-border certification (i.e. declaration as free from harmful pest species). The tools mentioned above are thus inadequate for effectively screening and targeting potentially invasive plant pathogens, and a new tool is needed.

This paper describes and applies an integrated framework for screening and targeting pest species for detailed biosecurity risk assessment: the Pest Screening and Targeting (PeST) framework. First, we explain how the PeST framework design integrates qualitative, quantitative and semi-quantitative methods and review the rationale for the assessment criteria chosen. Then, we demonstrate the use of the PeST framework in screening the biosecurity risks from plant-parasitic nematode species to Australia: these are the same examples as used in three previous investigations which provide some data used in the current study (Singh, Hodda & Ash 2013; Singh et al. 2013a, Singh et al. 2013b). We explain how the framework could also be used for other groups of pests and geographical locations as well. The PeST framework follows the International Standards for Phytosanitary Measures (ISPM) and should strengthen biosecurity systems by dealing effectively with the cryptic, small and difficult-to-detect plant pathogens which currently impose considerable challenges to prioritizing, devising and implementing biosecurity measures (Ferris et al. 2003; Rajan 2006; Okabe et al. 2012).

5.3 Methods
5.3.1 Selection of species
From the database of the 250 PPN species of highest potential phytosanitary importance (Singh, Hodda & Ash 2013), the 97 economically important species exotic to Australia but most likely to establish were selected. The likelihood of establishment was determined from a study modeling their global distribution using self-organising maps (Singh et al. 2013b).

5.3.2 Formulation of multi-criteria evaluation scheme
The criteria for evaluating biosecurity risks from PPN species were determined based on an expert opinion survey and literature review. The experts were members of the Australasian Association of Nematologists, who were surveyed via an emailed questionnaire asking the most important characteristics for evaluating biosecurity risks from exotic PPN. Ten nematologists working in Australia and New Zealand participated in the survey and each person nominated several criteria. Collation of these expert opinions yielded ten criteria for evaluating the biosecurity risks from PPN:
These ten criteria were critically analysed by carrying out a literature review and further refined by defining a scoring scheme for each and assigning weights based on their relative importance (Table 5.1). Of the ten criteria, biogeography was assigned the greatest weight (0.2) because it included the likelihood of establishment, which was consistently rated as important by the experts. Furthermore, likelihood of establishment can be estimated for most species objectively and quantitatively by modelling species distributions (Guisan & Zimmermann 2000; Pearce & Boyce 2006). Pathways, survival adaptations, pathogenicity, host range, emerging pest status, species identification and uncertainty due to knowledge base, were all rated as of lesser but equal importance by the experts and generally do not have objective measures as readily available as biogeography. Because the measures were less objective and certain, and the lower importance assessed by the experts, each of these criteria were assigned a weight of 0.1. Disease complexes and pathotypes were assigned the lowest weight of 0.05. These criteria were rated lowest by the experts, are applicable to some species only, and are the most difficult to define: authorities are divided on whether they exist for some species, and there are quite divergent definitions for other species, which affect their distribution and hence risks. Altogether the weights sum to 1.

For each species, biogeographic information was obtained from the PPN distribution dataset (Singh et al. 2013b). For the other nine criteria, information was gathered from published literature. A score was assigned for each according to the evaluation scheme described in Table 5.1. The scores for each species were recorded in a spreadsheet linked to a profile page documenting the reasons and references used. A conceptual representation of the framework is given in Figure 5.1. [Detailed information on each species is given as supplementary information 5.1].
Table 5.1 Multi-criteria evaluation scheme

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Scoring scheme (Maximum score: 1 minimum score: 0)*</th>
<th>Reasons for choosing criterion based on expert opinion survey responses and literature review</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biogeography</td>
<td>Self organising map modeling of PPN species distributions worldwide (presence and absence) was used to determine the likelihood of establishment of a species in Australia. The highest likelihood of establishment (SOM index values) for each of the 97 PPN species exotic to Australia was used.</td>
<td>There are patterns in the distribution of species defined by anthropogenic movement, biological and ecological adaptations of a species. The likelihood of exotic pest species establishing in foreign locations can be estimated using species distribution models.</td>
<td>0.2</td>
</tr>
<tr>
<td>Pathways</td>
<td>Associated directly with a traded commodity such as tubers, rhizomes, bulbs, propagative materials either seeds, nursery stock, ornamentals or bonsai plants; Three or more pathways: greater than 0.8. Two pathways: scored between 0.7 and 0.8. One pathway: scored between 0.6 and 0.7. Not directly associated with a traded commodity but intercepted as contaminant; Of seed or propagative material: scored between 0.5 and 0.6. In baggage, packaging material, container, footwear or machinery: scored between 0.4 and 0.5. In items other than those specified above: scored between 0.3 and 0.4. Pathways/published interception records not known: scored less than 0.3.</td>
<td>The means available for the introduction of a species is an important contributor to the chances of a species being moved and eventually arriving to a location away from its native range.</td>
<td>0.1</td>
</tr>
<tr>
<td>Survival adaptations</td>
<td>Known to survive longer than a year in absence of host e.g. cysts: scored greater than 0.8. Survive by undergoing anhydrobiosis or cryptobiosis: scored between 0.7 and 0.8. Eggs or juveniles with adaptations for surviving for a few months: scored between 0.6 and 0.7. Survive as endoparasites and lay eggs inside plant tissue: scored between 0.5 and 0.6. Live as semi-endoparasites and could survive attached to bare rooted plant material: scored between 0.4 and 0.5.</td>
<td>Species with specialised adaptations to cope with biotic and abiotic stresses are more likely to survive transit and also persist in a new environment which may aid in their adaptation, persistence, and establishment of a self propagating population.</td>
<td>0.1</td>
</tr>
</tbody>
</table>
### Pathogenicity

<table>
<thead>
<tr>
<th>Description</th>
<th>Score Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migratory endo/ecto-parasites which could survive associated with roots or</td>
<td></td>
</tr>
<tr>
<td>soil: scored between 0.3 and 0.4.</td>
<td></td>
</tr>
<tr>
<td>Without any of the above mentioned survival adaptations but likely to</td>
<td></td>
</tr>
<tr>
<td>survive for a month: scored less than 0.3.</td>
<td></td>
</tr>
<tr>
<td>Major pest with more than 10 damage reports: scored greater than 0.8.</td>
<td></td>
</tr>
<tr>
<td>Known to cause damage with 5-10 damage reports: scored between 0.7 and 0.8.</td>
<td></td>
</tr>
<tr>
<td>Not a major pest and less than 5 damage reports: scored between 0.6 and 0.7.</td>
<td></td>
</tr>
<tr>
<td>Cause damage only when present in combination with another pathogen:</td>
<td></td>
</tr>
<tr>
<td>scored between 0.5 and 0.6.</td>
<td></td>
</tr>
<tr>
<td>Cause damage at very high population densities or in combination with other</td>
<td></td>
</tr>
<tr>
<td>abiotic stress: scored between 0.4 and 0.5.</td>
<td></td>
</tr>
<tr>
<td>Known to damage plants but damage has not been quantified: scored</td>
<td></td>
</tr>
<tr>
<td>between 0.3 and 0.4.</td>
<td></td>
</tr>
<tr>
<td>Information on damage potential was not available, or confirmed as of</td>
<td></td>
</tr>
<tr>
<td>negligible impact to host plant through field and lab experiments: scored</td>
<td></td>
</tr>
<tr>
<td>less than 0.3.</td>
<td></td>
</tr>
<tr>
<td>The economic and environmental impacts of a pathogenic species depend on</td>
<td></td>
</tr>
<tr>
<td>their aggressiveness towards their host. The more aggressive a pathogen</td>
<td></td>
</tr>
<tr>
<td>is; the greater are the chances it will adversely affect its host and in</td>
<td></td>
</tr>
<tr>
<td>doing so also affect the yield of the host and also indirectly impact other</td>
<td></td>
</tr>
<tr>
<td>organisms dependent on the same host.</td>
<td></td>
</tr>
</tbody>
</table>

### Host range

<table>
<thead>
<tr>
<th>Description</th>
<th>Score Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four or more plant families or if host plant is widely cultivated worldwide:</td>
<td></td>
</tr>
<tr>
<td>scored greater than 0.8.</td>
<td></td>
</tr>
<tr>
<td>Three families: scored between 0.7 and 0.8.</td>
<td></td>
</tr>
<tr>
<td>Two families: scored between 0.6 and 0.7.</td>
<td></td>
</tr>
<tr>
<td>More than 20 hosts from one family: scored between 0.5 and 0.6.</td>
<td></td>
</tr>
<tr>
<td>10-20 hosts from one family: scored between 0.4 and 0.5.</td>
<td></td>
</tr>
<tr>
<td>Less than 10 hosts from one family: scored between 0.3 and 0.4.</td>
<td></td>
</tr>
<tr>
<td>Only one plant host which is not widely cultivated: scored less than 0.3.</td>
<td></td>
</tr>
<tr>
<td>Pathogenic species capable of parasitising and reproducing on a wide range</td>
<td></td>
</tr>
<tr>
<td>of plant hosts have better chances of finding a suitable host in a new range</td>
<td></td>
</tr>
<tr>
<td>than species with a narrow host range. The only exception is species which</td>
<td></td>
</tr>
<tr>
<td>parasitise cosmopolitan hosts which are widely cultivated.</td>
<td></td>
</tr>
</tbody>
</table>

### Emerging pest status

<table>
<thead>
<tr>
<th>Description</th>
<th>Score Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reports of damage from new places and evidence of spread in last ten years:</td>
<td></td>
</tr>
<tr>
<td>scored greater than 0.8.</td>
<td></td>
</tr>
<tr>
<td>Species with increased pathogenicity and parasitism of plants with resistance</td>
<td></td>
</tr>
<tr>
<td>genes in last ten years: scored between 0.7 and 0.8.</td>
<td></td>
</tr>
<tr>
<td>Frequent reports (1 or more per year) of damage from places where a pest</td>
<td></td>
</tr>
<tr>
<td>exists in last ten years: scored between 0.6 and 0.7.</td>
<td></td>
</tr>
<tr>
<td>Species recorded from 10 or more new plant hosts in last ten years: scored</td>
<td></td>
</tr>
<tr>
<td>between 0.5 and 0.6.</td>
<td></td>
</tr>
<tr>
<td>Species able to overcome host defences can be used as a proxy for underlying</td>
<td></td>
</tr>
<tr>
<td>genetic and phenotypic capacity to co-evolve and adapt. This combined with</td>
<td></td>
</tr>
<tr>
<td>evidence of recent spread is a good indicator for species with expanding</td>
<td></td>
</tr>
<tr>
<td>ranges.</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Species recorded from 5-10 new plant hosts in last ten years: scored between 0.4 and 0.5.
Species recorded from less than 5 new plant hosts in last ten years: scored between 0.3 and 0.4.
PPN species which have not spread into new areas but caused infrequent damage in areas where they are present in last ten years: scored less than 0.3.

<table>
<thead>
<tr>
<th>Species identification</th>
<th>Uncertainty due to knowledge base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular information not available and taxonomic expertise is required; From a Genus with more than 50 species: scored greater than 0.8. From a Genus with 20-50 species: scored between 0.7 and 0.8. From a Genus with less than 20 species: scored between 0.6 and 0.7. Limited molecular information; both morphological and molecular methods are required for reliable species identification: scored between 0.5 and 0.6. Positive control not widely available (e.g. species is not a well known quarantine pest) and require some taxonomic expertise for identification: scored between 0.4 and 0.5. Molecular markers and sequence information are available and species can be identified based on molecular methods: scored between 0.3 and 0.4. Well known quarantine pest, rapid diagnostic tools and positive controls are available (i.e. with molecular information and protocols to accurately identify a species without requiring taxonomic expertise): scored less than 0.3.</td>
<td></td>
</tr>
<tr>
<td>Less than 10 published studies, biology and ecology of species not well known: scored greater than 0.8. 10-20 published studies, very limited information on species biology and ecology: scored between 0.7 and 0.8. 20-30 published studies, but lacking some basic information required for risk assessment: scored between 0.6 and 0.7. 30-40 published studies, but lacking some information required for risk assessment: scored between 0.5 and 0.6. 40-50 published studies, but lacking some information required for risk assessments: scored between 0.4 and 0.5. More than 50 published studies, but lacking some information required for risk assessments: scored between 0.3 and 0.4.</td>
<td></td>
</tr>
</tbody>
</table>

Species identification is crucial for precise assessment of biosecurity risks. The ability to accurately identify a species can impact on the measures used to prevent their entry. If a species is misidentified, then the biosecurity risks are also likely to be misrepresented. Hence a measure of the skills required to identify a species indicates the practical challenges.

The lack of information on a species is a major challenge to assessing the biosecurity risks. By quantifying the available information on a species, we can estimate the uncertainty associated with a species.
Detailed studies on species biology and ecology are available and provide most of the basic information required for risk assessment: scored less than 0.3.

<table>
<thead>
<tr>
<th>Pathotypes</th>
<th>Disease complexes</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>More than 10 recognised races: scored more than 0.8.</td>
<td>Pathotypes of a species can have different levels of aggressiveness and impacts. Where countries do not have specific pathotypes, the biosecurity risks from exotic pathotypes can be assessed.</td>
<td></td>
</tr>
<tr>
<td>6-10 recognised races: scored between 0.7 and 0.8.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-6 recognised races: scored between 0.6 and 0.7.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-3 recognised races: scored between 0.5 and 0.6.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Races not recognised but considered as species complexes: scored between 0.4 and 0.5.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-species differences in host range and pathogenicity are published: scored between 0.3 and 0.4.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of races controversial or uncertain: scored less than 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Act as vector of virus and forms disease complex: scored greater than 0.8.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forms disease complex with fungi or bacteria: scored between 0.7 and 0.8.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Causes lesions which can predispose plant to attack by other pathogens: scored between 0.6 and 0.7.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Associated with disease complex but mechanism not known: scored between 0.3 and 0.6.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evidence on disease complex not available: scored less than 0.3.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* When assigning scores, when there are exceptions, an expert may assign higher or lower values than those described in the scheme and provide reasons for assigning the score.
Figure 5.1 Conceptual representation of the PeST framework

Stage 1: Qualitative method
(Selection of species, formulation of assessment criteria and weights)
A. Systematic selection of species of phytosanitary importance
B. Expert opinion - open ended questionnaire used to determine assessment criteria and weights
C. Literature review - validation of criteria and weights
Iterate and update following peer review and new information

Stage 2: Quantitative method (Biogeography)
Analysis of pest distribution data using Self Organising Map (SOM). The SOM index based on species assemblages and biogeography is used to rank and prioritise species for further analysis. Iterate and update following changes in species distributions.

Stage 3: Semi-quantitative method
(Species biology and ecology)
Expert evaluation of published information using the following 10 criteria and scores combined using weighted averages.
Threat index= SOM index from stage 2 *(0.2) + Pathogenicity* (0.1) + Host range* (0.1) + Disease complex *(0.05) + Pathotypes*(0.05) + Emerging pest status*(0.1) + Species identification*(0.1) + Uncertainty due to knowledge base*(0.1) + Pathways*(0.1) + Survival adaptations*(0.1)
Iterate and update following peer review and new information

Circular arrows represent iteration to accommodate changes as a result of peer review or new information (Stages 1 and 3), and changes in species distribution (Stage 2).
5.3.3 Statistical methods

The linear opinion pool method (Stone 1961) was used for aggregating the scores because it adjusts individual scores to account for differences in scales and scaling. The scores for each criterion \( (x) \) and its weight \( (w) \) were combined using weighted averages to obtain an overall risk index from a species i.e.

\[
\bar{x} = \sum_{i=1}^{n} w_i x_i
\]

Hence, risk index = Likelihood of species establishment*0.2 + pathways*0.1 + survival adaptations*0.1 + pathogenicity*0.1 + host range*0.1 + emerging pest status*0.1 + species identification*0.1 + uncertainty due to knowledge base*0.1 + pathotypes*0.05 + disease complex*0.05.

The scores for the 97 species for each of the ten criteria, plus the overall risk (weighted mean values obtained from the linear opinion pool method, above) were incorporated into a 97 by 11 data matrix. Spearman’s correlation coefficient was then used to statistically compare the interrelationships between each criterion and the overall risk. Principal Component Analysis (PCA) was also used to assess the variability of scores between species for each criterion and the contribution of each criterion to the overall risk index. The tests were conducted in R (R Development Core Team 2010) using the “pspearman” package (Savicky 2009) and “princomp” function (RDevelopmentCoreTeam 2010), respectively.

5.4 Results

Over a third of species (34% from 14 genera), were classified as emerging pests (i.e. with scores greater than 0.5; after assessing information on damage reports from new areas or increased pathogenicity or ability to overcome host plant resistance). More than three-quarters of species (86% from 28 genera with scores greater than 0.5) were also known to cause economically significant damage in areas where they had been recorded. More than half the species (51% from 20 genera) could form disease complexes with other soil microorganisms such as bacteria, fungi or acted as vectors of viruses, and nearly two thirds (65% from 25 genera) also had wide host ranges or cosmopolitan host species. When assessed for pathways, more than three-quarters of species (78% from 22 genera) were associated directly with traded produce (e.g. tubers, rhizomes or with propagative material such as seeds, nursery stock, ornamental, bonsai...
plants): hypothetically, any PPN inhabiting soil could be carried via soil as a contaminant but there were few published interception records for such a pathway. Nearly half of the PPN species assessed (47% from 13 genera) had adaptations to survive in soil or with their host plant as endoparasites, or as semi-endoparasites for several months, and some for many years in cysts, or in a quiescent state. Rapid species identification protocols not needing taxonomic expertise were only available for 18% of species (6 genera), but for most species (82% from 29 genera), specialist taxonomic expertise was required for their identification: about a third (32% from 19 genera) did not have any published genetic sequence information available in GenBank. Only a small percentage of species (13% from 6 genera) had pathotypes, but all these species had high risk indices and were ranked highly overall (see below): none had rapid identification protocols for pathotypes available. Species identification had a significant positive correlation ($\rho = 0.41$) to ‘uncertainty due to knowledge base’.

For the criterion of uncertainty in knowledge, no species was investigated in depth as an invasive species, so all species had general uncertainty scores greater than 0.5. Because there are few studies of nematodes as invasive species, the number of other studies, their objectives and the breadth of information (i.e. knowledge base) on a species were used as proxies for estimating general uncertainty. This knowledge base on PPN species was highly variable, with both geographic and taxonomic biases. The well-known and economically-important species recorded from countries with nematological expertise (e.g. *Bursaphelenchus xylophilus*, *Globodera pallida*, *Heterodera glycines*) were the best studied of all PPN, but still lacked much of the knowledge or data necessary for predicting risks, and so had scores above zero for this criterion. PPN species not well known (i.e. present in localised areas and not yet widespread e.g. *Paratylenchus minutus*, *Meloidogyne indica*, *Scutellonema unum*) were relatively less studied and had relatively higher scores on general uncertainty. Comparison of the scores for uncertainty against the overall risk index was used to determine species where research to fill in critical knowledge gaps will be most beneficial. For example *Globodera tabacum*, *Heterodera cajani*, *H. filipjevi*, *Meloidogyne ethiopica*, *Pratylenchus fallax* and *P. sudanensis* had risk index in the range of 0.52-0.63 and also had high uncertainty with scores over 0.6 and lacked basic information required for biosecurity risk analysis thus are good candidates for further research.
Overall risk indices ranged from 0.64 to 0.30 (Table 5.2). The ten top-ranked exotic PPN species for Australia had risk indices from 0.64 to 0.62 and were *Heterodera zeae, Meloidogyne graminicola, Meloidogyne enterolobii, Heterodera filipjevi, Ditylenchus destructor, Scutellonema bradys, Heterodera glycines, Globodera pallida, Bursaphelenchus xylophilus* and *Meloidogyne chitwoodi*. There was a small gap below 0.62 with the next highest score being 0.59, then another 22 species with slightly lower indices between 0.5 and 0.59. The majority of species (N= 65 or 67%) had indices lower again, between 0.3 and 0.49 (Table 5.2).

*Heterodera* (cyst nematodes) and *Meloidogyne* (root-knot nematodes) were by far the genera with the most high-ranked species, with 3 species each in the top 10 in addition to 11 and 9 species, respectively, in the top 50 (Figure 5.2).

The criteria with the strongest positive correlations to the risk index were emerging pest status ($\rho = 0.80$) and pathogenicity ($\rho = 0.77$). The top ranked species *Heterodera zeae, Meloidogyne graminicola* and *M. enterolobii* were all classified as emerging pests. Most of the other criteria also had significant positive correlations to the risk index, but with lower correlation coefficients. In descending order of coefficients they were: emerging pest status > pathogenicity > host range > SOM index > disease complexes > pathways > pathotypes > survival adaptations (Table 5.3). The criterion of uncertainty due to knowledge base was negatively correlated to the risk index, while the criterion on species identification did not have a significant correlation (Table 5.3). Each of the criteria contributed differently to the risk index of PPN species according to the PCA, which also indicated emerging pest status and pathogenicity as major components with the greatest variability contributing to the risk index (Figure 5.3).

Generally, the risk index was always greater than the SOM index except for *Hirschmanniella oryzae*, which had a slightly lower risk index than its SOM index (Figure 5.4).

There are similarities and differences in the ranked list of plant-parasitic nematodes produced by the PeST framework and the current High-Priority Pest (HPP) list for Australia. Five of the six top-ranked species were not on the HPP list: *Heterodera zeae, Meloidogyne graminicola, Meloidogyne enterolobii, Ditylenchus destructor* and *Scutellonema bradys*. However, most of the HPP list ranked in the top 15 in our analysis: *Heterodera filipjevi* (ranked 4th with a score of...
0.634186), *H. glycines* (ranked 7th with a score of 0.631558), *Globodera pallida* (ranked 8th with a score of 0.624176), *Bursaphelenchus xylophilus* (ranked 9th with a score of 0.62028), *H. latipons* (ranked 13th with a score of 0.56405). Of the species on the HPP list, only *H. carotae* was not near the top of the rankings (ranked 45th with a score of 0.466634). Some species currently not on the HPP list for Australia were ranked as posing risks greater than some of the species on the HPP list: these included *Meloidogyne chitwoodi, Nacobbus aberrans, Scutellonema clathricaudatum, Zygoltylenchus guevarai* and *Pratylenchus sudanensis* (Table 5.2).

**Figure 5.2** Risk index range and number of species per plant parasitic nematode genus

![Risk index range and number of species per plant parasitic nematode genus](image)

*Based on the top 50 species listed in Table 5.2*
Table 5.2 Plant parasitic nematode species rankings based on overall risk index

<table>
<thead>
<tr>
<th>Top 50 Species</th>
<th>Overall risk index</th>
<th>Species ranked 51-97</th>
<th>Overall risk index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Heterodera zeae</td>
<td>0.642916</td>
<td>51. Bursaphelenchus mucronatus</td>
<td>0.458422</td>
</tr>
<tr>
<td>2. Meloidogyne graminicola</td>
<td>0.641942</td>
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1PPN species indicated in bold are already on the high priority pest list for Australia.
Table 5.3 Spearman’s correlation values for criteria used in PeST framework

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<th>Pgen</th>
<th>Host</th>
<th>Dise</th>
<th>Ptyp</th>
<th>Emer</th>
<th>Sp.ID</th>
<th>Uncert</th>
<th>Pway</th>
<th>Surv</th>
<th>Risk</th>
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<td>0.496021*</td>
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</tr>
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<td>0.58543*</td>
<td>0.352649*</td>
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</table>

Criteria abbreviations: SOM = SOM index; Pgen = Pathogenicity; Host = Host range; Dise = Disease complexes; Ptyp = Pathotype; Emer = Emerging pest status; Sp.ID = Species identification; Uncert = Uncertainty due to knowledge base; Pway = Pathways; Surv = Survival adaptations. * indicates significant correlation at 95% confidence.
**Figure 5.3** Principle component analysis biplot of plant parasitic nematode species vs. criteria in PeST framework

Criteria abbreviations: SOM = SOM index; Pgen= Pathogenicity; Host= Host range; Dise= Disease complexes; Ptyp = Pathotype; Emer= Emerging pest status; Sp.ID = Species identification; Uncert = Uncertainty due to knowledge base; Pway = Pathways; Surv= Survival adaptations. For abbreviations of species, please refer to supplementary information 5.2.

**Figure 5.4** Plot of self organising map index and risk index of 97 plant parasitic nematode species
5.5 Discussion

5.5.1 Plant-parasitic nematodes

Of the ten criteria used to assess the risk from potentially invasive PPN, emerging pest status (determined by reports of recent spread of PPN species into new areas, ability to overcome host plant resistance or increase in pathogenicity) was most strongly correlated to the overall risk index. Studies of taxa other than nematodes have also suggested that previous invasion success is a good predictor of future invaders (Daehler & Strong Jr 1993; Ricciardi & Rasmussen 1998; Daehler & Carino 2000; Kolar & Lodge 2001; Mack et al. 2002; Marchetti, Moyle & Levine 2004; Hayes & Barry 2008; Philibert et al. 2011).

The pathogenicity of PPN species was also strongly positively correlated to the risk index. This criterion was based on current recorded levels of damage to host plants (as distinct from increase in damage as for emerging pest status, above). Of course, pathogenicity in an existing range does not necessarily equate exactly to pathogenicity and impacts in a new area because pathogenicity in any particular situation is based on a complex interaction between the particular nematode, host plant(s) edaphic factors and the environment (Acosta 1982; Trudgill 1991; Griffin 1996). Hence, although pathogenicity in existing areas is a worthwhile criterion, additional information on host range, likelihoods of establishment (SOM index), disease complexes and existence of pathotypes, as used together in the PeST framework, offer better estimation of the risk from a species than any criterion on its own. Additional information, such as the distribution of suitable host(s) and models forecasting economic and environmental impacts of a species, improve estimates of pathogenicity in potentially invaded areas even more, but require considerable time and effort to collect or formulate, and so are incorporated in the more detailed risk assessment stage (Hodda & Cook 2009; Cook et al. 2011b; Baker et al. 2012).

Pathways were another major positive contributor to the overall risk. Reports of interceptions are reasonably common (Grousset et al. 2012) and association with many pathways should therefore be a good estimator of potential to be moved either in direct association with produce, packaging material etc, or with contaminants such as soil. However, movement along a pathway does not mean that it will necessarily be a route for an invasion: successful invasions are rarely traceable to a specific event or pathway. Introduction via contaminants was rated as contributing less to overall risk than established pathways even though some known PPN invasions may
have resulted from contaminants (Ferris et al. 2003; Okabe et al. 2012). The lower rating reflected that fewer substrates and smaller amounts of contaminant materials are involved. Transmission may also be more intermittent than on directly traded materials. Partly balancing this is the fact that contaminant—principally soil in the case of plant-parasitic nematodes—may be transported in many ways, for example on footwear, clothing, machinery, with seeds and plant products etc. (Lal & Lal 2006; McNeill et al. 2011). Pathways have been found a good predictor of invasive success of exotic species in taxa other than PPN (Wilson et al. 2009; Pysek, Jarosik & Pergl 2011).

Survival adaptations, was a criterion contributing substantially to the overall risk index. Scores were based on the likelihood of species surviving transit via any known pathway. Species with better survival adaptations pose greater risks than those without: cyst-forming nematodes from the genera Globodera, Heterodera and Punctodera survive long periods in cysts without hosts; species from Aphelenchoides, Bursaphelenchus, Ditylenchus and Subanguina can enter cryptobiosis enabling them to survive for several months; and. endoparasitic and semi-endoparasitic nematodes in the genera Meloidogyne, Pratylenchus, Nacobbus, Rotylenculus, Scutellonema and Zygotylenchus can survive transit in bare rooted plant materials. Data for all these species and genera come from field studies rather than nematodes in transit, so may be only indicative of the true biosecurity situation. Actual survival periods vary depending on life stage (Womersley, Wharton & Higa 1998), so which stage—egg, adult or juvenile—is associated with a particular pathway is important to assess the real risks (Singh et al. 2013a). Furthermore, survival in transit may differ from that in field soils, but experimental data on this is not available for most PPN. Nonetheless survival of PPN in transit is a very real possibility considering the evidence of live PPN extracted from intercepted material (Tenente et al. 1996; Plumas, Taboada & Gandarilla 2002; Lal & Lal 2006). Information on survival periods in transit is a potentially important data gap which requires further research. The linkage of survival adaptations and pathways was evident in the positive correlation between the two criteria. Nevertheless, the correlation was far from perfect, and data available can vary substantially in both quantity and sources, so including both independently seem advisable.

Species identification was the one criterion that did not have a significant correlation to the overall risk index. Nevertheless, it is included because it is essential for the risk assessment and biosecurity decision making process. The species identity is the tag used for all the information
on a species (Mayr & Ashlock 1991). In the biosecurity context, this means whether the species is exotic to the region of interest or not, its risk profile and the appropriate biosecurity measures are all dependent on the species identity. An example is where biosecurity responses to an incursion can be quite different depending on the species identification (Carnegie & Cooper 2011; Elith et al. 2012). The capability and expertise required for accurately identifying species need to be factored into risk estimation because species which are difficult to identify are also the most poorly studied. (Here, difficult to identify meant requiring specialist taxonomic expertise and lacking molecular information for rapid diagnostics.) The association between species identification and general uncertainty was shown by the positive correlation between the species identification criterion and the knowledge base criterion. Indeed, scores for these criteria could be compared to the overall risk index to prioritise species for further research aimed at reducing the uncertainty and developing the diagnostic tools most needed.

There was a negative correlation between ‘uncertainty due to knowledge base’ and overall risk index: species with high general uncertainties had relatively lower overall risk indices than well-studied species with less uncertainty. The risks from species with little information (or high uncertainty) are the least predictable. Combined with geographic and taxonomic biases, lack of information and uncertainty limit estimation of risks (Pysek et al. 2008; Venette et al. 2010). This applies to many risk assessment methods for alien species (Baker et al. 2008; Dahlstrom, Hewitt & Campbell 2011; Leung et al. 2012) whereas the PeST framework explicitly recognizes species with high general uncertainty to avoid underestimation of biosecurity risks. The economic importance of a species, the species distribution, and the residence or availability of nematological expertise are all underlying factors which influence how well a PPN species has been studied (Singh, Hodda & Ash 2013), so species with high uncertainty may need some evaluation by experts before exclusion from detailed analysis. Species with the highest uncertainty may also be targets for further research.

5.5.2 PeST framework features and usability

The general requirements for a pest screening system to predict potential invaders (as outlined by Mack et al. 2002; Daehler et al. 2004) are:

- scientifically justifiable components;
- a logical framework that incorporates factors important in the invasion process (based on critical observations, experimentation or both);
• transparency of the processes leading to the outcome;
• a process open to peer review; and
• minimum use of subjective opinions such that assessments are repeatable and lead to the same conclusion.

The PeST framework satisfies these requirements using a three stage approach to screen and prioritise exotic species in a coherent way. Below we discuss how each of these requirements is achieved.

All three stages in the PeST framework are clearly documented and retraceable, thus providing transparency necessary for biosecurity risk assessments under ISPM. In the first stage of the PeST framework, a systematic global list of species of potential phytosanitary importance is generated, and the species considered and the scientific reasons for screening are documented (Singh, Hodda & Ash 2013). A systematic selection of species also ensures that species are not overlooked. To maintain transparency, the database can be constructed from publicly accessible, peer reviewed articles and databases like CABI abstracts, Web of Knowledge and CABI crop protection compendium, CABI invasive species compendium and the EPPO plant quarantine data retrieval system. These resources are used universally for invasive species research by scientists and policy makers (Bayliss et al. 2012).

The inclusion of a global pest list (in stage one) and SOM analysis of global distribution dataset (in stage two) of PeST framework, allow its application across geographies and species. The SOM index provides reliable preliminary estimate of biosecurity risks from a species to any country or region (Worner & Gevrey 2006; Paini et al. 2010; Paini et al. 2011; Morin, Paini & Randall 2013). Using a quantitative measure (SOM index) for prioritizing species minimises subjectivity, avoids bias due to expert opinion, and is a rapid means of ranking a large number of species (Paini et al. 2010). The SOM analysis also offers flexibility to select the region of interest for further analysis in stage three. Any other country or region in the dataset could be selected instead of Australia for further analysis.

Review of the literature and expert opinion are often used to estimate the characteristics of invasive species for incorporation in the prediction of future invaders (Pheloung, Williams & Halloy 1999; Mack et al. 2002; Daehler et al. 2004; Copp, Garthwaite & Gozlan 2005). Biological characteristics can be difficult to measure on quantitative, comparable scales, but the
third stage of PeST framework overcomes this by using weighted averages to integrate the SOM index with semi-quantitative scores from the other nine criteria. Using this method both biotic and abiotic factors important in the invasion process can be included.

The framework allowed incorporation of expert opinion in two ways: first, when it was used to assign weights to the assessment criteria consistently across all species and reflecting their relative importance; and second, when expert judgement was used to assign scores for each species. The PeST framework sets conditions for scoring each criterion that guide different assessors to score consistently between 0-1 for each criterion. In addition, the reasons for assigning a score should be documented as part of the process, thus becoming available for reference later and also open to peer review. This use of numerical quantitative or semi-quantitative assessment assists in refining assessments of risks, even those involving great complexity and uncertainty (North 1995; MacDiarmid & Pharo 2003). These features of the framework should facilitate more precise conclusions than those reached after qualitative reasoning alone.

The PeST framework also incorporates heterogeneous data and diverse expertise. Generally, these features improve the assessment quality, efficiency and predictive capability (Munns et al. 2003; Suter et al. 2003; Cook & Proctor 2007; Schrader et al. 2010; Holt et al. 2012; Kumschick et al. 2012; Leung et al. 2012; Schrader et al. 2012). Integration of multiple components for assessing the risks from alien species was also recommended in a review of over 300 pest risk assessments (Leung et al. 2012). Ideally the PeST framework should be used with multiple assessors, but when not available, a single assessor can be used (as was the case in this study). The scores from multiple experts can be combined either using simple averages or weighted averages when the expertise of assessors are highly variable (Hammitt & Zhang 2013). Weighted averages, as used here, avoid extreme scores for one criterion leading to either underestimation or overestimation of the overall risks (Zhu et al. 2000; Holt 2006; Holt, Black & Abdallah 2006). Also weighted averages allow explicit definition of the contribution of each criterion to the overall score (Holt 2006).

The Weed Risk Assessment (WRA) model (Pheloung, Williams & Halloy 1999) and Fish Invasiveness Scoring Kit (FISK) model (Copp, Garthwaite & Gozlan 2005) are two other examples of pest screening systems which have been adopted internationally for screening
potential invasive species. Both of these screening systems were designed mainly for evaluating intentional import requests. By comparison, the PeST framework is designed for species which are not imported intentionally and can be well-known pests or poorly known. The WRA and FISK models provide a score for deciding whether to accept or reject deliberate importation, while PeST ranks species based on their potential risks. Calibration may be necessary before the PeST framework can be used for screening intentional imports, because criteria such as pathways and survival adaptations may be redundant.

The PeST framework differs from the WRA and FISK models in several other ways. It systematically selects numerous species for screening instead of targeting particular species selected after import requests. It uses a quantitative estimate (the SOM index) for the suitability of a species for a particular region instead of a semi-quantitative estimate based on biogeography. It uses weights based on relative importance of the different criteria instead of equal weights. Finally, it uses a consistent scoring scale between 0-1 for all criteria instead of variable scoring scales between criteria or questions as in WRA and FISK. All these features make it more suitable for the highly uncertain situation in biosecurity for plant pests and diseases.

A similar decision-support scheme, the computer-assisted pest risk analysis (CAPRA) has been developed by the EPPO for evaluation of pest species (EPPO 2011). The CAPRA has four modules and requires answers for several questions on the biology and ecology of a pest species to categorise the risks and decide whether a full risk assessment is necessary (EPPO 2011). Compared to CAPRA, the PeST framework also assesses information on the biology and ecology of a species. However it incorporates quantitative establishment likelihood (SOM index) and uses a semi quantitative weighted multi-criteria scheme to determine a quantitative overall risk index which can be used to rank and prioritise multiple species simultaneously. The PeST framework is not equivalent to a detailed risk assessment as it is designed to assess multiple species and identify species for detailed risk analysis. Meanwhile the CAPRA scheme is designed to assess one species at a time and when a full risk assessment is necessary, CAPRA can be used to conduct a detailed risk analysis. This is a major difference from the PeST framework.
5.5.3 Recommendations and future use of PeST framework

The PeST framework provides the means to document and organise information and data as well as analysing multiple species using a consistent framework. It also has the advantage that, once compiled for a particular group of organisms, the list of species and their global distributions become a comprehensive resource that can be used for future risk analyses and updated as needed. The species list can also be amended in response to feedback from peer review, as new information becomes available, or as new species are recognised. The design allows for integration of feedback in all three stages. Indeed, we suggest that the information and data are organised using a database management system so that it can be updated easily. Risk assessments are frequently iterative, so hosting the framework on a database system also facilitates iterative improvements. Having the data on a single database can also make the process transparent and accessible to multiple stakeholders, including those working on risk analysis (e.g. academic and research organisations) as well as risk managers (e.g. diagnosticians, biosecurity officers, industry and policy makers). The framework is also aligned with the ISPM pest risk assessment processes so that species profiles compiled using the PeST framework can be used in later, more detailed risk assessment and risk management stages. All these characteristics should make the PeST framework maximally desirable for use on plant pathogens in biosecurity situations.

5.6 Acknowledgements

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5.7 References


Cross-validation of biosecurity risks from plant-parasitic nematodes to Australia using CLIMEX modelling

Sunil K. Singh, Darren J. Kriticos, Gavin J. Ash and Mike Hodda

Manuscript is under peer review in: Australasian Plant Pathology
6.1 Abstract
Climate is one of the most important factors affecting the chances of a species establishing in a new, foreign location. CLIMEX models are a proven way of estimating the eco-climatic suitability and growth potential of plants and animals, but have seldom been applied to microorganisms like plant-parasitic nematodes. CLIMEX models were developed for three plant-parasitic nematode species of biosecurity concern to Australia: *Heterodera zeae*, *Hirschmanniella oryzae* and *Meloidogyne graminicola*. The models were carefully parameterised with species phenology and global distributions obtained from peer reviewed literature only. Rain-fed and irrigated scenarios were included to simulate possible different field conditions. The projected species distributions from CLIMEX were concordant with all available experimental and field observations with only a few exceptions. All three species had high eco-climatic and growth index values in Australia (>30), suggesting the biosecurity risks identified by other criteria are real. Maps of host crops (maize, rice, wheat) and irrigation areas in Australia were compared with projected growth index maps of each nematode species to identify areas at risk. Much greater areas were conducive for the growth of the three species under irrigation than under rain-fed conditions, both globally and in Australia. When assessing the establishment potential and biosecurity risks from exotic species, presence of modified conditions such as irrigation need to be taken into consideration.

6.2 Introduction
Projecting the likelihood of an exotic pest species establishing in a new area is of fundamental importance to biosecurity risk analysis (Venette *et al.* 2010; Baker *et al.* 2012). Climate is one of the main influences on the suitability for establishment (Sutherst & Maywald 1985; Sutherst 2003). To estimate the climatic requirements for species, existing species distributions are modelled. There are many different models available, each with their own strengths and limitations (Guisan & Zimmermann 2000; Guisan *et al.* 2006; Dupin *et al.* 2011; Eyre *et al.* 2012). Mechanistic species distribution models use data on phenology (temperature preferences, growth rate, mortality) from laboratory or field experiments, while correlative models use known species distributions (Eyre *et al.* 2012). However data on species distribution are often incomplete and for some species data on phenology are not available (Eyre *et al.* 2012; Kriticos *et al.* 2012a). CLIMEX, a semi-mechanistic integrated approach modelling climatic preferences avoids these limitations by integrating information on species phenology, known distribution and eco-climatic preferences with climatic data to project the potential distribution of a species (Sutherst & Maywald 1985; Sutherst 2003; Kriticos *et al.* 2012a). It assumes that species have
particular climatic preferences for growth and survival so that the potential range of a species can be estimated by matching the species preferences to climates (Sutherst & Maywald 1985; Sutherst 2003).

Defining the parameters for species preferences (temperature, soil moisture and climatic stresses affecting species growth) in a CLIMEX model requires detailed analysis of distribution and understanding of how climatic factors affect geographic distribution (Sutherst 2003). A thorough search of all literature is necessary to gather as much information relating to a species biology and ecology is required for the model. Hence, detailed analysis is feasible on a small subset of species only. Species may be selected based on expert opinion or as a result of regular interceptions at borders, or because new pathways are identified (Petter, Brunel & Suffert 2010). Such data are not available for many exotic species and especially for cryptic and microscopic organisms (Perez et al. 2012; Chapple et al. 2013).

Biosecurity risks from 250 plant-parasitic nematode species to Australia were systematically prioritized by analysing global distributions using a Self-Organising Map (Singh et al. 2013a), then integrated with a semi-quantitative multi-criteria evaluation of additional information on host range, pathogenicity, emerging pest status, survival adaptations, pathways, disease complexes, pathotypes, species identification, and species knowledge base (Singh et al. unpublished data [Chapter 5]). The multi-criteria evaluation ranked Heterodera zeae Koshy, Swarup & Sethi, 1971 and Meloidogyne graminicola Golden & Birchfield, 1965 as the highest risk while Hirschmanniella oryzae (van Breda de Hann, 1902) Luc & Goodey, 1964 had the highest SOM index but ranked 20th using the multi-criteria evaluation for Australia. All three species are economically important pests of maize, rice, and wheat -ricecropping systems (Babatola & Bridge 1979; Srivastava & Sethi 1985; Soriano & Reversat 2003; Singh, Sharma & Singh 2010) and have not been recorded in Australia. In this study we develop CLIMEX models for these three species for Australia. The models further corroborate the other methods of risk assessment.

6.3 Methods
6.3.1 Species distributions and geo-referencing of records
All information on the worldwide distribution of H. zeae, H. oryzae and M. graminicola were gathered from primary peer reviewed literature sources and where accessible, from national surveys and nematode collections. For places where a species was recorded, the locality
name(s) was extracted and used for geo-referencing the species distributions, together with as much metadata as was available. The locality information was then searched in Google Earth (Google Corporation 2013) and the metadata matched to the hybrid Google Earth satellite image map (Google Corporation 2013) for attributes such as altitude, distance to nearest town or city, river or irrigation area. The approximated species occurrences were marked using pushpins and the coordinates were extracted. The coordinates of species occurrences were plotted using DIVA GIS (Hijmans et al. 2005) as a shape file and used for building the CLIMEX models.

### 6.3.2 CLIMEX models

Temperature and ecological preference data were also gathered from peer reviewed literature only. Details of the basis for the values of each parameter for each species are provided in Supplementary Table 6.1.

In the CLIMEX model, ‘CliMond 10’ spatial resolution long-term average meteorological dataset (Kriticos et al. 2012b) and the ‘Compare Locations’ function were used to calculate the suitability of geographical locations globally based on the species parameters. The CLIMEX user’s guide provides detailed descriptions of how the model works (Sutherst, Maywald & Kriticos 2007). Values of the model output—the growth index (GI)—above 30 were regarded as indicating high suitability for a climate. Between 30 and 15, the index was taken to indicate suitability, and values between 15 and 5 were regarded as indicating marginal suitability.

All models were run with naturally occurring moisture and with an irrigation scenario. For *H. zeae* this was 2 mm per day; for *H. oryzae* this was 5 mm per day; and for *M. graminicola*, this was 3 mm per day. These figures are based on the normal irrigation regimes for the main crop hosts for each species as detailed in Supplementary Table 6.1.

### 6.3.3 Comparison of nematode distributions with host crop growing areas

The main crop host production area maps for maize, rice and wheat were obtained from the SPAM global database (You et al. 2012) and layered over the projected distributions for *H. zeae, H. oryzae* and *M. graminicola* using DIVA GIS (Hijmans et al. 2005). The projected nematode distributions (growth index – GI) were then compared with their respective host crop production areas in Australia, to determine where the nematodes posed the greatest biosecurity risk.
6.4 Results
A total of 243, 481 and 304 coordinates were obtained for *H. zeae*, *H. oryzae* and *M. graminicola*, respectively. All were used for fitting species parameters in the CLIMEX models and are summarised in Table 6.1. CLIMEX model parameters obtained for each species for rain-fed and irrigated scenarios are listed in Table 6.2.

Table 6.1 Current worldwide distribution of *Heterodera zeae*, *Hirschmanniella oryzae* and *Meloidogyne graminicola*

<table>
<thead>
<tr>
<th>Species</th>
<th>Current worldwide distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. zeae</em></td>
<td>Asia: Afghanistan, India, Indonesia, Nepal, Pakistan, Thailand.</td>
</tr>
<tr>
<td></td>
<td>Africa: Egypt.</td>
</tr>
<tr>
<td></td>
<td>Europe: Greece, Portugal.</td>
</tr>
<tr>
<td></td>
<td>North America: USA.</td>
</tr>
<tr>
<td><em>H. oryzae</em></td>
<td>Asia: Bangladesh, China, India, Indonesia, Japan, Malaysia, Myanmar, Nepal, Pakistan, Philippines, South Korea, Sri Lanka, Taiwan, Thailand, Vietnam.</td>
</tr>
<tr>
<td></td>
<td>Africa: Cote d’Ivoire, Egypt, Gambia, Ghana, Guinea, Madagascar, Mauritania, Niger, Nigeria, Senegal, Sierra Leone.</td>
</tr>
<tr>
<td></td>
<td>Europe: Portugal.</td>
</tr>
<tr>
<td></td>
<td>Central America and Caribbean: Costa Rica, El Salvador.</td>
</tr>
<tr>
<td></td>
<td>South America: Argentina, Brazil, Guyana, Venezuela.</td>
</tr>
<tr>
<td></td>
<td>North America: USA.</td>
</tr>
<tr>
<td></td>
<td>Africa: South Africa.</td>
</tr>
<tr>
<td></td>
<td>South America: Brazil, Columbia, Ecuador.</td>
</tr>
<tr>
<td></td>
<td>North America: USA.</td>
</tr>
</tbody>
</table>

1The record for *H. zeae* in Afghanistan, Indonesia, Greece and Portugal are recent and not shown in CABI map 851 (2002).
2The record for *H. oryzae* in South Africa shown in CABI map 813 (2000) is invalid and could not be traced to any published primary source (L. McGillivray, CABI, Pers. comm. 2013). The status of *H. oryzae* in South Africa has been confirmed by nematologists from South Africa (Drs E. Van den Berg and M. Marais, Pers. comm. 2013).
3The record for *M. graminicola* in Ecuador is recent and not shown in CABI map 826 (2001).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>H. zae</th>
<th>H. zae</th>
<th>H. oryzae</th>
<th>M. graminicola</th>
<th>M. graminicola</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moisture</strong></td>
<td></td>
<td>Rain-fed</td>
<td>Irrigated</td>
<td>Irrigated</td>
<td>Irrigated</td>
<td>Rain-fed</td>
</tr>
<tr>
<td>SM0</td>
<td>Lower soil moisture threshold</td>
<td>0.10</td>
<td>0.10</td>
<td>0.20</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>SM1</td>
<td>Lower optimal soil moisture</td>
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<td>0.20</td>
<td>0.60</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>SM2</td>
<td>Upper optimal soil moisture</td>
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<td>1</td>
<td>2</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>SM3</td>
<td>Upper soil moisture threshold</td>
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<td>1.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DV0</td>
<td>Lower temperature threshold</td>
<td>15</td>
<td>15</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
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<td>DV1</td>
<td>Lower optimal temperature</td>
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<td>28</td>
<td>27</td>
<td>26</td>
<td>26</td>
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<tr>
<td>DV2</td>
<td>Upper optimal temperature</td>
<td>33</td>
<td>33</td>
<td>32</td>
<td>30</td>
<td>30</td>
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<tr>
<td>DV3</td>
<td>Upper temperature threshold</td>
<td>40</td>
<td>40</td>
<td>38</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td><strong>Cold stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTCSA</td>
<td>Cold stress temperature threshold average</td>
<td>13</td>
<td>13</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>THCSA</td>
<td>Cold stress accumulation rate average</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.001</td>
</tr>
<tr>
<td><strong>Heat stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TTHS</td>
<td>Heat stress temperature threshold</td>
<td>40</td>
<td>40</td>
<td>38</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>THHS</td>
<td>Heat stress accumulation rate</td>
<td>0.005</td>
<td>0.005</td>
<td>0.01</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Dry stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMSD</td>
<td>Soil moisture dry stress threshold</td>
<td>0.10</td>
<td>0.10</td>
<td>0.15</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>HDS</td>
<td>Dry stress accumulation rate</td>
<td>-0.001</td>
<td>-0.001</td>
<td>-0.005</td>
<td>-0.001</td>
<td>-0.001</td>
</tr>
<tr>
<td><strong>Wet stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMWS</td>
<td>Soil moisture wet stress threshold</td>
<td>1.5</td>
<td>1.5</td>
<td>Not used</td>
<td>Not used</td>
<td>Not used</td>
</tr>
<tr>
<td>HWS</td>
<td>Wet stress accumulation rate</td>
<td>0.005</td>
<td>0.005</td>
<td>Not used</td>
<td>Not used</td>
<td>Not used</td>
</tr>
<tr>
<td><strong>Day degree</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DVCS</td>
<td>Temperature threshold used to calculate degree-days for cold stress functions; default = DV0</td>
<td>15</td>
<td>15</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>DVHS</td>
<td>Temperature threshold used to calculate degree-days for heat stress functions; default = DV3</td>
<td>40</td>
<td>40</td>
<td>38</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>PDD</td>
<td>Annual heat sum threshold</td>
<td>450</td>
<td>450</td>
<td>580</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td><strong>Irrigation mm/day</strong></td>
<td></td>
<td>Not used</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>Not used</td>
</tr>
</tbody>
</table>
6.4.1 *Heterodera zeae* projected distribution compared to known distribution

Majority of the presence records for *H. zeae* (98%) were in concordance with the projected growth index (GI) when using the 2 mm top-up irrigation model (Figure 6.1). The few exceptions included records from high altitude areas in Kashmir of northern Pakistan. The marginally suitable areas (GI range 6-13) where *H. zeae* has been recorded included Sind (Pakistan) and northern India. The best suited locations (GI > 40) where *H. zeae* has been recorded included southern India, southern Pakistan, Thailand, Indonesia and the Nile River delta areas in Egypt (Figure 6.1). *H. zeae* could grow all year in tropical countries while in the subtropics and temperate areas summer months were optimal for growth.

**Figure 6.1** Current and projected global distribution of *Heterodera zeae* (2 mm/day top-up irrigation)

Blue dots indicate places where *H. zeae* has been recorded and red shading represents areas with growth index of 6 or greater; the darker the shading the more climatically suitable.

6.4.2 *Hirschmanniella oryzae* projected distribution compared to known distribution

The projected distribution of *H. oryzae* using the 5 mm/day top-up irrigation model encompassed all known distribution records. Locations projected as marginally suitable (GI range 13-19) where *H. oryzae* is known included northern China (near Beijing) and in the north of Japan (Hokkaido). The countries projected as most suitable (GI >50) where *H. oryzae* already occurs included Indonesia, Philippines, Malaysia, Vietnam, Thailand, Burma, Bangladesh, Southern China, Southern India, Sri Lanka, Nigeria, Ghana, Cote d’Ivoire, Guinea,
Guyana, Venezuela, Costa Rica and El Salvador (Figure 6.2). *H. oryzae* could grow all year in tropical areas but summer months were optimal for the growth in subtropical and temperate areas.

**Figure 6.2** Current and projected global distribution of *Hirschmanniella oryzae* (5 mm/day top-up irrigation)

Blue dots indicate places where *H. oryzae* has been recorded and red shading represents areas with growth index of 6 or greater; the darker the shading the more climatically suitable.

### 6.4.3 *Meloidogyne graminicola* projected distribution compared to known distribution

All known records of *M. graminicola* fell within the projected distribution under the irrigation model. Marginally suitable locations (GI 8-12) where *M. graminicola* is present included northern India (Haryana and Uttar Pradesh) and a location near Sheikhupura, Pakistan. The most favourable locations (GI >50) where *M. graminicola* is known to occur included Indonesia, Philippines, Singapore, Malaysia, Vietnam, Laos, Thailand, Myanmar, Bangladesh, Southern India, Sri Lanka, South Africa, Columbia, Ecuador and the states of Louisiana and Florida in USA (Figure 6.3). *M. graminicola* could grow all year in tropical countries, but summer months were optimal in the subtropics and temperate countries.
6.4.4 Suitability of areas in Australia for *Heterodera* zae

northern Australia, including most crop growing areas in Queensland, parts of the Northern Territory and the northern parts of Western Australia, were the most suitable for growth of *H. zae* under rain-fed conditions, together with smaller areas in northeast New South Wales (GI >30, Figure 6.4a). In these areas 4-9 generations per year were projected. In Victoria, South Australia, Tasmania and southern parts of Western Australia, under rain-fed conditions, only 1-2 generations per year were projected, with growth indices <15.

Under the 2 mm/day top-up irrigation scenario, areas along the border of New South Wales and Victoria (Riverina, Murray and Murrumbidgee) where irrigation is used and maize is also grown (Figure 6.4 b and c), became more conducive for the growth of *H. zae* (GI 20-30) with a projected 3-4 generations per yr.

The optimal period for growth of *H. zae* in Australia was projected as October to March. In the southern parts of Australia including Tasmania, cold stress limited growth over the cooler months (April until September), while dry stress limited growth in central Australia and arid parts of Western Australia.
**Figure 6.4** CLIMEX projection of areas in Australia suitable for growth of *Heterodera zae*: (a) under rain-fed conditions; (b) under 2 mm/day top-up irrigation; and (c) maize-growing areas

### 6.4.5 Suitability of areas in Australia for *Hirschmanniella oryzae*

Under the irrigated scenario, most of the crop growing areas along the east coast of Australia (New South Wales, Queensland), northern Australia and south of Western Australia were suitable for the establishment of *H. oryzae* (GI 30-100; Figure 6.5a). The Riverina, Murray and Murrumbidgee irrigation areas where the majority of the rice in Australia is produced (Figure 6.5b), were also suitable for the establishment of *H. oryzae* (GI 30-45), with the warmer months from October to April optimal for growth (2-5 generations per year). In northern Australia the conditions were optimal for growth of *H. oryzae* all year round and 6-10 generations per year were projected.

The eco-climatic conditions in southern Australia—including Tasmania—were less suitable during the cooler months (May to September) for growth of *H. oryzae* (GI <30) because of cold
stress. The conditions in central Australia and the drier parts of Western Australia were less favourable for growth (GI <15) due to dry stress.

**Figure 6.5 (a) CLIMEX projection of areas in Australia suitable for growth of *Hirschmanniella oryzae* under 5 mm/day top-up irrigation; and (b) rice growing areas**

![CLIMEX projection of areas in Australia suitable for growth of *Hirschmanniella oryzae* under 5 mm/day top-up irrigation; and (b) rice growing areas](image)

**6.4.6 Suitability of areas in Australia for *Meloidogyne graminicola***

The east coast of Australia and parts of northern Australia were most favourable for the establishment of *M. graminicola* (GI 30-80, Figure 6.6a). In these areas, growth all year round and 9-12 generations per year were projected. Relatively smaller areas in southern Australia, Tasmania and Western Australia were also suitable for establishment of *M. graminicola* (GI 20-30). In southern Australia (including Tasmania), 2-8 generations per year were projected, and October to April was the most suitable period for growth. Dry and heat stress limited growth in the central and arid parts of Western Australia, while cold stress limited growth in southern Australia during the cooler months.

Under the 3 mm per day top-up irrigation scenario, greater areas in Queensland, New South Wales, Victoria, South Australia and Western Australia (Figure 6.6b) become more conducive for the establishment of *M. graminicola*. The majority of the rice (Figure 6.5b) and wheat cropping areas (Figure 6.6c) were suitable for the establishment of *M. graminicola* under these conditions.
**Figure 6.6** CLIMEX projection of areas in Australia suitable for growth of *Meloidogyne graminicola*: (a) under rain-fed conditions; (b) under 3 mm/day top-up irrigation; and (c) Wheat growing areas

6.5 Discussion

The projected distributions for *H. zea*, *H. oryzae* and *M. graminicola* matched 98-100% of their known records. The few exceptions were records of *H. zea* in the high altitude areas of Pakistan, and could be due to microclimatic variation such that suitable conditions for growth and persistence of *H. zea* are locally present in small, deep valleys where maize is grown (Ahmed & Qasim 1990).

Records of occurrences in small, isolated places away from major agricultural areas were, however, rare. Much more common are incomplete species distribution data. Areas not known to have been sampled are frequently encountered during biosecurity risk assessments. This
happens particularly often in nematodes because of dwindling taxonomic expertise and lack of thorough surveys (Coomans 2002; De Waele & Elsen 2007). The records of *H. zea*, *H. oryzae* and *M. graminicola* used were mainly from places where economically significant damage was occurring. It is plausible that each may be present in other areas which have not been reported. In these cases, CLIMEX modelling has been shown to better predict range expansion for invasive species than logistic regression methods (Sutherst & Bourne 2009), because species preferences are inferred from both known current distributions and biological information (Sutherst *et al.* 2004).

Distribution maps for the three species investigated are available from CABI (CABI 2013), but the maps show national and state level records only and are not recent. CLIMEX requires precise latitude and longitude data (Sutherst, Maywald & Kriticos 2007), so all precise species distribution coordinates were used. In CLIMEX, the climate matching database uses long term average monthly maximum and minimum air temperatures to match the species distributions with corresponding climate data (Sutherst, Maywald & Kriticos 2007). Since nematodes live mostly in soil, the inclusion of soil temperatures instead of air temperatures in the meteorological database would have been ideal. However due to the unavailability of long term soil temperature data on a comparable global grid; the air temperatures database was used. There is a need for global soil temperature data for modelling the distributions of soil borne pests and diseases.

Soil types are an important factor affecting PPN distributions (Norton, 1978). Although the CLIMEX models do not explicitly include data on distribution of soil types; it is indirectly taken into consideration in the model by using all the coordinates of known PPN species distributions. Given that the known species distributions are the result of a complex interaction of factors such as soil types, temperature, soil moisture, availability of host plants, cultivation history, etc. The soil types also affect the type of crops which can be grown and the crop distribution. The CLIMEX model projections were supported by comparing the distribution of host crops with the projected PPN distributions.

Relying only on a subset of records can lead to underestimation of the projected range of invasive species (Beaumont *et al.* 2009). However, by including data from studies of species survival and performance, CLIMEX at least partially circumvents this problem. Temperature
and soil moisture have been shown to affect the life cycles (survival and growth) of many plant-parasitic nematodes (Griffin, Asay & Horton 1996; Todd, Blair & Milliken 1999; Bakonyi & Nagy 2000; McSorley 2003). Although such ecological conditions may have indirect effects, geographic distributions are strongly influenced by how well nematodes survive and grow (Freckman & Caswell 1985). Furthermore, in CLIMEX the preferences of a species are used to calculate location-specific weekly and annual indices describing eco-climatic suitability, growth and stresses, so that unfavourable extremes are considered, not just annual averages (Sutherst 2003).

The CLIMEX model projections agree with independent field observations on growth of all three species. Laboratory studies were used to parameterise the model. The model predictions of the warmer months as most favourable for *H. zeae* agree with peak population levels observed on maize recorded during the warmer months in Egypt and India (Ismail, Abadir & Kheir 1993; Aruna & Siddiqui 1997). Model predictions of warmer months as most favourable for *H. oryzae* similarly agree with observations of peak populations in rice fields during summer in China, Japan, Egypt, Myanmar and India (Sato, Koyama & Koshihara 1970; Korayem 1993; Ramakrishnan 1995; Youqing, Xuebaio & Jianwen 1997; Maung et al. 2012). Likewise, model predictions of warmer months as most favourable for *M. graminicola* agree with observations of peak populations in rice fields during summer in Myanmar, Bangladesh and India (Rahman 1990; Dabur, Taya & Bajaj 2004; Win, Kyi & De Waele 2011). Such agreement of independent field observations with model predictions indicates that the models are robust in estimating the periods or seasons most favourable for growth as well as the suitability of locations.

CLIMEX projections also enabled spatial comparisons of risks by mapping GI, cropping and irrigation areas together. This further refines the estimation of risk. For instance, Queensland and Northern Territory in northern Australia are very favourable for establishment of *H. oryzae*, but rice—the main host—is not grown on large scales in these areas currently. However, there is considerable interest in growing rice in northern parts of Australia to meet projected increasing demands (Eastick 2012; Tapp 2013). *H. oryzae* is therefore a high risk quarantine pest for northern Australia because of both the suitability of the region for its establishment and its potential impact on future development of rice agriculture.
The results from the irrigation scenarios for all species investigated showed that relatively greater areas were suitable for establishment and growth under irrigation than under rain-fed conditions. This applied both in Australia and globally. This supports suggestions that when crops are grown under modified conditions like irrigation or in a glasshouse, the modification will often favour growth of associated pests as well, meaning that the modifications need to be included in biosecurity risk assessments (den Nijs, Brinkman & van der Sommen 2004; Gamon & Lenne 2012; Kriticos et al. 2012a). Establishment of plant-parasitic nematodes in irrigated areas may be an important consideration because these areas are particularly vulnerable to incursions from nematodes spread in irrigation water (Roccuzzo & Ciancio 1991; Hugo & Malan 2010).

The model predictions of where seasonal factors are likely to be major influences on nematode populations should enhance the efficiency of surveillance. Chances of early detection are better when nematode sampling is conducted during seasons most suitable for nematode growth. Often this is synchronised with the optimum growth conditions for the host plant(s) (Norton 1978; Neher 2010). After arrival in a foreign location, populations may take some time to build up to detectable levels (Singh et al. 2013 b). Lag times may be up to 4-20 years (Banks et al. 2012). Early detection, however, can vastly improve the success of eradication campaigns and decrease their cost (Hodda et al. 2008; Pluess et al. 2012). In the cases of the species investigated, this means that *H. zeae*, *H. oryzae* and *M. graminicola* could be surveyed at any time of the year in the tropical areas of Australia, but in temperate areas, the optimum time for surveying is from October to April.

In previous investigations *H. zeae*, *H. oryzae* and *M. graminicola* were identified as high biosecurity risk to Australia based on their history of spread, good survival adaptations, evidence of economically significant damage to host and the availability of host plants (Singh et al. unpublished data [Chapter 5]). The present study confirms that they could establish and grow well in local eco-climatic conditions if they arrive. The majority of Australian cropping areas of each species main host were suitable for their establishment. The establishment of nematodes may even be facilitated by the presence of alternative crop and weed hosts (Ringer, Sardanelli & Krusberg 1987; MacGowan 1989; Soriano & Reversat 2003; Abd-Elbary, Eissa & Youssef 2012).
Our modelling suggests that the biosecurity risks to Australia are scientifically justified for *H. zeae*, *H. oryzae* and *M. graminicola*. With the risks justified, biosecurity agencies and relevant industries can now decide on the appropriate counter–measures, such as development of detection and diagnostic capacity, certification requirements and closer scrutiny of pathways.

### 6.6 Acknowledgements
The authors acknowledge the support of the Australian Government Cooperative Research Centres program.

### 6.7 References


7

General discussion
7.1 General discussion

The studies presented in this thesis have shown how plant pests could be prioritised systematically for detailed biosecurity risk assessment. A new framework for the process has been developed, and applied to generate a prioritized list of the biosecurity risks from plant-parasitic nematodes to Australia. The results have been tested using detailed climatic modelling on three highly-ranked species. In doing this the characteristics of plant-parasitic nematodes which may result in them becoming invasive have been surveyed and synthesised, a data set of the most relevant characteristics has been developed, and several methods used to refine and prioritize the species in the data set, including the novel PeST framework method. The data and methods presented should provide a scientific basis for assessment of biosecurity risks; particularly to Australia from plant-parasitic nematodes, but also more generally and in other circumstances for other small, cryptic plant pathogens and pests.

The initial selection of species using broad criteria was important because a wide range of biosecurity risks from PPN species could be included in the screening process, including association with economically important plant host, ability to act as vectors of viruses or to form disease complex with other pathogens, plus the regulatory status of a species. This is a more proactive approach than the common current practice of initiating pest risk assessments after a pathway is identified, a pest is intercepted, records of incursions are released, new scientific information becomes available, or as biosecurity policies are revised (FAO-ISPM-11 2013).

A proactive approach to selecting species for screening biosecurity risks is necessary because many plant pests are introduced unintentionally or accidentally (Schrader & Unger 2003; Baker et al. 2008). This means that the pests can be transported by many pathways, including with traded goods, plants, seeds, nursery stock, ornamentals, cut flowers, packaging materials or soil (Roques & Auger-Rozenberg 2006; Kenis et al. 2007; Hulme et al. 2008). Based on their known or likely biology, many of the 250 PPN species originally identified in Chapter 3 could use many of these pathways, but only a few have actually been recorded within the pathways (Chapter 2). Despite the biases in the interception data, it can be very useful when assessing the available pathways. Interception data from several different countries were considered in Chapter 2 to determine the various pathways available for the international movement of PPN. Information on pathways is totally lacking for some other species: without targeted sampling and laboratory examination to detect their presence, microscopic organisms like nematodes can
be missed easily (Ward & Hockland 1996; Hockland et al. 2013). Interception data can also be biased towards particular pathways or groups of organisms depending on the sampling method and intensity (Bacon, Bacher & Aebi 2012). In the absence of information on pathways and due to biases in the interception data, PPN species may be overlooked when deciding which species are chosen for detailed risk analysis. This is a major potential pitfall that was avoided by systematically selecting PPN species for further analysis.

Another potential pitfall was avoided by using the likelihood of a species’ establishment for preliminary prioritization, rather than likely impacts. Even though information on the main hosts, yield loss and regulatory status were gathered during the preliminary screening stage, likelihood of establishment is a better indicator of biosecurity risks. The impacts and the economic value of the impacts caused by PPN are not easily determined, differ between the native and invaded ranges and are not available for many species (as explained in Chapters 2 and 3). Using likely impacts from a species during the preliminary prioritization means that a species whose impacts are not known may be eliminated in the preliminary stage even though it may have a high likelihood of establishing and later becoming invasive. In fact, the impacts from some invasive species become apparent and are recorded only long after they have established (Mack et al. 2000; Crooks 2005; Brasier 2008; Simberloff 2011).

Nonetheless, the likely impacts from a species is an important factor in the overall risk assessment and is the most common influence on policy decisions regarding invasive species (Heikkila 2011; Kumschick et al. 2012; Leung et al. 2012). Thus, the likely impacts from PPN species in the form of pathogenicity were included in the later, integrated assessment stage. Inclusion at this stage was feasible because fewer species were involved, given that gathering and evaluating information on pathogenicity is time consuming. The process by which self organizing maps (SOM) used potential for establishment to rank the initial 250 species considered, is described in Chapter 4.

The SOM modeling of global distributions of the 250 PPN enabled quantitative estimation of the establishment likelihoods for each species in Australia as a whole as well as for the individual states and territory. This involved a global species distribution dataset, and is advantageous because a large number of species were simultaneously analysed. This approach drastically reduces the time taken to determine the species establishment likelihoods compared
to other methods which model one species at a time. Indeed, gathering species presence and absence information was the most time consuming component in Chapter 4. However for pest groups where distribution datasets are more readily available from global databases such as the CABI Crop Protection Compendium (example datasets used by Worner & Gevrey 2006 and Paini et al. 2010), the SOM analysis could be carried out in a day or two, depending on the size of the dataset. The interpretation of the results from the SOM analysis and statistical analysis was the next most time consuming aspect.

The time and data requirements are both important factors in the choice of methods for biosecurity risk analysis (Baker et al. 2008; Magarey, Colunga-Gracia & Fieselmann 2009). The methods as outlined in Chapter 4 were a very efficient and effective way of ranking a large number of species and selecting species for further analysis. In addition, the species distribution datasets once gathered become a valuable resource for future pest risk assessments and can be used globally.

Pest risks are thought to vary for different geographical locations (Venette et al. 2010). For large countries such as Australia there is considerable variation among different bioclimatic regions (Hutchinson et al. 2005) and so pest risks would be expected to vary between states and territories within Australia. Different risks were identified by the SOM for the different jurisdictions, with the different rankings allowing prioritisation of species for both national and domestic quarantine (Chapter 4). The likelihood of establishment for a species in each jurisdiction is good preliminary measure of biosecurity risk which can be used to determine which species need further evaluation at the state rather than national level. Biosecurity is a shared responsibility between federal and state governments in Australia (Beale et al. 2008), so prioritisation of species at each level is desirable for administration of biosecurity measures and for optimal allocation of resources (Cook et al. 2011; Rout et al. 2011).

The biosecurity risks from 97 PPN species absent from Australia and of potential importance nationally were selected for further analysis using the PeST framework in Chapter 5. Species already present in Australia but with restricted distributions and of biosecurity importance locally (i.e. for each of the jurisdictions), were identified, but not analysed further in the present study due to time constraints. However, they could be evaluated very effectively using the PeST framework.
The PeST framework, as described in Chapter 5, was used to integrate the likelihoods of species establishment from Chapter 4 with multiple other criteria to determine an overall risk index encompassing species biogeography, biotic factors and abiotic factors. The multi-criteria evaluation was included after an expert opinion survey revealed that experts consistently used multiple criteria when assessing the biosecurity risks from exotic PPN. This conclusion from the expert opinion survey was supported by opinions and data in the published scientific literature (Chapter 2). Thus the scheme proposed for evaluating biosecurity risks formulated in Chapter 5 includes multiple criteria. In practice, expert opinion is widely and routinely used in biosecurity risk analysis (Leung et al. 2012), but the PeST framework uses expert opinion in a structured and transparent way to evaluate information and assign scores when evaluating the biosecurity risks from multiple species.

The PeST framework also uses weighting to ensure that different perceptions or measurement scales used in different criteria do not result in biased evaluations. Using multiple criteria should also lessen biases potentially caused by different quantities and qualities of data being available for different criteria and species. The PeST framework uses quantitative and semiquantitative scores to assess and prioritise multiple species for detailed risk assessments using limited resources. The PeST framework systematically estimates the risks but is not equivalent to a detailed risk assessment. Detailed risk assessments require significantly greater information and resources to determine the biosecurity risks from pest species more precisely.

The evaluation of the 97 species using the PeST framework ranked species differently than the SOM analysis in Chapter 4. The incorporation of additional information on species biology, ecology and uncertainty changed the overall rankings for species. For instance, *Hirschmanniella oryzae* ranked 1st based on SOM analysis but was ranked 20th using the PeST framework. *Heterodera zae* ranked 25th based on SOM analysis but was ranked 1st using the PeST framework. The species rankings based on SOM analysis are based on species distributions only, and so are recommended for use complementing more detailed assessments and expert evaluations (Worner et al. 2006, Paini et al. 2010). The multi-criteria evaluation in the PeST framework adds the other data explicitly, and so extends the application of the SOM analytical technique for biosecurity risk analysis.
In the present study, a person experienced and proficient in the taxonomic group (Masters Degree) gathered the information and data, scored the species using the multi-criteria scheme, and used the PeST framework. Users less experienced in a particular group of organisms could equally use the multi-criteria evaluation scheme proposed, but input from experts on the group would ensure that information and data are reliable prior to assigning scores. Rigorous review of data by experts is recommended if possible, but is often not practicable.

Information technology and database systems are integral tools for biosecurity risk assessments globally (Ricciardi et al. 2000; Graham et al. 2008; Magarey, Colunga-Gracia & Fieselmann 2009). The information and data on a species once gathered, evaluated and documented using the PeST framework could be a valuable resource for future risk analysis or for risk analysts in other geographical locations. Apart from the likelihoods of establishment, which is specific to a particular country or jurisdiction, the scores for the other nine criteria are applicable universally to PPN. As suggested in Chapter 5, hosting the PeST framework on a database management system to document all the information would allow iterative improvements as new information or data on a species becomes available. Such architecture of the PeST framework also provides transparency, satisfies international guidelines for pest risk assessment and the datasets generated would save time for future risk analysis.

The multi-criteria evaluation scheme was designed considering the characteristics of PPN, but could be used for other groups of plant pests with minimal adaptation. Most of the criteria are general characteristics, such as pathogenicity, survival mechanisms and so on.

To validate the species prioritization obtained using the PeST framework, *Heterodera zaeae*, *Meloidogyne graminicola* and *Hirschmanniella oryzae* were chosen for CLIMEX assessment. These species were ranked 1st, 2nd and 20th respectively in the PeST framework. CLIMEX models were developed to quantitatively determine the climatic suitability for the three species in Australia and also get a spatial representation of the risks. CLIMEX was chosen over correlative and mechanistic species distribution models because of its semi-mechanistic model building platform (Sutherst, Maywald & Kriticos 2007) which allowed integration of species distribution, phenology and irrigation scenarios relevant to the selected species. Only three species were chosen for CLIMEX modeling because of time constraints of this study. The data required (coordinates of species distributions and species phenology) for CLIMEX models were
not readily available and had to be gathered from various published literature as described in Chapter 6. *Meloidogyne enterolobii, Scutellonema bradys* and *Meloidogyne chitwoodi*, identified as potential new risks in Chapter 5 and currently not on the high priority pest list in Australia, would have been other good candidates for CLIMEX modeling.

Compared to the SOM analytical technique, CLIMEX models use coordinates of species distribution and phenology, and also model one species at a time. CLIMEX modelling required greater time per species and is therefore only suitable for species a few species of high priority. Commensurate with the greater information and time requirements, a more detailed representation of the biosecurity risks was achieved using CLIMEX than when using the results from SOM analysis. The inclusion of detailed species information in the CLIMEX models for *H. zeae, H. oryzae* and *M. graminicola* allowed spatial representation of the likelihoods for establishment in Australia (see maps in Chapter 6), the seasons and locations most favourable for growth. The CLIMEX models developed here could also be used on a global scale to determine the biosecurity risks from these species elsewhere in the world but due to the objectives of this study; we limited our analysis to Australia only.

The inclusion of irrigation scenarios in the CLIMEX models enabled calculation of risks from the species under irrigated conditions. *H. zeae, H. oryzae* and *M. graminicola* could all establish and grow in irrigated areas much better than under non irrigated conditions (see maps in Chapter 6). The mapping of irrigation areas overlaid on the projected growth index from the irrigation scenario CLIMEX models enabled a more realistic representation of the biosecurity risks. Pest species distributions under climate change scenarios can also be modelled in CLIMEX (Sutherst, Maywald & Kriticos 2007; Sutherst *et al.* 2011) however were not included in the current models because of time constraints.

Since the CLIMEX model output (growth index, eco-climatic index, cold, wet, heat, and dry stresses) have a spatial resolution (each value has a coordinate), they can be mapped using geographical information system tools: the maps produced using DIVA GIS in Chapter 6 are examples. The mapping of biosecurity risks from pest species enables decision makers to visualise areas at risk and use the information in conjunction with maps of crop hosts, transport networks for analysis of potential impacts and spread (Mack, Von Holle & Meyerson 2007; Venette *et al.* 2010; Magarey *et al.* 2011). Thus the results from CLIMEX model can be used to
support biosecurity decision making (Sutherst et al. 2004) or be used in more detailed biosecurity decision support models (Eyre et al. 2012).

7.2 Future directions
The list of PPN of phytosanitary importance, species distribution database and species profiles from PeST framework have been designed so that they can be updated as new information becomes available. The conclusions of the present studies should be robust to small additions to the data set, but our understanding of PPN is increasing all the time. Over time, sufficient additional information may accumulate to alter the current results but such is the nature of continual improvement in understanding through scientific study. The datasets are general enough to be used in future risk prioritization using the methods and techniques outlined in this study or else with other methods requiring similar datasets.

The prioritised lists of PPN for Australia could be used by biosecurity agencies in Australia for acting on species identified as potential new risks. Heterodera zea, Meloidogyne graminicola and Hirschmanniella oryzae could be included on the pest priority list for Australia as biosecurity risks are scientifically justified. Meloidogyne enterolobii, Scutellonema bradys and Meloidogyne chitwoodi are other species identified as great risks. Detailed evaluation of eco-climatic and growth suitability in Australia of these species was, however, beyond the resources available in the present study.

Several knowledge gaps and uncertainties became apparent during these studies. Further investigations of lag times during invasions, mechanisms used by PPN to adapt to new environments, and impacts in both agricultural and non agricultural systems are all necessary to reduce uncertainty and to obtain a better understanding of biosecurity risks from PPN. An important part of biosecurity is knowing how exotic pests could affect agriculture, environment and human health in a new country, thus collaborative research with countries where particular threats already occur could be most beneficial to fill in these gaps.

Conceptually, the methods used in this study could be used with modification for other pest groups and geographical locations. The criteria for initial selection of species of phytosanitary importance and the multi-criteria evaluation scheme would require modification to suit other pest groups. The PeST framework could be used for gathering data and organising information
on species for prioritizing biosecurity risks for other pest groups. The application of the PeST framework on other pest groups and geographical locations would further test the efficiency and effectiveness of the PeST framework.

7.3 Conclusions
The results from first gathering comprehensive data, then applying SOM, and the newly-developed integrated assessment framework (PeST), together with the detailed climatic modelling of species identified as high risks, are an effective way to prioritise and target species for biosecurity risk assessments.

The screening of the widest range of species using broad selection criteria in the initial phase was vindicated by the identification of several species that may not have been identified otherwise. These species were shown in the subsequent analyses, using additional data, to have high probability of entry and establishment. *Meloidogyne graminicola* is an example of such a species. The systematic approach, too, was vindicated by the differences in risks apparent when multiple criteria were used together in a robust way with weighting and scaling. The subsequent climatic modelling and matching with cropping regions also showed the approach was useful.

The SOM analysis of global species distributions was shown to be an effective and efficient technique for determining the likelihood of establishment for a species in a particular country. It ranked a large number of species in the present studies and could be used in any country or jurisdiction.

The biosecurity risks of the species identified by SOM as likely to establish is improved by using multiple criteria to include heterogeneous data and information on a species when estimating the biosecurity risks. The PeST framework provides such a multi-criteria scheme for evaluating pests and integrating the species establishment likelihoods with semi-quantitative scores to determine an overall risk index.

The PeST framework can be used to target species for more detailed risk assessments if desired. Modeling the climatic suitability of a region for the establishment of targeted species and mapping of areas which are at greatest risk is the next logical step, but this requires additional data. When applied to three highly-ranked species the climatic modelling showed the efficiency
of the integrated approach by confirming that the climatic conditions in Australia were indeed suitable for the establishment of these species should they arrive.

Furthermore, the detailed climatic modelling can be used to inform biosecurity decision making on a finer spatial scale by including detailed data of species distributions and phenology. Irrigation and other scenarios can further improve spatial representation of the eco-climatic suitability and growth potential of species and may prove very useful for making informed decisions on the appropriate biosecurity measures. However, this sort of detailed analysis is only feasible for a few species because of the greater data requirements.

The PeST framework is thus a useful addition to plant biosecurity for screening the largest numbers of species in a science-based way which conforms to international standards, and for producing ranked lists of plant pests and diseases which are themselves valid, but can also be analysed further if required.

7.4 References


Appendices

The supplementary information and Tables from Chapters 4, 5 and 6 are included in appendices as digital files on a CD because of their large volume in print form.

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