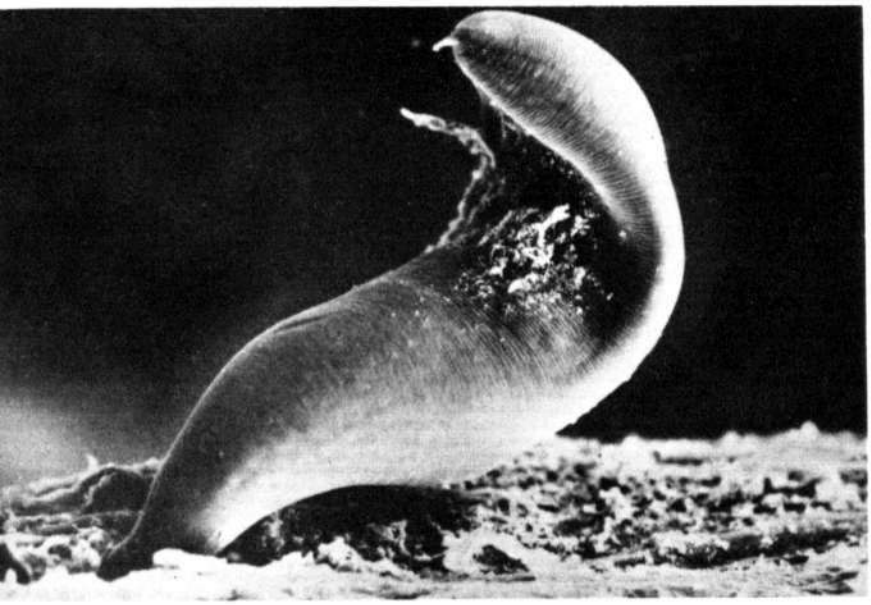

Diagnosis of Key Nematode Pests of Chickpea and Pigeonpea and their Management

International Crops Research Institute for the Semi-Arid Tropics

Citation: Sharma, S.B. (ed.) 1997. Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 112 pp. ISBN 92-9066-383-9 Order code CPE 115.

Abstract

Plant parasitic nematodes, the hidden enemies of crops, are important constraints to crop productivity and yield stability in developing countries where farmers are generally unaware of the presence of these microscopic organisms in their soils. Developing countries do not have adequate trained nematologists to detect the presence of these harmful nematodes and to make management recommendations to farmers. The subtle nature of nematode damage is one of the major reasons why so few efforts have been made to control them. This training course addresses technology transfer to encourage and promote research on nematodes in parts of the semi-arid tropics. Future courses, with participation from researchers in the African region, are envisaged.

Résumé

La diagnose et la maîtrise des nématodes importants nuisibles au pois chiche et au pois d'Angole: comptes rendus d'un programme de formation régional, 25–30 novembre 1996, ICRISAT-Patancheru, Inde. Les nématodes parasites des plants sont des contraintes importantes à la productivité et la stabilité du rendement agricole dans les pays en développement où les paysans ignorent la présence de ces organismes microscopiques dans les sols. Ces pays n'ont pas aussi de nématologistes formés pour détecter ces nématodes et pour recommander des méthodes de lutte aux paysans. Les dégâts causés par les nématodes étant trop subtils, on a fait peu d'efforts pour maîtriser ces ravageurs. Ce stage vise à combler cette lacune et encourage les chercheurs des zones tropicales semi-arides d'entreprendre des études sur les nématodes. On envisage de conduire de tels stages avec la participation des chercheurs des régions africaines.

These research activities were partially supported by the Commisssion of the European Communities, the Islamic Republic of Iran, and Spain. The cost of this publication was partially supported by the Asian Development Bank.

Cover: Female reniform nematode feeding on pigeonpea root (electron micrograph x 400).

Diagnosis of Key Nematode Pests of Chickpea and Pigeonpea and their Management

Proceedings of a Regional Training Course

25-30 Nov 1996
ICRISAT, Patancheru, India

Edited by
S B Sharma

ICRISAT

International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India

1997

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Foreword

Nematodes represent a unique challenge to agricultural research, in that they combine the potential for serious reductions in growth and yield in a wide range of crop plants, often with rather nonspecific and easily misdiagnosed symptoms. Equally, nematode populations themselves are comparatively difficult to identify and quantify, and relationships between nematode population size and crop damage are subject to many environment- and crop-specific factors. Finally nematodes are system pests, rather than single crop pests—particularly in the tropics, where cropping sequence may support similar species when cropping intensity is high and conditions favorable for large population buildup. Control or management systems are therefore likely to be much more complex in tropical agriculture than monocrop temperate agriculture. The fact that nematodes have received much less attention and funding than more readily visible, if not more damaging, crop diseases and arthropod pests is therefore not surprising.

The first requirement in the design of pest management systems for nematodes is the ability to identify species and to estimate population sizes in a relatively inexpensive and efficient manner. Conventional techniques for estimating population sizes have improved in recent years, but are still largely manual and time consuming. There is a much greater promise in new techniques for species identification, based on the unique DNA characteristics of different species, and potentially, of different populations. These should be much less tedious and permit much better estimations of species composition of specific populations than do present morphological techniques.

It is ICRISAT's hope that this workshop, through the sharing of methodology for identifying nematode species and estimating population sizes, will further research on the management of this neglected, but very important, constraint to the increase of productivity and profitability of semi-arid tropical agriculture. The design of better management systems relying on choice of crop species or variety and specific crop rotation is especially important for small farmers who do not have access to the chemical control methods that are the mainstay of the "management" on this problem in more developed agricultural systems. I sincerely hope that this Workshop will provide you better tools to pursue research on appropriate nematode management systems for these farmers.

FR Bidinger
Acting Executive Director
ICRISAT Asia Region

Introduction

S B Sharma[1]

Plant parasitic nematodes are hidden enemies of crops. The symptoms pf the damage they cause are not easily recognizable. Their microscopic size further reduces the chances of being recognized as the causal organisms of any damage. Some nematodes predispose plants to other pathogens, while other nematodes act as virus vectors. On a worldwide basis, these, worms are estimated to cause crop yield losses of over US$ 78 billion. These losses are estimated to be over 14% in the developing countries and about 9% in the developed countries. The nematode-caused damage is much higher in the tropical regions than in the temperate regions. Scarcity in nematological expertise and lack of awareness, widespread occurrence of many highly pathogenic nematode species, temperatures conducive for reproduction during most of the year, and longer crop growing seasons are some of the major reasons for greater damage in the tropics. The developing countries in the semi-arid tropics, with few exceptions, have inadequate numbers of nematologists to identify, demonstrate, and manage the crop damage caused by nematodes.

pigeonpea *(Cajanus cajan)* and chickpea *(Cicer arietinum)* are important grain legumes in the semi-arid tropics. They provide a necessary protein supplement to the largely cereal-based diets of the population, and enrich the agricultural soils by fixing atmospheric nitrogen. These crops are attacked by a large number of species of plant parasitic nematodes, and some of these species cause serious damage. On a worldwide basis, nematodes cause economic losses in the vicinity of US$ 177 million to pigeonpea and US$ 328 million to chickpea. Despite their econpmic importance, these microscopic pests have evaded the attention of farmers because they do not produce any tell-tale symptoms on the. above-ground plant parts; while their effect on crop growth is not dramatic, it is still certain and cumulative. The root-knot nematodes (*Meloidogyne incognita* and *M. javanica*) are important nematode pests of chickpea and pigeonpea in the tropics. The cyst nematode (*Heterodera cajani*) and reniform nematode (*Rotylenchulus reniformis*) are important nematode pests of pigeonpea.

This training workshop pn diagnosis and management pf nematode pests of chickpea and pigeonpea was designed to acquaint the participants from Bangladesh, India, Myanmar, Nepal, Pakistan, and Sri Lanka with the important nematode pests of chickpea and pigeonpea as well as provide

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training in general diagnosis of key nematode pests, introduce the application of newer tools in molecular biology in nematology, and principles of nematode management. One of the objectives was to demonstrate the use of some of the newer diagnostic tools and host-plant-resistance screening techniques developed at ICRISAT. For example, to test the presence of the reniform and root-knot nematodes, the roots of plants are dipped in a 0.25% solution of trypan blue. The roots are washed with water after two minutes and, if the nematodes are present, the nematode eggsacs are stained dark blue and they become clearly visible without aid of a microscope. Presence of the cyst nematodes can be easily detected while plants are at the seedling stage when the nematode females appear as white bead-like pearls on the roots. The "pearly root" is a characteristic sign of cyst nematode attack.

Our collaborators from USA (Dr Sam Anand, University of Missouri, Portageville, Missouri), Canada (Dr Theirry Vrain, Pacific Agri-Food Research Center, Summerland, BC), United Kingdom (Dr Keith Davies, Rothamsted, Harpenden), and India (Drs S K Midha, Indian Council of Agricultural Research, New Delhi; D D R Reddy, Acharya N G Ranga Agricultural University, Hyderabad; J S Prasad, Directorate of Rice Research, Hyderabad; and K S Varaprasad, National Bureau of Plant Genetic Resources, Hyderabad) who very kindly joined us in delivering lectures and participating in discussions on important topics such as cropping-systems-based nematode management, resistance and tolerance to nematodes in crops, integrated management of nematodes, nematode quarantine, and protein and nucleic-acid-based nematode identification and management.

The national agricultural research institutions in Africa and Asia do not have adequate expertise in nematology. It is hoped that organization of similar training workshops will greatly assist in enhancing the capabilities of researchers in these regions in diagnosing and managing the nematode-induced constraints to crop production.

1. Nematode Identification

Identification and Quantification of Plant Parasitic Nematodes Using Immunological Techniques

K G Davies[1]

Introduction

The management of plant parasitic nematode pests relies upon the identification and quantification of these pests in the soil. Plant parasitic nematodes are microscopic animals with a highly conserved morphology, and are, therefore, notoriously difficult to identify even by highly trained personnel. New management strategies will require simple, rapid, and reliable methods for the identification and quantification of nematode pests that will enable the routine monitoring of nematode populations. The advent of molecular biology has brought with it a number of new techniques for analyzing nucleic acids, protein, carbohydrates, and lipids that can be helpful in the identification of pests; of these, allozyme, monoclonal antibody, and DNA-based systems are the most well developed for nematodes and have been reviewed extensively (Curran 1991; Curran and Robinson 1993; Davies 1994; Esbenshade and Triantaphyllou 1985; Forrest 1994; Schots et al 1990; Williamson 1991). However, each technique has its strengths and weaknesses and the author refers readers to these comprehensive reviews for comparisons of the different methods. Qualitative diagnostic tests that require the detection of nematodes present in small numbers are essential for quarantine and certification schemes but one of the most intractable problems in nematology is the development for use by agricultural advisers of routine quantitative assays that are able to combine a high degree of both specificity and sensitivity Quantitative assays using molecular techniques have to rely on diagnostic probes and, as the technology for enzyme linked immunoabsorbent assays (ELISA) is more highly developed than for quantitative DNA-based assays (Miller and Martin 1988), the present paper will concentrate on ELISA. To date, most research has been aimed at developing assays for root-knot (RKN) (*Meloidogyne* spp) and potato cyst (PCN)

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Davies, K.G. 1997. Identification and quantification of plant parasitic nematodes using immunological techniques. Pages 3-11 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

(*Globodera spp*) nematodes It is the purpose of this paper to highlight the considerations that must be taken into account in the development of a quantitative immunoassay for these nematodes with special reference to research at the Institute of Arable Crops Research, (IACR)-Rothamsted.

Immunoassay Development

A number of practical and theoretical considerations are necessary before a quantitative immunoassay is developed (Fig. 1). One of the first questions to ask is simply whether or not an immunologically based assay would represent an improvement over presently available techniques; immunoassays are costly to develop and if the nematode problem, e.g., the presence of cysts on roots, can be assessed, for example, by eye with the help of a magnifying glass, then this is preferable to the development of a sophisticated assay. Once it has been established that an assay is required, its stringency will be determined by how widely it is envisaged the assay will be used. One developed for a crop grown in a particular locality, e.g., potatoes (*Solanum tuberosum*) within the UK, and with few other nematodes with which the pest can be confused, will be much less rigorous than if the assay is to be used on a world-wide basis. Having decided the geographical scale to be used in the assay, it is necessary to identify the needs of the end users at an early stage; a laboratory undertaking quarantine and plant health certification will have very different requirements to an agricultural extension or advisory service. Similarly, assays developed as a research tool for screening nematode-resistant germplasm or for nematicide screening trials will have requirements very different from those of the two users mentioned above. An important consideration is also the type of matrix (soil, water, roots, etc.) in which the nematode assay will be performed; this is related to the extraction and antigen release procedures that will be undertaken prior to performing the assay. Having decided on the geographical locality and the stringency with which the assay has to perform, a number of criteria have to be assessed concerning the specificity of the immunoassay. It is here that decisions have to be made regarding the requirements of the type and specificity of the antibody(ies) to be used. The level of specificity required will depend on the level of discrimination required for the purposes of the assay. Four factors will need to be addressed interdependently and the best possible compromise made. These are taxon specificity, nematode stage specificity, antibody specificity, and the type of management which is possible (Fig. 1).

Taxon specificity refers to the taxonomic level of the nematode group at which diagnosis has to operate and the other organisms that will also be present in the sample to be assayed; these will be dependent on the nematode extraction procedure and the matrix from which the nematodes have to be discriminated (it should be remembered that in root/shoot assays the antibodies must not cross-react with the plant material). Stage specificity is also

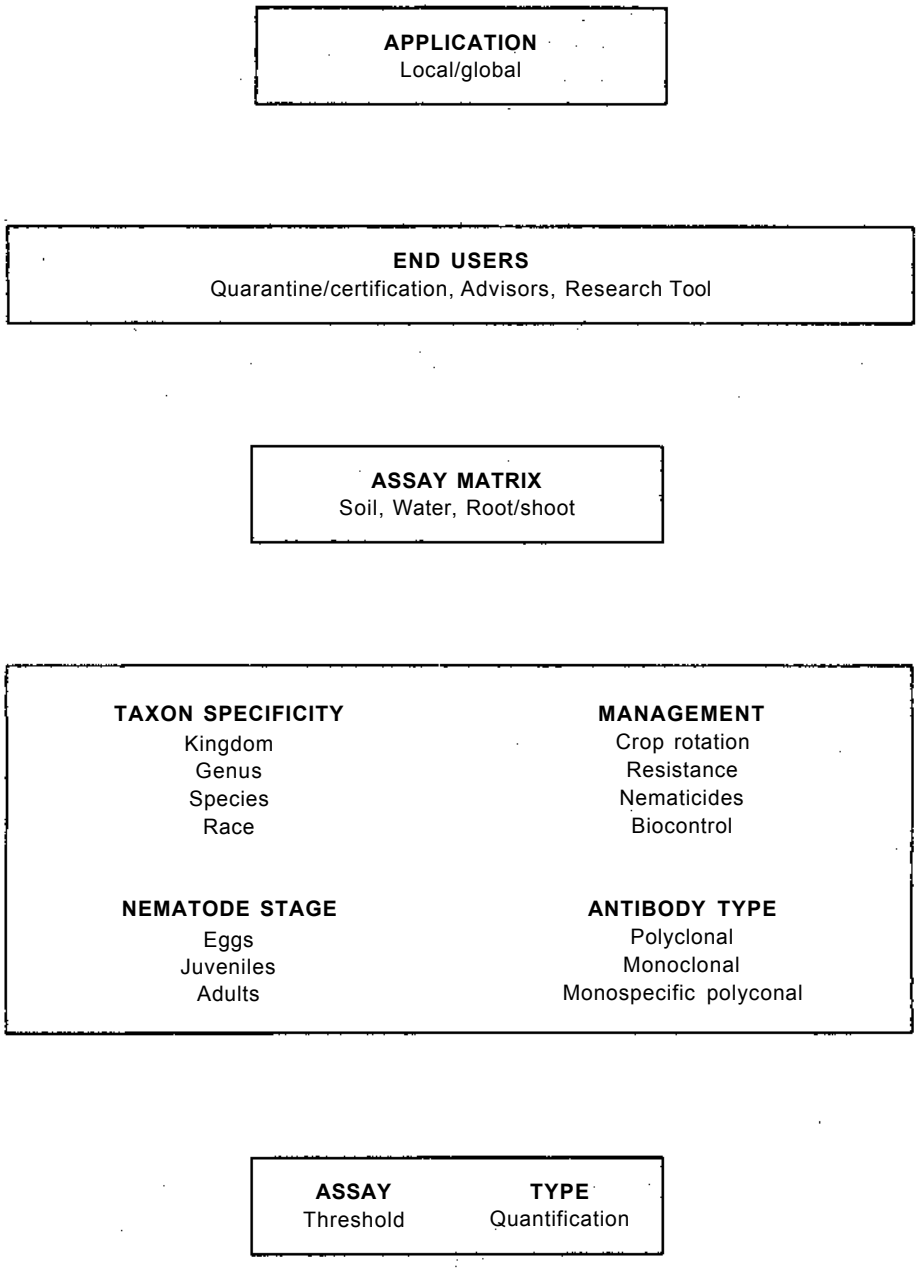

Figure 1. Practical and theoretical considerations in the development of an immunoassay for the diagnosis and quantification of plant parasitic nematodes.

an important parameter that needs to be taken into account. Plant-parasitic nematodes undergo development from an egg stage, through four moults, to an adult, and during this growth and development there is differential expression of antigens (Atkinson and Harris 1989; Davies 1994); this must therefore be taken into account When developing the assay. The preceding factors must also be assessed in the light of management strategies that can be employed and the work that is required to obtain a suitable antibody. The application of a broad-spectrum nematicide requires a less rigorous level of diagnosis than the implementation of planting a resistant cultivar that carries resistance, the latter requiring identification of a particular pathotype or race. Once all this is done, all that remains is to decide on the strategy to obtain an antibody that meets the requirements of the assay. Polyclonal antibodies tend to have high levels of sensitivity but are not usually discriminative, as they are highly cross-reactive across nematode genera. Although they are relatively cheap to produce they have the disadvantage that different batches of antisera can react differently in the same assay. An intermediate approach is to use monospecific polyclonal antibodies, which, if made to a diagnostic protein, can combine a high degree of discrimination together with relatively high sensitivity. This latter approach obviously has the disadvantage that a diagnostic protein needs to be identified and then purified; also, antibodies produced in this manner may contain cross-reactive epitopes. Monoclonal antibodies, which can be selected for any particular level of discrimination, are highly reproducible but tend to be less sensitive and are very expensive to produce. Before embarking on the costly and time-consuming process of making a new antibody it is well worth checking whether suitable antibodies are already available; screening monoclonal antibody libraries that have already been produced could identify suitable antibodies without the need for time-consuming immunization schedules. The last aspect of developing a quantitative immunoassay concerns the definition of the upper and lower thresholds of the assay; a threshold assay, developed as either positive or negative above and below a certain threshold respectively, is simply a very restricted form of quantitative assay.

Identification

The most recent developments for the identification of plant parasitic nematodes involve polyacrylamide gel electrophoresis (PAGE) and this research has mainly focused on cyst and root-knot nematodes. The differentiation of *Globodera rostochiensis* from *G. pallida* is usually performed by homogenizing cysts containing eggs and second-stage juveniles of PCN and using isoelectric focusing (IEF) techniques to identify species-specific markers (Fleming and Marks 1983). The identification of RKN involved the homogenization of female RKN and analysis by isozyme electrophoresis and staining for esterase

(Esbenshade and Triantaphyllou 1985). For the initial production of antibodies, the antigens selected for the immunizations were eggs and juveniles for PCN and females for RKN, as these stages had been found previously to be useful in distinguishing species of PCN (Fleming and Marks 1983) and RKN (Esbenshade and Triantaphyllou 1985). The production of monoclonal antibodies (Mabs) was then done using standard techniques (Davies and Lander 1992; Robinson et al 1993). The initial screening of the antibodies produced by the hybridoma cell lines was done on protein homogenates of the species and stages of nematode that needed to be differentiated. For PCN this involved screening Mabs against *G. rostochiensis* and *G. pallida,* as the assay was designed for use locally in Europe and these were the two species that represented a problem for which there were a variety of management options available. In the case of RKN, the Mabs were screened against the three major species, the basis of which was that species differentiation was linked to the differential host range test (Sasser 1979).

The use of Mabs for the immunological differentiation of the two species of PCN has been described (Robinson et al 1993; Schots et al 1992). The Mabs produced by Schots et al (1992) were not species specific because they not only cross reacted with the two species of PCN but also with other species of nematodes; however, by combining a panel of antibodies they could differentiate the two species as each antibody had a different affinity for the antigens of the different nematodes. The two Mabs produced to PCN by Robinson et al (1993) did not have the problem of cross-reactivity since they only recognised species of the genus *Globodera*. Because *Globodera* species from the *S. tabacum* complex are not present in Europe the Mabs can be considered specific to *G. rostochiensis* and *G. pallida.* It was fortuitous that further research showed that these antibodies recognized the same diagnostic markers at pI 5.7 for *G. pallida* and pI 5.9 for *G. rostochiensis* as those identified by Fleming and Marks (1983). Western blotting after sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that these two proteins, with different pIs, had the same molecular weight of 34 kD and that the antibodies were weakly cross reactive. Further immunizations with the purified diagnostic markers at pI 5.7 and pI 5.9 produced Mabs with similar levels of specificity. Subsequent characterization of these diagnostic markers has shown that they only recognize viable eggs (Dunn, Curtis, and Evans, unpublished data). For RKN three Mabs were selected which could, in an assay using females, differentiate the species by ELISA and dot blot. These Mabs were highly cross reactive and it was impossible to separate *M. incognita* from *M. javanica* by Western blot analysis in a qualitative test (Davies and Lander 1992). Western blotting of IEF gels and the probing of blots with each of the Mabs did not show differences in isoelectric points of the antigens between the different species of RKN (Davies and Fargette, unpublished data). In an attempt to differentiate *M. incognita* from *M. javanica,* species-specific esterase bands have been purified and Mabs produced; although it is now possible to differentiate these two species, the

Mabs also cross react with other RKN. As the levels of cross-reactivity of the Mabs used to identify the species of PCN were sufficiently low as to make them irrelevent, it was possible to identify samples containing mixed populations of PCN (Dunn, Curtis, and Evans, unpublished data); in contrast, this was not an approach that was considered for RKN as the levels of cross-reactivity were substantially higher.

Quantification

An important aspect of plant parasitic nematode management is the testing of a particular site for presence of nematodes prior to the planting of a crop. Traditional methods of quantification rely on laborious extraction techniques together with manual counting using a microscope. Quantification could normally be carried out only at the level of genus prior to the development of electrophoretic techniques (Fleming and Marks 1982). However, electrophoretic techniques are not easy to adapt for quantitative purposes. Immunoassays now offer the potential to combine identification and quantification in one single step and also have the potential for automation. Hitherto, immunoassays for diagnosis have been developed on readily identifiable stages of the nematode that have been relatively uncontaminated with other nematodes and other soil and root material. The challenge of a soil assay is to identify the desired nematode and quantify it amongst an array of other nematodes and soil fauna and flora. It is useful to consider the assay from two aspects, first identifying the desired nematode and then the release of the target antigen in a manner that is proportional to the number of nematodes present. As seen above, several antibodies have been selected as useful in diagnosis, which may be useful in quantification assays.

Several attempts have been made to quantify PCN and RKN nematodes directly in soil homogenates, but this approach has so far proved unsuccessful and some level of nematode extraction from soil has proved necessary. It was possible to quantify second-stage juveniles of RKN with genus-specific Mabs following the extraction of the nematodes by and the subsequent use of Ballotini beads as a method of antigen release centrifugation (Davies and Carter 1995). However, even though several different types of ELISA format were tested, no assay was sufficiently sensitive to quantify juveniles at the required threshold of one juvenile per gram of soil The diagnostic antigens that had been selected were either not present in sufficient quantity or were not being released satisfactorily. The Mabs produced by Schots et al (1992) were used to quantify the two species of PCN from clean extracts of cysts, whilst the Mabs produced by Robinson et al (1993) were successful in quantifying PCN from soil extracts (Evans et al 1995). Although the use of Balottini beads wasnot successful at releasing antigens from either PCN cysts or eggs, both sonication and homogenization of crude Fenwick can (Fenwick 1940) extracts containing

cysts proved that enough antigen was released to detect approximately 3 x 10^{-3} cysts g^{-1} soil (about 0.9 eggs g^{-1} soil). Therefore, the abundance and ease of release of diagnostic antigen from cysts were important factors in the development of the quantitative assay for PCN.

Future Prospects

The research discussed here shows that the development of an immunological assay to identify and quantify PCN is much more advanced than that for RKN, and it is instructive to compare and contrast the nematodes as this brings into focus some of the important factors to be considered in the development of an assay. PCN are not indigenous to Europe and the two species present were shown to be serologically distinct even before they were identified as separate species on morphological grounds (Davies 1994). Whether or not the assay would be useful in the area of the South American Andes, which is the centre of origin for PCN where they co-evolved with their host plants (*Solanum* spp) would seem rather doubtful (Evans et al 1975). PCN are genetically much more heterogeneous than RKN, which by comparison have maintained a high degree of homogeneity through their parthenogenetic mode of reproduction (Trudgill et al 1995) and this heterogeneity has greatly helped in the development of the immuno-diagnosis of the two nematode species. Another great help was that eggs and juveniles in cysts could be removed from the soil with relative ease. Added to this was the advantage that the diagnostic markers were abundant and easily released. These factors collectively facilitated the development of a successful assay.

The polyphagous lifestyle of RKN and their genetic homogeneity suggests that diagnosis and quantification at the level of genus is sufficient for practical purposes, as no morphological or biochemical markers that are predictive of host range have yet been identified (Davies 1994). This contrasts with PCN where the diagnostic antigens that separate *G. pallida* from *G. rostochiensis* are useful in predicting whether a particular population of PCN in the UK will be virulent or avirulent on potatoes containing the H1 gene. This gene confers resistance to some populations of *G. rostochiensis.* There is much interest in understanding the molecular mechanisms by which nematodes establish feeding cells (Sijmons et al 1994) and it is likely that such studies will eventually lead to the production of Mabs and DNA probes that will be predictive of host range; these probes will be the basis of producing diagnostic tests that will be able to identify nematode pathotypes and races.

The basis of a quantitative immunoassay for PCN has now been established at the species level and an assay for RKN at the genus level is not far away. However, to date, no research has been aimed at developing assays for migratory ecto- and endo-parasitic nematodes. For such nematodes, samples are likely to contain mixed stages of the nematode and the development of an assay for this group of nematodes will present a new set of challenges.

Acknowledgements

IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council and the Ministry of Agriculture, Food and Fisheries.

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A Summary of Basic Immunology for Nematologists

KG Davies[1]

1. The production of antibodies occurs when "non-host" molecules enter an animal (reptiles, birds, mammals).
2. The reason an animal produces antibodies is to stop possible infections by bacteria, viruses, fungi, and other infectious agents including nematodes.
3. Antibodies, after they have been produced, are involved in the elimination of infectious agents as they circulate in the blood.
4. The production of antibodies is stimulated even when the "non-host" molecules have been introduced via non-viable infectious agents or even organisms that are not pathogenic to the host.
5. These antibodies (which are proteins) can be used as tools for studying and solving problems in plant nematology.
6. Molecules that produce an immune response are called antigens—one part of the immune response is the production of antibodies.
7. Antibodies are produced by cells in the blood called B cells—a B cell produces an antibody that will bind to a single molecular configuration (i.e., shape) like a particular key will open a particular lock.
8. Each molecular configuration to which an antibody binds is called an epitope.
9. Each antibody binds to its corresponding epitope at a region of the antibody which is variable (Fig. 1).
10. Antibodies consist of a whole class of different types of molecules called immunoglobulins (Ig). The different types of antibody are called IgG, IgM, IgE, IgA, and IgD—the ones most commonly used as experimental tools are IgG immunoglobulins.
11. Nematodes have many antigens, each of which will contain one or more epitopes (Fig. 2).
12. Antibody A will recognize and bind to antigens X, Y, and Z;
    Antibody B will recognize and bind to antigens X and Y;

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Davies, K.G. 1997. A summary of basic immunology for nematologists. Pages 12-14 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

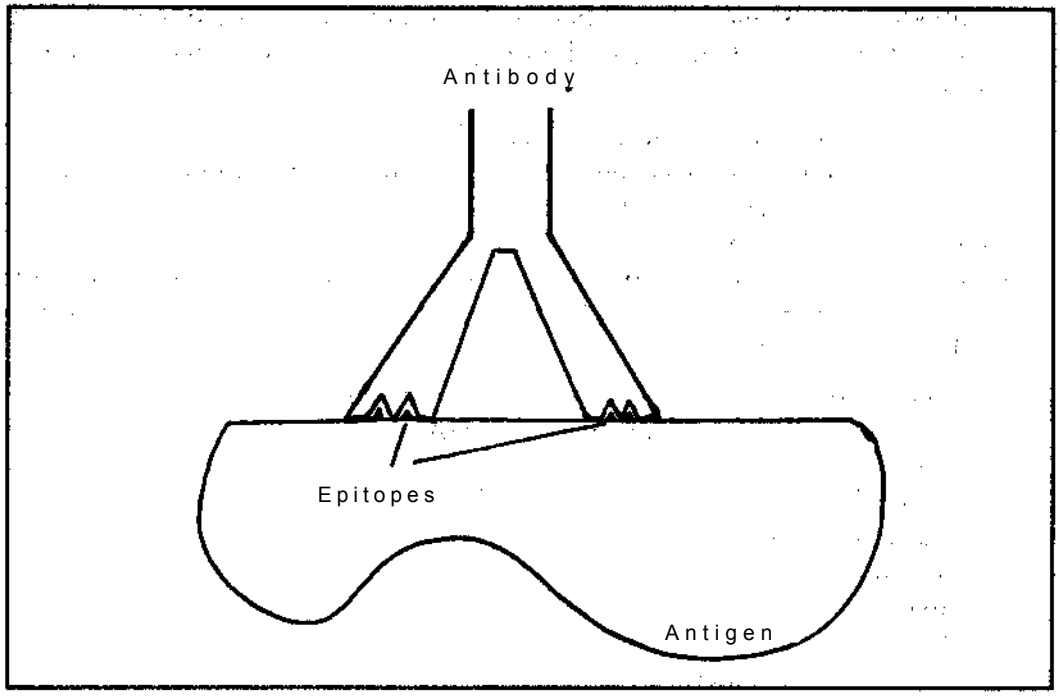

Figure 1. An antibody binds to an antigen at the epitope sites.

Antibody C will recognize and bind to antigens Y and Z;
Antibody D will recognize and bind to antigen Z.

13. Antibodies A, B, and C are said to be cross-reactive;
Antibody D is specific to antigen Z.

14. Antigen X has 2 epitopes, a and b;
Antigen Y has 3 epitopes, a, b, and c;
Antigen Z has 3 epitopes, a, c, and d.

15. Each B cell, and its descendants, produce only one type of antibody that will recognize one epitope configuration (i.e., the variable region on this B cell and all its descendants are the same).

16. An animal immunized with a mixture of nematode antigens will therefore produce an immune response in which a mixture of antibodies will be produced that recognize all the different epitopes to the different antigens. Antibodies collected from such a series are called polyclonal antibodies.

17. Polyclonal antibodies tend to be crossreactive as they contain many antibodies that recognize many different epitopes.

18. An antibody produced from a single B cell, and its descendants, is called a monoclonal antibody. Monoclonal antibodies are specific to one epitope and are less cross reactive.

19. Antibodies can be made to antibodies. For example: an antibody made in a rabbit can be purified and injected into a goat. This is called a goat anti-rabbit antibody.

20. Antibody conjugates: In order to see when an antibody has bound to a particular epitope, various chemicals can be bound to the antibody to help in visualization. Enzymes can be bound (conjugated) to an antibody which catalyze reactions that can lead to the production of a color or a precipitate. Fluorescent molecules (FITC) can also be coupled to antibodies for visualization with a fluorescent microscope. Gold and silver can be used for electron microscopy.

21. Enzyme linked immunoabsorbant assay (ELISA) (Indirect)
    (a) Antigens can be bound to a plastic ELISA plate.
    (b) Wash excess antigen away.
    (c) Antibodies (made in rabbits) can be then bind to the antigen.
    (d) Wash excess antibodies away.
    (e) Anti-rabbit antibodies (raised in goats) conjugated to an enzyme can be used to direct the rabbit antibodies.
    (f) Wash excess antibodies away.
    (g) Add substitute and color will develop.

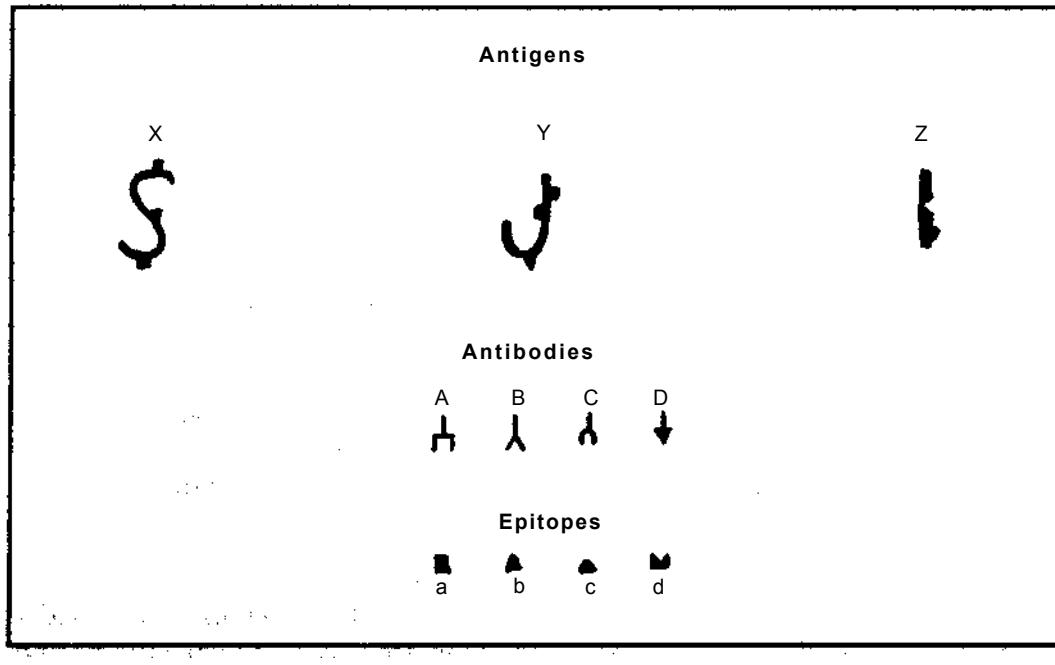

Figure 2. Antigen-antibody reaction.

Restriction Fragment Length Polymorphism: a Molecular Technique to Identify Nematodes

T C Vrain[1]

Introduction

Molecular biology techniques can be applied to investigate nematode taxonomy. These techniques depend on preparing pure high-molecular-weight DNA. There are several protocols that have been published for the extraction and purification of genomic DNA from nematodes. First the content of nematodes is released, usually by crushing the nematodes in liquid nitrogen. The DNA solution is extracted from the nematode extract with a phenol/chloroform/ isoamyl alcohol mixture to remove protein contaminants, then precipitated with 100% ethanol. The DNA is pelleted after the precipitation step, washed with 70% ethanol to remove salts and small organic molecules, and resuspended in buffer at a concentration suitable for further experimentation. An alternative to this method is to use glass beads, which works well in my laboratory when we extract DNA from single nematodes.

The DNA fragments are then separated by gel electrophoresis. A variety of important variables affect the migration of DNA fragments on gels. These include the conformation of the nucleic acid, the pore size of the gel, the voltage gradient applied, and the salt concentration of the buffer. The most basic of these variables is the pore size of the gel, which dictates the size of the fragments that can be resolved. Larger-pore agarose gels are used to resolve fragments 100 to 2000 bp and smaller pore acrylamide or sieving agarose gels are used for fragments smaller than 100 bp. Frequently it is desirable to identify an individual DNA fragment in a complex mixture that has been resolved by gel electrophoresis. This is accomplished by a technique termed Southern blotting, in which the fragments are transferred from the gel to a nylon or nitro-cellulose membrane and the fragment of interest is identified by hybridization with a labelled nucleic acid probe.

1. Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC V0H 1Z0, Canada.

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Phenol Extraction and Precipitation of DNA

First you will need to freeze nematodes (suspended in a very small Volume of water, perhaps 0.1 to 0.2 ml) in liquid nitrogen in a mortar. You will need enough nematode DNA (from several hundred to several thousand nematodes) to be purified in 0.1 to 0.4 ml. Crush to a powder, and transfer the nematode extract to a microcentrifuge tube. Add an equal volume of phenol/chloroform/ isoamyl alcohol to the DNA solution. Vortex vigorously for 10 sec and microcentrifuge 15 sec at room temperature. Remove the top (aqueous) phase containing the DNA using a 200-µl pipettor and transfer to a new tube, without removing the white protein precipitate present at the aqueous/organic interface. Repeat if necessary, until there is no more protein precipitate at the interface.

Add 1/10 vol of 3 M sodium acetate, pH 5.2, to the solution of DNA. Mix by vortexing briefly or by flicking the tube several times with a finger. Add 2 to 2.5 vol (calculated after salt addition) of ice-cold 100% ethanol. Mix by vortexing and place in crushed dry ice for 5 min or longer. Spin 5 min in a fixed-angle microcentrifuge at high speed and remove the supernatant. Add 1 ml of room-temperature 70% ethanol. Invert the tube several times and microcentrifuge at high speed. Remove the alcohol supernatant, drain the alcohol droplets out of the tube, and dry the pellet in a desiccator under vacuum or in a Speedvac evaporator, usually for 2 to 3 min. Dissolve the dry pellet in 20 µl to 50 µl of water if it is going to be used for further enzymatic manipulations requiring specific buffers. Dissolve in TE buffer, pH 8.0, if it is going to be stored indefinitely

Phenol must be of the highest quality, preferably shipped under nitrogen. For some purposes, fresh liquefied phenol (88% phenol) can be used without further purification. However, for purification of DNA prior to cloning and other sensitive applications, phenol must be redistilled before use because oxidation products of phenol can damage and introduce breaks into nucleic acid chains. Redistilled phenol for use in nucleic acid purification is commercially available. The phenol must be buffered before use. Place 500 ml of liquefied phenol or melted crystals of redistilled phenol (melted in a water bath at 65°C) into a 2-liter glass beaker. Add 0.5 g of 8-hydroxyquinoline. The phenol will turn yellow due to the 8-hydroxyquinoline, which is an antioxidant. Add 500 ml of 50 mM Tris base. Cover the beaker with aluminium foil. Stir for 10 min at low speed with magnetic stirrer at room temperature. Let phases separate at room temperature. Decant the top (aqueous) phase into a suitable waste receptacle. Remove what cannot be decanted with a 25-ml glass pipette and a suction bulb. Add 500 ml of 50 mM Tris-HCl, pH 8.0. Repeat two successive equilibrations with 500 ml of 50 mM Tris-HCl, pH 8.0. The pH of the phenol phase can be checked with indicator paper and should be 8.0. If it is not, steps 3 to 7 should be repeated until this pH is obtained. Add 250 ml of 50 mM Tris-HCl, pH 8.0, or TE buffer, pH 8.0, and store at 4°C in brown glass battles or clear glass bottles wrapped in aluminium foil. For use in DNA

purification procedure (basic protocol), mix 25 vol phenol (bottom yellow phase of stored solution) with 24 vol chloroform and 1 vol isoamyl alcohol. Phenol prepared with 8-hydroxyquinoline as an antioxidant can be stored for about 2 months at 4°C

Extraction of DNA Using Glass Powder

The use of a glass powder suspension allows the rapid separation of DNA from contaminating proteins, RNA, or organic solvents. DNA in solution is adsorbed onto glass in the presence of sodium iodide. The DNA-glass powder suspension is washed to remove solution contaminants, and DNA is subsequently eluted into water or a low-salt buffer. There are commercial kits available: Glas-Pac from National Scientific Supply; GeneClean from Bio101; and Qiaex Gel Extraction Kit from Qiagen.

Add 3 vol NaI solution to DNA in a 1.5-ml microcentrifuge tube. Add 5 µl glass powder suspension. Incubate 5 min at room temperature. Microcentrifuge DNA/glass powder complex for 5 sec. Remove and discard supernatant. Wash the DNA/glass pellet three times with 500 µl wash solution. Lightly vortex the mixture to resuspend, then microcentrifuge briefly to pellet the beads. Resuspend pellet in TE buffer, pH 8.0. Incubate 2 to 3 min at 45°C to elute DNA from the glass. Microcentrifuge 1 min and transfer the DNA-containing supernatant to a fresh tube. Store at 4°C until use.

Gel Electrophoresis

The DNA fragments can be visualized only after separation on an agarose gel. The gel is prepared with an agarose concentration appropriate for the size of DNA fragments to be separated. The DNA samples are loaded into the sample wells and the gel is run at a voltage and for a time period that will achieve optimal separation. The gel is then stained, or, if ethidium bromide has been incorporated into the gel, visualized directly upon illumination with UV light.

Prepare the electrophoresis buffer (TAE or TBE) to fill the electrophoresis tank and prepare the gel. Add the desired amount of electrophoresis-grade agarose to a volume of electrophoresis buffer sufficient for constructing the gel. The buffer is normally made up as a 10x stock solution containing 20% Ficoll, 0.1 M Na_2EDTA, pH 8, 1.0% sodium dodecyl sulphate, and two tracking dyes, 0.25% bromophenol blue and 0.25% xylene cyanol. Melt the agarose in a microwave oven or autoclave and swirl to ensure even mixing. Agarose gels typically contain 0.8 to 2% agarose in 1x TAE or TBE depending on the size of the fragments you want to resolve. Electrophoresis grade agarose powder is added to lx gel buffer and melted by boiling for 2 to 3 min. Check that all agarose particles are completely melted. Melted agarose should be cooled to

55°C in a water bath before pouring onto the gel platform. This prevents warping of the gel apparatus.

To facilitate visualization of DNA fragments during the run, ethidium bromide solution can be added to the electrophoresis buffer to a final concentration of 0.5 μg ml^{-1}. DNA fragments can be seen during and after the separation by adding 0.5 μg ml^{-1} of ethidium bromide in the gel. Make a 1000x stock solution (0.5 mg ml^{-1}, 50 mg ethidium bromide in 100 ml H_2O). Protect from light by storing in an amber bottle or a bottle wrapped with aluminium foil. Always wear gloves when handling ethidium bromide solutions or an agarose gel containing ethidium bromide.

Gels are typically poured between 0.5 and 1.0 cm thick. Remember to keep in mind that the volume of the sample wells will be determined by both the thickness of the gel and the size of the gel comb. Seal the gel casting platform if it is open at the ends. Pour in the melted agarose and insert the gel comb, making sure that no bubbles are trapped underneath the combs and all bubbles on the surface of the agarose are removed before the gel sets. Most gel platforms are sealed by taping the open ends with adhesive tape. After the gel has hardened, remove the tape from the open ends of the gel platform and withdraw the gel comb, taking care not to tear the sample wells. Place the gel casting platform containing the set gel in the electrophoresis tank. Add sufficient electrophoresis buffer to cover the gel to a depth of about 1 mm (or just until the tops of the wells are submerged). Make sure no air pockets are trapped within the wells. DNA samples should be prepared in about 10 μl, a volume that will not overflow the gel wells, by addition of the appropriate amount of 10x loading buffer. Load the samples into the wells with a pipettor or micropipette. You will need to include appropriate DNA molecular weight markers to compare the size of your DNA fragments.

Be sure that the leads are attached so that the DNA will migrate into the gel toward the anode or positive lead. Set the voltage to the desired level, typically 1 to 10 V cm^{-1} of gel, to begin electrophoresis. Depending on the voltage, a run can normally take 30 min to several hours. For normal runs, use 60 to 80 volts for 1 h. The progress of the separation can be monitored by the migration of the dyes in the loading buffer. Turn off the power supply when the bromophenol blue dye from the loading buffer has migrated a distance judged sufficient for separation of the DNA fragments. You can check the migration of the DNA bands during the run if you have incorporated ethidium bromide into the agarose gel.

Whether during or at the end of the separation, the DNA can be visualized by placing on a UV light source and can be photographed directly. DNA can be photographed in agarose gels stained with ethidium bromide by illumination with UV light. A UV transilluminator is typically Used for this purpose, and commercial models are available designed specifically for DNA visualization and photography, UV light is damaging to eyes and exposed skin. Protect your eyes while using a UV light source,

The Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a rapid procedure for in vitro enzymatic amplification of a specific segment of DNA. Like molecular cloning, PCR has spawned a multitude of experiments that were previously impossible. The number of applications of PCR seems infinite. They include direct cloning from genomic DNA or cDNA, in vitro mutagenesis and engineering of DNA, genetic fingerprinting of forensic samples, assays for the presence of infectious agents, prenatal diagnosis of genetic diseases, analysis of allelic sequence variations, analysis of RNA transcript structure, genomic footprinting, and direct nucleotide sequencing of genomic DNA and cDNA.

There are three kinds of DNA fragments in the PCR reaction: the double-stranded DNA to be amplified and two single-stranded oligonucleotide primers flanking it. Additionally, there is a protein component (the enzyme DNA polymerase), deoxyribonucleoside triphosphates (dNTPs), a buffer, and salts. The double-stranded DNA to be amplified is denatured by heating the sample at 94°C. In the presence of DNA polymerase and excess dNTPs, oligonucleotides that hybridize specifically to the target sequence can prime new DNA synthesis. The first cycle is characterized by a product of indeterminate length; however, the second cycle produces the discrete "short product," which accumulates exponentially with each successive round of amplification. This can lead to the many million-fold amplification of the discrete fragment over the course of 30 to 40 cycles. The primers hybridize to opposite strands of the DNA and are oriented with their 3' ends facing each other so that synthesis by DNA polymerase (which catalyzes growth of new strands 5'~3') extends across the segment of DNA between them. One round of synthesis results in new strands of indeterminate length that, like the parental strands, can hybridize to the primers upon denaturation and annealing. These products accumulate only arithmetically with each subsequent cycle of denaturation, annealing to primers, and synthesis.

However, the second cycle of denaturation, annealing, and synthesis produces two single-stranded products that together compose a discrete double-stranded product that is exactly the length between the primer ends. Each strand of this discrete product is complementary to one of the two primers and can therefore participate as a template in subsequent cycles. The amount of this product doubles with every subsequent cycle of synthesis, denaturation, and annealing, accumulating exponentially so that 30 cycles should result in a 270 millionfold amplification of the discrete product. In practice you will only obtain a few millionfold amplification, which is still enormous, and gives you plenty of the particular sequence you are amplifying.

The first step of PCR simply entails mixing template DNA, two appropriate oligonucleotide primers, Taq DNA polymerase, deoxyribonucleoside triphosphates (dNTPs), and a buffer in a small volume (20 to 100 µl). Use only sterile, distilled or deionized water to prepare all reagents. The amplification buffer is often supplied with the Taq polymerase, and usually contains 500 mM KCl, 100

mM Tris-HCl, pH 8.4,1 mg ml^{-1} gelatin, and 15 mM $MgCl_2$. The PCR reaction is extremely sensitive to the concentration of $MgCl_2$. The optimal concentration can be first determined empirically using a 10x amplification buffer minus $MgCl_2$, with a home-made solution of $MgCl_2$.You will add 2 mM of each dNTP, 50 pM of each oligonucleotide primers, the template DNA to be amplified (1 ng to 10 µg of genomic DNA depending on the sequence), 2.5 U of Taq DNA polymerase, and finally 100 µl (a drop or two) of sterile mineral oil.

Once assembled, the mixture is cycled repeatedly (usually 30 to 40 times) through temperatures that permit denaturation (94°C), annealing (45 to 65°C depending on the primers sequences), and synthesis (72°C). Each step in the cycle requires a minimal amount of time to be effective, while too much time at each step can be both wasteful and deleterious to the DNA polymerase. If the amount of time in each step can be reduced, so much the better. It is critical that complete strand separation occur during the denaturation step. This is a unimolecular reaction that itself is very fast. The standard 90 sec used in the protocol ensures that the tube spends enough time in the water bath or automatic thermal cycler to reach 94°C. If PCR is not working, it is well worth checking the temperature inside a tube containing 100 µl water. If G-C content is extremely high, higher denaturation temperatures might be necessary; however, Taq DNA polymerase activity falls off quickly at higher temperatures.

Primers with relatively low G-C content (<50%) may require temperatures lower than 55°C for full annealing. On the other hand, this may also increase nonspecific products. For primers with high G-C content, higher annealing temperatures may be necessary. It can be worthwhile to experiment with this parameter. As with denaturation, the time for this step is based mainly on the time it takes to reach the proper temperature, because the primers are in such excess that the annealing reaction occurs very quickly.

The extension temperature of 72°C is close to the optimal temperature for Taq DNA polymerase, yet prevents the primers from falling off. Indeed, primer extension begins during annealing, since Taq DNA polymerase is partially active at 55°C. The time of extension depends mainly on the length of the sequence to be amplified. The 3 min suggested in the protocol should be ample for extension of several kbp. For longer distances, increasing the time to 15 min can improve yield, but longer times do not seem to help. Certain protocols complete the PCR with a long final extension time in an attempt to try to make products as complete as possible. This is especially important if perfectly blunt-end products are required for cloning or sequencing.

Ramp time is the time it takes to change from one temperature to another. Using water baths and moving samples manually from temperature to temperature probably gives the shortest ramp times, which are mainly the time required for the tube's contents to change temperature. Different thermal cyclers have different ramp times; basically, the shorter the better.

In most of the references listed below where RFLPs are used to identify nematode species or races, the next step would be to digest the amplified product with restriction enzymes to try to find specific restriction sites. Then the

restriction digest is analyzed (the restriction fragments are visualized) on an agarose gels.

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II. Nematode Management

Developing Nematode-resistant Cultivars of Pigeon pea and Chickpea

S B Sharma[1]

Introduction

Pigeonpea (*Cajanus cajan*) and chickpea (*Cicer arietinum*) provide a necessary protein supplement to the largely cereal-based diets of people of the semi-arid tropics; in addition, they enrich the soil by fixing atmospheric nitrogen. Yields of these crops are as such poor, and pests and diseases further reduce yield potential. Plant parasitic nematodes cause significant damage to these crops; however, due to the covert nature of the damage and their microscopic size and subterranean habitat, nematodes are usually successful in evading the attention of growers and plant protection practitioners. On a worldwide basis, plant parasitic nematodes are estimated to cause annual losses in the vicinity of US$ 177 million to pigeonpea and US$ 328 million to chickpea. There is a compelling need to halt the depredation being caused by these hidden enemies; and it is imperative that every effort be made to protect pigeonpea and chickpea crops from assault by these tiny worms. Since nematicides to control nematode pests are either too expensive or unavailable to farmers in subsistence farming systems, development of nematode-resistant cultivars is one of the most effective, viable, and environmentally safe options to curb the losses caused by nematodes. Although pigeonpea and chickpea cultivars with resistance to nematodes have not yet been developed, efforts are now being made in this direction.

Development of Resistant Cultivars

Nematodes as a Constraint to Production

The process of development of resistant cultivars ideally involves the synergistic team efforts of a nematologist, plant breeder, agronomist, and cell

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Sharma, S.B. 1997. Developing nematode-resistant cultivars of pigeonpea and chickpea. Pages 25-27 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

biologist; each one has a well-defined role in the developmental process. The first step is to emphasize the importance of nematodes as a constraint to chickpeaandpigeonpeaproduction.Forexample,basedonsurveysandcrop loss estimation trials, it is evident that *Heterodera cajani, Rotylenchulus reniformis,* and *Meloidogyne* spp are important nematode pests of pigeonpea in India, while *Meloidogyne* spp and *Pratylenchus* spp are important on chickpea. Generally, population densities of greater than one nematode cm^{-3} soil at the time of sowing are harmful for plant biomass and seed yield; susceptible varieties may suffer heavy damage when they are grown in soil infested with greater than three nematodes cm^{-3} soil. These nematodes suppress the formation of *Rhizobium* nodules and enhance the severity of fusarium wilt of chickpea and pigeonpea. In some wilt-resistant chickpea and pigeonpea genotypes, resistance mechanisms do not operate effectively in the presence of nematodes. As evident from various publications, it seems logical to conclude that, at least in India, the first step has nearly been completed.

Development of Techniques, Methodology, and Facilities

The second step consists of such activities as developing facilities for growing plants and maintaining nematode cultures, standardizing procedures for screening plants for resistance and tolerance, and developing rating scales for assessing the levels of resistance. Good progress has been made in these areas: facilities for maintaining nematode cultures and preparation of nematode inocula have been developed, and simple and reliable screening techniques have been standardized. For example, a technique has been developed to identify resistance to *R. reniformis* without using a microscope. Roots of the test genotypes are dipped in 0.25% trypan blue stain and rinsed in water. The nematode eggsacs on roots are selectively stained blue and can be easily counted. Similarly, a simple method has been developed to screen pigeonpea for resistance to *H. cajani* at the seedling stage, using the white cyst index without the aid of a microscope. Techniques have also been standardized for *Meloidogyne* spp, to quickly assess root damage and number of eggsacs produced on chickpea and pigeonpea. A damage index based on the size and number of galls and percent galled area of the root has been developed. These parameters are intrinsic parts of the damage caused by the root-knot nematodes. The damage index is a useful criteria for identification of resistance to the damage caused by the root-knot nematodes. Although it is not clear how extensively these standardized methodologies are being used at present, their development signifies the progress made so far in this critical second step.

Identification of Sources of Resistance

The third step includes identification of sources of resistance and tolerance, confirmation of results in repeat tests, and multilocational testing of these

promising sources. A perusal of the literature indicates that many sources of resistance and tolerance have been identified, but in most cases results have not been confirmed in repeat tests. However, it is apparent that, in pigeonpea germplasm, sources of resistance to the cyst nematode (*H. cajani*) and reniform nematode (*R. reniformis*) are not as readily available as for the root-knot nematode (*Meloidogyne* spp). Fortunately, resistances to all these species are available in the genepool of wild relatives of pigeonpea. Accessions with multiple resistance to the three major nematodes have been identified and resistance to the cyst nematode in *Cajanus platycarpus* accessions has been purified; it can be transferred to the cultivated pigeonpea by newer molecular biology techniques. Unfortunately, while wild relatives of chickpea do not seem to have resistance to root-knot nematodes, nematode-tolerant genotypes have been identified in the chickpea germplasm. Three chickpea cultivars (N 31, N 59, and ICCC 42) and a promising chickpea breeding line (ICCV 90043) have been identified as tolerant to the root-knot nematode, and two promising short-duration pigeonpea breeding lines (ICPL 83024 and ICPL 85045) and selections from medium-duration lines (ICPLs 8357, 85068, 85073, 89050, 89051, and 90097) have been identified as tolerant to the reniform nematode. Tolerance to the cyst nematode in pigeonpea has been identified in preliminary tests and is presently being confirmed. Multilocational testing of all of these promising genotypes has been started. The third step needs a boost to expedite the overall process.

Incorporation of Resistance

The fourth step encompasses incorporation of resistance into high yielding cultivars. Some of these identified sources of resistance have not yet been utilized in breeders' crossing blocks. This step has not yet gained momentum, and greater interaction among team members is necessary to make rapid progress. It seems reasonable to believe that it is not an insurmountable task; if we try a little harder in this mission, within the next 5-10 years we can develop and produce cultivars of chickpea and pigeonpea with the ability to combat the damage caused by nematodes. The future seems to be brighter!

Engineering Resistance in Crops Parasitized by Nematodes

TC Vrain[1]

Introduction

Various research programs are using engineering technology to transfer resistance genes against nematodes across species barriers from wild relative species to common agricultural crops. Until recently we studied the physiology, behavior, and population dynamics of nematodes and their interactions with host plants. The new molecular technologies allow us to identify essential processes that can be tampered with, thereby limiting feeding, development, or reproduction. As with all other management strategies, we are using this new knowledge to disrupt the life cycle of plant parasitic nematodes. For general reading on this subject, I recommend two books I have listed in the section on Additional Reading, Aldridge 1996 and Ohl et al 1997.

For the transfer of genetic information into a plant, we use a natural process where a pathogenic bacteria (*Agrobacterium tumefaciens*) integrates into the chromosomes of a plant cell its own bacterial genetic message. The bacteria carries on a large plasmid a small number of genes making a small number of proteins that take over the cell machinery. These bacterial proteins turn the adjacent tissue into a gall, and if this reminds you of certain sedentary nematodes, you must appreciate that the bacteria learned to do that long before the nematodes. Some of the bacterial genes integrated into the tumor cells also code for proteins that alter the biochemical cycles that result in the synthesis of sugars. The bacteria needs the plant to make some very unplant-like sugars strictly to support bacterial growth.

Twenty years ago we discovered that the genetic material transmitted by the bacteria into the plant was always in the same place on a bacterial plasmid, between two short but very constant borders. We found that we could extract the plasmid from the bacteria, remove the bacterial genes between the borders and replace them with other genes, and place the plasmid back into the bacteria. The bacteria now integrates that new genetic material into the

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Vrain, T.C. 1997. Engineering resistance in crops parasitized by nematodes. Pages 28-36 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

chromosomes of the plant cells. So we remove the bacterial genes that cause the *Agrobacterium* tumors, the genes that make the plants synthesize Sugars for the bacteria, and we replace them with genes for resistance.

Like conventional breeding, these genetic engineering technologies represent an environmentally and socially acceptable means to control nematode pests. Once incorporated into a crop, genetic resistance can decrease the use of nematicides, and improve standards of health, safety, and environmental quality in agriculture.

Resistance Genes with Unknown Products

The last 50 years have seen resistance genes effective against plant parasitic nematodes identified in a few wild species related to agricultural crops. Some commercial cultivars have been bred for resistance, for example cultivars of potato (*Solanum tubersonum*) with the gene H1 resistant to the potato cyst nematode, tomato (*Lysopersicon esculentum*) with the gene Mi resistant to some species of root-knot nematode, and soybean (*Glycine max*) resistant to the soybean cyst nematode.

The Mi gene comes from a wild species of tomato, *Lycopersicon peruvianum.* It has been introduced into commercial cultivars of tomato and imparts resistance to several major species of root-knot nematode. When these nematodes start feeding on plants containing the *Mi* gene, the cells that would normally transform into giant feeding cells show instead a hypersentive response and become necrotic within a few hours. With several research groups involved, it is expected that this gene could be isolated and cloned by 2000. The cloning strategy is map-based and identifies DNA fragments, from a cDNA library of the tomato genome, that cosegregates with the Mi gene (Williamson et al 1992). The aim is to transfer this gene, and confer resistance to root-knot nematodes, into crops other than tomato in which no effective resistance genes are available.

Another gene from tomato (*Hero*), and a gene from potato (*Gro1*), that confer resistance against all pathotypes of the potato cyst nematode, *Globodera rostochiensis*, are being isolated (Ballvora et al 1995; Ganal et al 1995). The gene *H1* that has been transferred from a wild species, *Solanum tuberosum* ssp *andigena*, to many commercial potato cultivars in the last 30 years because it confers resistance to pathotypes Ro1 and Ro4 of *G. rostochiensis,* is also being isolated (Gebhardt et al 1993; Pineda et al 1993). Resistance genes against soybean cyst, sugar beet cyst, and cereal cyst nematodes are also being mapped with RFLP (Jung et al 1992; Vierling et al 1996; Williams et al 1994).

The first gene for resistance against nematodes was mapped and isolated by three European research teams (Cai et al 1997). It has been known for some time that a wild relative of sugar beet (*Beta procumbens*) did not support development of the sugar-beet cyst nematode (*Heterodera schachtii*). The juveniles could invade the tissue and establish a syncytium, but this feeding

structure quickly degraded or did not provide for the proper development of female nematodes. The $HS1^{pro-1}$ resistance gene was cloned with the use of genome-specific satellite markers and chromosomal breakpoint analysis. When transferred to a susceptible sugar-beet (*Beta vulgaris ssp cicla*), this gene made it resistant to the beet cyst nematode.

Interfering with the Formation of Syncytia, Giant Cells, and Nurse Cells

One of the more obvious interactions between the plant and the nematode is the feeding process, where the nematodes inject plant cells with their salivary gland secretions (presumably rich in digestive enzyme to partially liquefy the cytoplasm), and then ingest some or most of the cell content. However, as soon as they start feeding, the salivary secretions from many sedentary nematodes have a drastic effect on gene regulation and the affected cells undergo radical changes. They become very active metabolically, enlarge considerably, and contain many nuclei. Different nematodes induce somewhat different specialized feeding cells, referred to as giant cells, syncytia, or nurse cells according to their origin and morphology. The nematodes inducing these specialized feeding cells cause much more damage than other nematodes, so a large effort is underway to interfere with the formation of these feeding cells, or at least to impede their performance. One avenue being explored is to sytematically identify genes expressed in the feeding cells (Bird 1997; Van der Eycken et al 1996; Williamson et al 1992).

If one or more of the proteins in the salivary secretions begins the cascade of events that eventually ends with the formation of giant cells, then interfering with this particular protein may abort the formation of these cells. Several research groups in Europe and North America are identifying salivary gland proteins from cyst and root-knot nematodes and raising monoclonal antibodies against them. Expression in plant cells of antibodies (plantibodies) binding to nematode salivary gland secretions may interfere with the induction, development, or maintenance of feeding cells, and result in resistance to sedentary nematodes (DeBoer et al 1996). Single chain antibodies are constructed from first strand cDNAs of hybridoma cells using PCR. They are expressed in E. coli and their activities on nematodes salivary secretions is compared to the monoclonal antibodies, before being transformed into plants. Efforts are underway to analyze their expression in plants and their ability to impede the development of cyst and root-knot nematodes. Hoewever, the first plantibody to be expressed as a fully functional immunoglobulin in tobacco plants did not affect nematode development or reproduction (Baum et al 1996).

The identification of gene expression in nematode feeding cells and the manipulation of their control elements provide the means to alter their development or maintenance (Bird 1997; Gheysen et al 1996; Van der Eycken et

al 1996; Williamson et al 1992). These studies are determining molecular mechanisms amenable to such manipulation by characterizing a large number of genes with regulatory functions, and other genes sustaining the biochemical make-up of root-knot nematode feeding cells.

Anti-nematode proteins (plantibodies, proteinase inhibitors, etc.) can be expressed constitutively in the plant, or they can be expressed in the feeding cells only under the control of a nematode-inducible promoter. A promoter that regulates a gene coding for a water channel protein in tobacco (*Nicotiana tabacum*), TobRB7, was tailored to contain only such a highly specific nematode responsive element. When a series of 5' deletions of this promoter were fused to ß-glucuronidase (GUS) and transferred to tobacco infected with root-knot nematodes, GUS activity was found only in the feeding cells and nowhere else in the plants (Opperman et al 1994). This feeding cell specific promoter was fused to a RNAse (barnase) gene and transformed into tobacco. Nematodes attempting to infect these transgenic tobacco plants switched on the RNAse gene that aborted the development of feeding cells. Nematodes were successfully controlled in the roots, but unfortunately the RNAse gene was minimally expressed in other parts of the tobacco plants, blocking normal development (Opperman et al 1997). Another strategy would be to disrupt plant gene expression in the feeding cells using antisense constructs to target specific transcripts. The nematode-inducible promoter derived from TobRB7 was used to control the expression of an antisense construct of the putative water channel gene TobRB7 into tobacco. Nematodes attempting to induce giant cells by feeding initiate the expression of these antisense constructs, resulting in the degeneration and necrosis of the cells. Field trials showed 70% less nematodes on these plants compared to the control.

Interfering with Nematode Feeding and Digestion

Another strategy is to interfere with the digestion of cell content ingested by nematodes. A strategy developed against insects in the 1980s, at the same time as the Bt strategy (Hilder et al 1987), has now been adapted to combat nematodes. Many plants contain defence proteins that can block various enzymatic activities in insects or other herbivory feeding on the plant tissues (Ryan 1990). Proteinase inhibitors are small proteins common in plants, especially seeds and other reproductive parts, that inhibit proteolytic enzymes and suppress protein digestion, resulting in slow starvation, and delay in development and reproduction of the pests. Hilder et al (1987) characterized a trypsin inhibitor (CpTI) from cowpea (*Vigna unguiculata*), and engineered its expression in tobacco. The CpTI protein blocked the development of several insects feeding on transgenic tobacco leaves. As early as 1975, Dasgupta and Ganguly showed a trypsin-like activity in a root-knot nematode, *Meloidogyne incognita*, and indeed when the CpTI gene is expressed in transgenic potato (Hepher and Atkinson 1992), it impedes reproduction of this root-knot

nematode, and it also interferes with the development of a cyst nematode (*Globodera pallida*).

This discovery has prompted in vitro studies of nematode digestive proteinases and their potential inhibitors. Another type of proteinase activity (cysteine) are also present in cysts and root-knot nematodes. These enzymes are inhibited in vitro by plant cystatins (cysteine proteinase inhibitors) such as oryzacystatins (OCI and OCII) from rice (*Oryza sativa*). Koritsas and Atkinson (1994) showed that females of *G. pallida,* a potato cyst nematode species, had one or more cysteine proteinases, but no serine proteinase activity. Michaud et al (1996) demonstrated cysteine proteinases in preparasitic juvenile stages of *M. incognita* and *M. javanica,* and in all feeding stages of *M. hapla,* but contrasting with the findings of Dasgupta and Ganguly in *M. incognita,* they could not show any serine proteinase activity in *M. hapla*.

OCI completely inhibits the proteolytic activity in all stages of *M. hapla* (thus confirming the absence of serine proteinases), but it is less effective in *M. incognita* and *M. javanica*. Another cystatin from rice (OCII) is more effective with these two latter species, but not so with *M. hapla* (Michaud et al 1996). Thus, particular inhibitors can be more potent (specific) towards some nematode proteinases than others. Many plant cystatins remain to be tested, and it is expected that some will be extremely effective against specific nematodes. Urwin et al (1995) demonstrated that amino acid deletions or insertions at the active site of OCI could enhance the inhibition of papain (a plant cysteine proteinase) or a cysteine proteinase from *Caenorhabditis elegans.* The engineered protein was also effective against *G. pallida.*

Bacillus thuringiensis

The crystal proteins produced in the spores of this bacterium are very toxic to many insects, and the expression of these proteins in transgenic crops is an effective protection against major insect pests. Plant parasitic nematodes cannot ingest bacteria or their spores because the size of the opening of their stylet through which they feed is too small. However, *Caenorhabditis elegans,* a bacterial feeding nematode, is killed when ingesting spores of the bacteria. As with insects, the toxicity is very specific; some bacterial strains are without effects, and among 15 species of bacterial feeding nematodes tested, only 2 species of *Caenorhabditis* are affected (Borgonie et al 1996). These results are extremely promising, since the mechanism of toxicity appear to be related or similar in nematodes and in insects, involving the destruction of intestinal membranes. We do not yet know enough about the structure and physiology of the intestine of nematodes to predict that plant parasitic nematodes should be affected by *Bacillus thuringiensis* endotoxins in the same manner as *C. elegans.* However, because of the relatedness between bacterial feeding and plant parasitic nematodes, there is a real possibility that the expression of certain Bt toxins in transgenic crops will affect nematode pests.

Lectins

Lectins are carbohydrate-binding proteins in plants that are toxic to insects, but unfortunately almost all lectins are also toxic to mammals, thus they cannot be used in transgenic food crops. However, a lectin from snow drop (*Galanthus nivalis*) with little demonstrated toxicity against mammals has been engineered in transgenic plants and gives protection against insects (Boulter et al 1990). This lectin is toxic to *Pratylenchus penetrans,* and others (Burrows 1997). A very important point is that if lectins are truly effective for engineering resistance against nematodes, they then provide a completely different mechanism of action, which will allow their use in combination with other toxins or enzyme inhibitors.

Conclusion

Wild relatives of our crops tend to have several mechanisms for resistance to any pest. The risks of losing the effectiveness of vertical resistance genes are the same regardless of the methods used, standard breeding or genetic engineering. It is therefore important to treat the resistance of transgenic crops just as another component of integrated pest management. The expression of more than one type of resistance gene increases the durability of the resistance and broadens the spectrum of activity against pests. With nematodes, the strategies impeding the induction of feeding cells, where promoters from genes normally involved in feeding cell development are turned against nematodes, have the advantage that nematodes cannot evolve to overcome the resistance.

Foods derived from engineered crops must be safe to eat, and for that reason many genes for resistance found in certain plants, even in commercial crops, cannot be expressed in other crops. For example, while several lectins from beans are very effective against many pests, these lectins are quite toxic and they should not be expressed in other crops such as tomato or strawberry. Trypsin proteinase inhibitors are effective against many insect and nematode pests, but some of them also affect mammalian digestion, making it doubtful that health regulatory bodies will allow their expression in transgenic crops. However, these toxic proteins will undoubtedly be used to protect nonfood crops, such as in floriculture or in forestry.

We can be pretty sure that genetic engineering will improve agricultural crops. These new technologies are not yet widely accepted, especially in developed countries where the public may not appreciate the need for these revolutionary techniques in agriculture, so it may be that their use will evolve at vastly different paces in different regions.

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Integrated Pest Management Strategies for Nematodes

D D R Reddy[1]

Introduction

Integrated pest management (IPM) of nematodes may be defined as the integration of management methodologies for all economically important nematodes of the agroecosystem with the objective of optimizing productivity, net returns, stability and environmental quality. IPM in its simplest form is accepted as being a control strategy in which a variety of physical, mechanical, cultural, biological, and chemical control measures are combined to give stable, long-term nematode control.

The IPM Concept

The IPM approach is both conceptual and methodological. Most of the tactics utilized in IPM are not new, but the deliberate effort to integrate the utilization of these tactics in a harmonious manner is unique. IPM implementation involves application of the following principles for the management of all key nematode pests in the agroecosystem:

1. **Identify the key pests to be managed.** Pests that cause or have the potential for causing economic loss in the ecosystem must be identified, and information on their biology and ecology must be assembled as the basis for developing management strategies.
2. **Define the management unit—the agroecosystem.** The migratory capacity of key nematode pests determines the boundaries of the agroecosystem that must be managed. If the capacity for migration is limited, the boundaries of the management unit may be restricted to a single field, but if the capacity is high the management unit may comprise a continent (e.g., seed-borne nematodes).

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3. **Develop the management strategy.** The management strategy must be directed at reducing the initial population levels of key nematode pests. It should be a strategy based upon management tactics that optimize pest reduction and crop productivity. Knowledge of nematode pest ecology and other fundamental ecosystem relationships is the principle basis for selecting and deploying existing management tactics and for the development of new ones.
4. **Establish economic thresholds.** The economic threshold is the density at which control measures should be applied to prevent an increasing nematode population from reaching the economic injury level.
5. **Develop assessment technique.** Nematode population assessment is essential to the selection and proper timing of management tactics.
6. **Evolve descriptive or predictive models.** The ultimate objective of IPM is the development of predictive models. This process entails the collection and integration of several sets of complex data, with each set relating to a dynamic biological, physical, meterological, or socioeconomic systems, for the development of a valid IPM system.

Aims of IPM

1. Use of nematicides for prophylaxis should be steered away from and directed towards optimized selective use, using the various IPM components to guide decision making.
2. It should be recognized that the noneconomic consequences of nematode control (including pesticide use) may be harmful to the environment; pesticide inputs should be reduced and natural control maximized in order to minimize side effects to the environment.
3. Effective control methods should be synthesized in such a way that the integrated components are capable of containing the nematode pest below the economic threshold levels. Before taking up an IPM program, knowledge of the pest's biological and behavioral characteristics is necessary to assess the most natural and effective methods of control. Such parameters must be determined as vulnerable stages of the pest, appropriate time of action, which is the most effective control method suitable for the particular stage, etc.

Components of IPM

The most important and effective components of IPM can be identified as:

1. Cultural methods;
2. Physical methods;
3. Mechanical methods;

4. Biological methods;
5. Resistant varieties;
6. Regulatory methods (plant quarantine); and
7. Chemical methods.

Practical Feasibility of IPM

Even though judicious use of nematicides is the slogan, in actual terms, the area and frequency of treatment with nematicide is rising. This is because the crop production systems, and the consequent level of nematode damage that can be tolerated, have changed radically. The other factor, probably the most important, is that cosmetic standards have increased to where the presence of feeding scars is often sufficient for crops to be rejected by processors, and even by the fresh market in many instances. The most important factor operating against the use of IPM is the demand for high quality produce. Because of the short-term relationship between pest and crop, natural enemies of the pest have insufficient time to establish their superiority before the crop is damaged and quality is lowered. There are ways, however, by which the amounts of nematicide applied can be reduced: e.g., band and spot treatment instead of broadcast treatment, and seed or seedling treatment.

Nematicides will continue to have a role in IPM of nematode pests but it is increasingly imperative that they can be used knowledgeably and responsibly, and only when other tactics are inadequate. Their use should be based on economic threshold values and predictive systems.

Recently there have been consistent attempts to promote research on and adoption of nematode IPM programs, both nationally and internationally. However, progress is less than had been expected or hoped for, due to:

1. Crop protection personnel with inadequate training in nematology;

2. Serious retention problems with technical personnel because of noncompetitive salaries, limited professional opportunities, and inability to recruit replacement professionals;

3. Inadequate operational funds for programs in research or extension;

4. Lack of continuity in government commitments to programs and in program leadership;

5. Consequently, lack of ability to develop long-range plans and to sustain their implementation.

The All India Coordinated Research Project on Nematode Pests and their Control: a Possible Model for Other Countries

S K Midha[1]

Introduction

Nematological investigations in India received some semblance of recognition during the 1960s with the discovery of the association of *Heterodera avenae* with the "Molya" disease in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) in Rajasthan and identification of the dreaded golden nematode in the potato (*Solanum tuberosum*) fields of the Nilgiris. At this time research work on nematode pests had been started at three institutes: the Indian Agricultural Research Institute (IARI), New Delhi; Aligarh Muslim University, Aligarh; and Tamilnadu Agricultural University, Coimbatore. These institutes documented the nematode fauna present in agricultural soils and their association with unhealthy growth of crops. The need to initiate an organized network throughout the Country for studies on nematode pests was recognized. With this background, and with the approval of the Indian Council of Agricultural Research (ICAR), IARI developed an All India Coordinated Research Project in 1977 for work on nematode pests. Initially, this project was funded by the Department of Science and Technology for 2 years (April 1977 to March 1979). Subsequently, the project was taken over by ICAR for further funding from the plan funds.

To strengthen the work on nematode problems of oilseed crops, a subcentre of Gujrat Agricultural University was added during the seventh plan period and two new centres at Ludhiana and Srinagar were added in the eighth plan period. The project now has 17 centres in addition to the Coordinating Cell, and are listed below.

Centres

- Indian Agricultural Research Institute, New Delhi (Coordinating Cell);
- Chowdhury Charan Singh Haryana Agricultural University, Hisar, Haryana;

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- Orissa University of Agriculture and Technology, Bhubneshwar, Orissa;
- Assam Agricultural University, Jorhat, Assam;
- Dr Y S Parmar University of Agriculture and Technology, Solan, Himachal Pradesh;
- Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh;
- Kerala Agricultural University, Vellayani, Kerala;
- Tamilnadu Agricultural University, Coimbatore, Tamilnadu;
- Rajasthan College of Agriculture, University of Udaipur, Rajasthan;
- Mahatma Phule Agricultural University, Rahuri, Maharastra;
- Gujrat Agricultural University, Anand, Gujrat;
- Rajendra Agricultural University, Pusa, Bihar;
- Chandra Sekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh;
- University of Agricultural Sciences, Bangalore, Karnatka;
- BC Krishi Vishwa Vidhalaya, West Bengal;
- Gujarat Agricultural University Subcentre, Junagarh, Gujarat;
- Punjab Agricultural University, Ludhiana, Punjab; and
- Sher-e-Kashmir University of Agriculture and Technology, Srinagar, Jammu and Kashmir.

Objectives

- Quantification of crop yield losses and development of forecasting models;
- Virulence identification surveys for major nematode groups, i.e., root-knot and reniform nematodes;
- Germplasm evaluation and identification of sources of resistance;
- Development of economically viable integrated systems of management, including biological control, solarization, and eco-friendly procedures; and
- Identification of new nematode problems that may arise from time to time.

Mandate

- To conduct coordinated trials for reducing crop losses caused by nematode pests through an integrated management approach; and
- To demonstrate those management technologies through on-farm trials.

Future Thrust Areas

- To use nematode management to enhance production potential in pulses, oilseeds, and vegetables;
- To integrate management technologies for nematode pests with those of insects and diseases;

- To study population dynamics of important nematode pests in different crop rotations under different agroclimatic regimes;
- To liaise with seed production farms to assess nematode pests and demonstrate management technologies to enhance seed yields; and
- To investigate new nematode problems and monitor the spread of major nematode pests.

Achievements in Brief

Identification of Nematode Problems

The most important achievement to date has been identification of several noxious nematode pests (Table 1). Predominant populations of the root-knot nematodes and the reniform nematodes have been found throughout the country and are important factors in limiting successful production of vegetables, pulses, oilseeds, fruit, and other crops. Similarly, rice-root nematode (*Hirschmanniella* spp.) has been recorded in high densities from all the rice (*Oryza sativa*)-growing areas. Citrus nematode has emerged as a major production constraint in citrus orchards. Survey programs have also identified some hitherto unknown nematode problems such as White Tip nematode on rice (*Aphelenchoides besseyi*) in Tamil Nadu, Orissa, and Assam; Ufra nematode (*Ditylenchus angustus*) from Assam; root-knot nematode (M.

Table 1. Varieties/lines found resistant to the root-knot nematode (*Meloidogyne incognita*) after multilocational testing.

Crop	Resistant cultivars/lines
Tomato	SL 120, Patriot, Healani, VFN-Bush, Bresch, Piersol, Ronita, Karnataka Hybrid, Punjab NR 7, NT 8, NT 3, and NT 12
Brinjal	Ghatikia White, Gachha Baigan, PBr-91-2
Chilli	C-17-A, C-1-B, C-70-A, Pusa Jawala, NP 46, Mohini, SF 6, P-6-3, X 235
Cowpea	81-1b and C 152
Mung	ML 80 and ML 62
Cotton	Bikaneri Nerma, Sharda, Pay Master
Grape	Khalili and Kishmish Beli, Early Muscat, Jasbeli Loose Pearlette

graminicola) from Assam, Orissa, and West Bengal; cyst nematode (*H. oryzicola*) on rice; mushroom nematode (*Ditylenchus myceliophagus*) on mushroom in Himachal Pradesh, Delhi, and Haryana; and other Heterodera spp on maize (*Zea mays*) in Himachal Pradesh, Rajasthan, Bihar, and Maharastra; (*H. cajani*) in all pulse-growing areas of the country; root-knot nematode (*Meloidogyne* spp) and lesion nematode (*Pratylenchus thornei*) on chickpea (*Cicer arietinum*); and root-knot (*M. arenaria* and *M. javanica*) on groundnut (*Arachis hypogaea*). Some new nematode problems like lesion and pin nematode (*Pratylenchus* spp and *Paratylemusa* spp) on stone fruits from Himachal Pradesh, *H. oryzicola* on banana (*Musa* spp) from Kerala, and *Ditylenchus myceliophagus* on mushroom have been identified.

Crop Losses

The avoidable crop yield losses as a result of important nematodes were determined: 20% in rice due to rice root nematode; 34-83% in vegetable crops; 7-65 % in mung (*Phaseolus aureus*); 30-40% in cowpea (*Vigna unguiculata*); 17% in pigeonpea (*Cajanus cajan*); 11-38% in groundnut; 11-34% in mung; 12-43% in cowpea, 19-40% chickpea; 20-49% in blackgram (*Phaseolus mungo*); and 6% in jute (*Corchorus* spp) due to root-knot nematodes (*Meloidogyne* spp). In pulses 20-23% yield losses were due to *R. reniformis;* 26% due to lesion nematode (*P. thornei*) on chickpea; and 17-25% in castor (*Ricinus communis*) due to *R. reniformis*. The losses invariably increased with an increase in nematode population density.

Management Technologies

Sources of resistance against nematode pests have been identified. Table 1 shows a number of such sources.

1. By using some of these source of resistance, cowpea variety GAU 1 and tomato varieties Hisar Lalit and PNR 7 with resistance to root-knot nematodes (*M. incognita*) have been bred and released for commercial cultivation.
2. Rice nursery raised in beds treated with carbofuran (1 kg a.i. ha^{-1}) gave protection against rice-root nematode (*Hirschmanniella* spp) and root-knot nematode (*M. graminicola*).
3. Application of carbofuran (3 kg a.i. ha^{-1}) plus neem cake (400 kg ha^{-1}) plus urea (24 kg ha^{-1}) was effective in reducing root-knot nematode infections in vegetables and increasing the yield at a cost-benefit ratio of 1:2.5.
4. Technology for clean cultivation of mushrooms (*Agaricus campestris*) free from nematode infection was developed. Such management practices as using 5% formalin for disinfection of trays and implements, maintaining bed

temperature in pasteurization at 60°C for 2 h, sterilizing the casing soil, steaming of mushroom houses at 70°C for 5-6 h and use of nematode-free irrigationwater.

5. For obvious reasons, the use of chemicals for nematode cohtrol has been restricted to nursery bed, root dip, and seed treatments. The results revealed that nursery bed treatment of rice with carbofuran 1 kg a.i. ha^{-1} and with Nemacur® and diazinon gave healthy nematode-free seedlings, which on being transplanted in the main field gave increased ear-bearing tillers and increased yield, ranging from 14-20%. It also controlled the root knot nematode infestation on rice with a cost-benefit ratio as high as 1:11.5 at some locations. A root dip of rice nursery in 0.2% solution of carbofuran/ oncol/chloropyriphos for 6 h also reduced the nematode population and enhanced the yield (the cost-benefit ratio with chloropyriphos was 1:3.5).

6. In the case of vegetables [tomato *(Lycopersicon esculentum),* brinjal *(Solanum melongena),* and chillies *(Capsicum frutescens)]* nursery bed treatment with carbofuran at 0.3 g a.i. m^{-2}, root dip in 0.2% solution of carbofuran/ phenamiphos/phorate for 12 h, or seed dressing with carbofuran at 3% a.i. w/w in the case of okra *(Hibiscus esculentus),* pointedgourd, bittergourd *(Mamordica charantia)* and mung effectively controlled the nematodes infecting these crops and enhanced the yield.

7. In fruit crops like citrus and grapes *(Vitus vinifera),* the use of carbofuran/ oncol each at the rate of 4 kg a.i. ha^{-1} or neem cake at 3 kg $plant^{-1}$ alone or 2 kg a.i. carbofuran plus 1 kg neem cake $plant^{-1}$ controlled the important nematodes. The residues of these chemicals in the ripe fruits were below the detectable level.

8. Root-dip treatment of tomato and brinjal seedlings with carbosulfan and monocrotophos each at 500 ppm before transplanting reduced root-knot nematode disease and increased yield significantly (yield increase of 26-59% with monocrotophos and 14-36% with carbosulfan).

9. Seed dressing with carbosulfone (25 ST) at 3% was most effective in reducing the root-knot nematode population in bold-seeded crops of vegetables and pulses.

10. Root-knot nematode disease in groundnut was effectively managed with an integrated method of polythene mulching plus neem cake application at 200 kg h^{-1}.

11. A combined treatment of paring and hot water to banana suckers at 55°C for 20 min followed by application of neem cake at 1 kg $plant^{-1}$ and carbofuran at 0.5g a.i. $plant^{-1}$ was found very effective in reducing the population of nematode complexes (R. *similis, M. incognita, Helicotylenchus multicinctus,* H. *dihystera,* and *P. coffeae).*

III. Nematology in South Asia

Status of Nematode Problems and Research in Myanmar

Aung Swe[1]

Introduction

Myanmar is located in Southeast Asia between latitudes 9°32' and 28°31'N, and longitudes 92°10' and 101°11'E. It has an area of 677 000 km^2, and has international boundaries with Bangladesh, India, China, Laos, and Thailand. Myanmar is a mountainous country drained by four major river systems: the Ayeyarwaddy, the Chindwin, the Thanlwin, and the Sittaung. A number of shorter rivers also drain the Rakhine and Taninthayi regions. The four broad geographic regions are the eastern highlands, the western Yoma, the Central Basin, and the Rakhine and Taninthayi coastal regions.

The Tropic of Cancer passes roughly near Mogok, to the north of Mandalay, and divides the country into two major climatic regions: tropical and subtropical, or temperate. The southern part is influenced by the Southwest monsoon from May to October, depending on the elevation and the distance from the coast. The period from October to February is typically cool and dry. In the north and mountainous areas, temperatures are substantially lower throughout the year. Myanmar can be divided into the following three zones, according to rainfall:

1. An adequate rainfall zone of 2500-4000 mm, e.g., in the Rakhine, Mon, Taninthayi, and Kayin States;
2. A moderate rainfall zone of 2000-3000 mm, with rainfall mainly during a period of 3-4 months; and
3. A low rainfall or "dry" zone where rainfall of less than 2000 mm occurs during a period of 5-6 months, e.g., Mandalay, Magwe, and Southern Sagaing Division, and Shan, Chin, and Kayah States.

1. Plant Protection Division, Myanma Agriculture Division, Bayintnaung Road, Gyogone, Insein, Yangon, Myanmar.

Swe, Aung. 1997. Status of nematode problems and research in Myanmar. Pages 47-52 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Agriculture in Myanmar

The economy of Myanmar is still predominantly agricultural, accounting tor nearly 40% of the gross domestic product, and provides employment to about 65% of the total work force. Besides meeting domestic food requirements, the agricultural sector also produces sizeable exports. Out of a total of 68 million ha, about 10 million ha are arable. The major crops cultivated are rice (*Oryza sativa*) (6.5 million ha), sesame (*Sesamum indicum*) (1.3 million ha), pulses (1.5 million ha), groundnut (*Arachis hypogaea*) (500 000 ha), sunflower (*Helianthus annuus*) (120 000 ha), and cotton (*Gossypium herbaceum*) (140 000 ha).

Plant Parasitic Nematodes Occurring in Myanmar

Nematodes are plant parasites of major economic importance predominantly in the tropics, but also of some importance in the temperate regions of Myanmar. Losses due to nematodes are frequently severe, depending on the species and the crop. Table 1 shows nematode species found on different crops in Myanmar, according to a survey by the Plant Pathology Department, Institute of Agriculture and Plant Protection Division, Myanma Agriculture Service. The most economically important nematodes in Myanmar are described below.

Nematodes of Rice

A number of genera of plant parasitic nematodes are associated with rice in Myanmar, but only three genera are of proven or potential economic importance: *Aphelenchoides besseyi, Ditylenchus angustus,* and *Meloidogyne* spp.

***Aphelenchoides besseyi* (White Tip).** This is a serious pest in Myanmar. It is widely distributed throughout the entire country because of its ability to spread from one place to another by seed. Yield losses can reach up to 50%, depending on the nematode population density and the susceptibility of the crop.

***Ditylenchus angustus* (Ufra disease).** In Myanmar, *D. angustus* occurs in both deepwater and lowland rice, and is a serious problem, especially in Ayerawady Division. It can cause yield losses in deepwater rice of 40-90%, but no serious infestation has been reported in irrigated rice (also known as "summer rice" in Myanmar).

***Meloidgyne* spp (root-knot).** In Myanmar, root-knot nematode on rice is not as serious as Ufra and White Tip nematodes. Only three species have been found on rice, including *M. graminicola, M. incognita,* and *M. javanica.*

Table 1. Plant parasitic nematode genera identified in Myanmar.

Crop	Nematode
Rice	*Criconema, Ditylenchus, Hirschmanniella, Hoplolaimus, Macroposthonia, Meloidogyne*
Wheat	*Anguina, Aphelenchoides, Ditylenchus, Helicotylenchus*
Maize	*Aphelenchus, Ditylenhus, Meloidogyne, Pratylenchus, Scutellonema, Tylenchorhynchus*
Banana	*Aphelenchoides, Helicotylenchus, Meloidogyne, Pratylenchus*
Papaya	*Aphelenchoides, Helicotylenchus, Pratylenchus, Scutellonema, Trichodorus*
Lemon	*Meloidogyne*
Potato	*Ditylenchus, Helicotylenchus, Meloidogyne*
Sweet potato	*Helicotylenchus, Meloidogyne*
Yam	*Meloidogyne*
Bittergourd	*Meloidogyne*
Bombey hemp	*Meloidogyne*
Bottle gourd	*Meloidogyne*
Cauliflower	*Meloidogyne*
Celery	*Meloidogyne*
Coriander	*Helicotylenchus*
Cucumbers	*Meloidogyne*
Eggplant	*Meloidogyne*
Garlic	*Criconema*
Chrysanthemum	*Aphelenchoides, Criconema, Ditylenchus*
Lettuce	*Meloidogyne*
Okra	*Meloidogyne*

continued

Table 1 continued

Crop	Nematode
Onion	*Meloidogyne*
Pepper	*Meloidogyne*
Pumpkin	*Meloidogyne*
Mustard	*Meloidogyne*
Radish	*Meloidogyne*
Tomato	*Meloidogyne*
Butter bean	*Hemicycliophora*
Cowpea	*Meloidogyne*
Kidney bean	*Meloidogyne*
Green gram	*Meloidogyne*
Mung bean	*Hirschmanniella*
Pigeonpea	*Meloidogyne*
Lima bean	*Meloidogyne*
Groundnut	*Tylenchorhynch us*
Sesame	*Meloidogyne*
Sunflower	*Meloidogyne*
Jute	*Meloidogyne*
Gladiolus	*Tetylenchus*
Sugarcane	*Aphelenchoides, Criconema, Helicotylenchus, Tylenchorhynchus*

Nematodes of Pulses

For a number of years in Myanmar, pulses have been cultivated as a winter crop on about 830 000 ha. However, in recent years the production of some peas and beans has noticeably increased; and since 1992-93, the area cultivated to pulses under multi-cropping systems has increased in response to rising prices. In one year's time, from the 1994-95 cropping season to 1995-96, the total area sown to black gram (*Phaseolus mungo*), green gram (*Phaseolus aureus*), chickpea, pigeonpea, soybean (*Glycine max*), and cowpea (*Vigna unguiculata*) increased from 1.49 million ha to 1.63 million ha, and total yields increased from 0.84 million t to 1.06 million t.

In Myanmar, pulses are important not only for local consumption but also for export. In addition, they are relatively easy to grow and inexpensive to produce. Out of the six major pulses mentioned above, chickpea is of major importance in Myanmar. In the 1995-96 cropping season, a total of 181 919 ha were sown to chickpea; Sagaing, Mandalay, Magway, Bago, and Ayeyarwady are the major chickpea-growing regions.

A few plant parasitic nematodes have been reported in association with pulses. Among them, *Meloidgyne* spp are of the only known economic importance. Systematic surveys must be done to determine the relevant importance of other species.

Research on Plant Parasitic Nematodes in Myanmar

The following research has been accomplished for plant parasitic nematodes in Myanmar.

- Observations have been made on rice root-knot nematode for identification and testing of control measures.
- The geographical distribution of White Tip nematode in monsoon rice has been studied.
- The effectiveness of the parasitic fungus Paecilomyces lilacinus has been tested on root-knot nematode in potato (*Solanum tuberosum*).
- Various nematicides have been tested in rice for the control of White Tip and Ufra diseases.

Scientists Working on Plant Parasitic Nematodes in Myanmar

Dr Tin Aung, PhD (Nematology), Plant Protection Division, Myanma Agriculture Service, Yangon;

Dr Ye Ye Myint, PhD (Nematology), Institute of Agriculture, Yezin;

U Aung Swe, MSc (Nematology), Plant Protection Division, Myanmar Agriculture Service, Yangon; and

Daw Pyone Pyone Kyi, MSc (Nematology), Central Agriculture Research Institute, Myanma Agriculture Service, Yezin.

Future Plans

So far, there have been no systematic surveys on plant parasitic nematodes of chickpea and pigeonpea. Survey work on nematode incidence on these crops in Myanmar is urgently needed. It is hoped that this research will soon be carried out with the assistance and collaboration with ICRISAT and international or national working groups on plant protection. Research is needed in several major areas, including a general survey on plant parasitic nematodes, identification of those nematodes, determination of sources of genetic resistance, and prevention and control methods.

Status of Nematode Problems and Research in Sri Lanka

G D S N Chandrasena[1]

Introduction

Sri Lanka is an island of over 6 million ha located in the Indian ocean. It is divided into three climatic zones according to the annual precipitation—wet, dry, and intermediate zones—arid further divided into seven agroecological zones on the basis of elevation and rainfall. Based on the topograpy and soil type, these are divided further into 22 subagroecological regions. Because of the variation in rainfall, elevation, and soil type, the diversity of vegetation on the island is very high; thus, there is great potential to grow many different kinds of cultivated crops in the country. Tea *(Camellia sinensis),* rubber, and coconut *(Cocos nucifera)* are the major plantation crops. Rice *(Oryza sativa),* the staple food crop in Sri Lanka, is grown predominantly in most of the low-lying and wet soil areas. Cardamom *(Elettaria cardamomum),* clove *(Eugenia caryophyllus),* nutmeg *(Myritica fragrans),* and coffee *(Coffea arabica)* are some of the export agricultural crops. Most of the horticultural crops are grown mainly in the wet and intermediate zones. Pepper (chilli) *(Capsicum frutescens)* and onion *(Allium cepa)* are the most important condiment crops grown in the dry zone. Pulses and oil crops are also grown traditionally under subsistence farming conditions, mainly in the dry zone. Pigeonpea *(Cajanus cajan),* which was introduced to the country in 1950s has also proved to be an ideal rainfed crop for the dry zone marginal uplands. Although chickpea *(Cicer arietinum)* is also a potential *maha* (rainy season) crop for some parts of the dry and intermediate zones, research efforts are still underway to introduce it to farmers.

Plant Parasitic Nematodes Occurring in Sri Lanka

Plant parasitic nematodes are one of the major constraints to cultivation of many crops grown in Sri Lanka. About 95% of horticultural crops are attacked by nematode pests. Tea, coconut, rice, black pepper *(Piper nigrum),* grain

1. Field Crops Research and Development Institute, Maha Illuppallama, Sri Lanka.

Chandrasena, G.D.S.N. 1997. Status of nematode problems and research in Sri Lanka. Pages 53-56 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

legumes, and sugarcane (*Saccharum officinarum*) are some of the other crops that have been detected as host crops for various nematode species. As pigeonpea and chickpea are still in the process of being adopted by Sri Lankan farmers, little investigation has been done on these crops. However, a root-knot nematode, *Meloidogyne incognita,* has been found in association with both of these crops. It has also been found that this species is the most harmful of all the species occurring in large population densities in soils cultivated to vegetables.

The presence of plant nematodes in Sri Lanka was reported for the first time in 1940. *M. brevicauda,* a species endemic to Sri Lanka, was the first root-knot nematode recorded. It has been determined that there are 47 plant parasitic nematode species belonging to 24 genera in association with economically

Table 1. Parasitic nematodes recorded in Sri Lanka.

Genus	Number of species
Xiphinema	9
Meloidogyne	6
Helicotylenchus	5
Pratylenchus	3
Hoplolaimus	2
Longidorus	2
Scutellonema	2
Aphelenchoides	2
Hirschmanniella	1
Dolichodorous	1
Globodera	1
Radopholus	1
Rotylenchulus	1
Tylenchulus	1
Paralongidorus	1
Paratylenchus	1
Trichodorus	1
Paratrickodorus	1
Ditylenchus	1
Macroposthonia	1
Aphelenchus	1
Hemicriconemoides	1
Rotylenchus	1
Tylenchus	1

important crops (Table 1). The frequency of occurrence of common nematodes associated with some of the important crops is shown in Table 2. Of these, *Meloidogyne* is the most predominant, widely distributed, and most destructive nematode associated with many crops grown in the island. As this nematode group has a wide host range, cultivation of different types of host crops throughout the year in farmers' fields facilitates the rapid multiplication and the distribution of the pest over the country. Two other genera, *Xiphinema* and *Helicotylenchus,* have also been detected in over 80% of the crops surveyed. The survey revealed that *M. arenaria,* M *incognita,* and *M. javanica* are widely distributed in all the agroecological regions, while *M. hapla is* confined to cooler areas in the hills. *M. graminicola* is restricted to a limited area cultivated to rice in the southern province, while M. *brevicauda* is confined to tea plantations in Kandy district only. Strict local quarantine regulations have played a major role in controlling the distribution of these species to other areas of the country. *M. arenaria, M. incognita,* and *Rotylenchulus reniformis* are the most widespread species on the island. *Globodera rostochiensis, Radopholus similis, Tylenchulus semipenetrans, Pratylenchus brachyurus, P. loosi, Aphelenchoides besseyi,* and *Hirschmanniella oryzae* are also considerea to be important nematode pests.

Extending more lands for agriculture, implementation of agricultural intensification projects, and imports of sowing materials into the country are the main factors that have paved the way to the introduction of plant parasitic nematodes, their establishment, and dissemination throughout the country.

Though there are diverse methods to control the nematode pests, farmers tend to use pesticides, which have proven their efficacy in minimizing crop losses according to their experiences. However, considering the human and

Table 2. Frequency of occurrence of common nematodes found associated with some important crops in Sri Lanka.

Nematode genera	Rice	Potato	Tomato	Cucurbits	Banana	Bean	Leafy vegetables
Helicotylenchus	1.4	2.8	10.3	7.1	22.5	11.7	5.1
Meloidogyne	0.4	3.7	59.3	29.9	11.2	10.2	4.1
Xiphinema	2.2	1.3	6.3	8.5	9.6	8.3	2.8
Hoplolaimus	3.1	0.7	2.4	2.2	2.2	10.6	2.2
Hirschmanniella oryzae	39.4	-	-	-	-	-	-
Globodera rostochiensis	-	13.4	-	-	-	-	-
Pratylenchus	1.1	0.5	-	2.1	3.1	-	0.4
Longidorus	11.8	-	1.1	-	-	6.5	-
Trichodorus	0.4	-	0.4	-	-	-	-

environmental hazards of pesticides, it has become essential to direct research on nematode control towards more environmentally sound methods.

Acknowledgements

Most of the facts and evidence in this report have been obtained from H M R K Ekanayake, Nematologist, Horticulture Research and Development Institute, Department of Agriculture, Peradeniya, Sri Lanka; and Yokio Toida, Japan International Research Centre for Agricultural Sciences, Tsukuba, Ibaraki, Japan.

Status of Nematode Problems and Research in Nepal

P B Karki[1]

Introduction

Nepal, a small land-locked country, is located between latitudes 26°22' to 30° 27'N and longitudes 80°12'E. The total area of the country is 147 181 km^2 with an estimated population of about 20 million people. Of the total land area, only 18% is arable.

Nepal is an agricultural country. This sector provides employment to about 90% of the population, and contributes about 60% to the gross domestic product. There is a wide variation in climate, ranging from alpine to cool temperate in the North, to subtropical in the South. This dramatic variation enables farmers to cultivate many species of crops.

Rice (*Oryza sativa*), wheat *(Triticum aestivum*), and maize (*Zea mays*) are the three most important crops of Nepal. Collectively, grain legumes occupy fourth place, both in terms of area and production. Among the grain legumes, lentil (*Lens culinaris*) is the major crop in Nepal.

The productivity of crops grown in Nepal is very low. Diseases are one of the major constraints to increased crop production in the country.

Plant Parasitic Nematodes Occurring in Nepal

A total of 1100 plant diseases have so far been reported from Nepal; about 91% are caused by fungi and the remaining 9% by nematodes, bacteria, and viruses (Amatya et al 1989). Most pathological work has concentrated on survey and identification until 1972. After the establishment of several commodity programs in different parts of the country in 1972, pathological work was strengthened, and now covers other areas such as loss assessment, host-plant resistance, and other control practices. However, even at the present time, most research work is concentrated on fungal diseases of major crops.

1. Grain Legumes Research Program, Rampur, Chitwan, Nepal.

Karki, P.B. 1997. Status of nematode problems and research in Nepal. Pages 57-60 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Limited surveys have led to the identification of 45 plant parasitic nematode species belonging to 24 genera (Amatya and Shrestha 1969; Bhatta 1967; Hogger 1981; Manandhar and Amatya 1987; Sharma et al 1990). Of the total genera, only *Aphelenchoides, Anguina, Hirschmanniella, Meloidogyne,* and *Tylenchulus* have been frequently reported by several authors. The well-established crop diseases induced by nematodes are presented in Table 1. Of them, *Meloidogyne* is the most important nematode and is a serious threat to such crops as tomato *(Lycopersicon esculentum),* chilli *(Capsicum frutescens),* okra *(Hibiscus esculentus),* brinjal *(Solanum melongena),* chickpea *(Cicer arietinum),* and cowpea *(Vigna unguiculata)* in all areas where they are grown.

Research on Nematodes in Nepal

There are very few reports on host-nematode relationships and chemical control. Chickpea, pigeonpea *(Cajanus cajan),* and tomato varieties were screened against *Meloidogyne* at Rampur, Chitwan, and some varieties with moderate resistance were reported (Manandhar and Amatya 1987). However, the research was not systematic and the identified resistant materials have not been utilized. Insecticides such as carbofuran, aldicarb, etc., are widely used in vegetables, and are reported to control nematodes also.

Table 1. Important diseases of crops in Nepal caused by nematodes.

Disease	Nematode	Important host	Distribution	Status/ prevalence
Ear Cockle	*Anguina tritici*	Wheat	Central and eastern Terai	Major/sporadic
White Tip	*Aphelenchoides besseyi*	Rice	Kathmandu	Major/sporadic
Root disease	*Hirschmanniella oryzae*	Rice	All areas	Minor/common
Root-knot	*Meloidogyne arenaria*	Cowpea and tomato	All area	Minor/common
Root-knot	*Meloidogyne hapla*	Tomato	High hills	Minor/common
Root-knot	*Meloidogyne incognita*	Brinjal, chickpea, chilli, cowpea, okra, and tomato	All areas	Major/common
Root-knot	*Meloidogyne javanica*	Brinjal, chickpea, chilli, cowpea, okra, and tomato	All areas	Major/common
Slow decline	*Tylenchulus semipenetrans*	Citrus spp	All areas	Major/common

Institutional Development

The Division of Plant Pathology of the Nepal Agriculture Research Council (NARC) is the main organization involved in research with plant diseases. The division, which was established in 1954, started active research only in 1963. Later, smaller plant pathological units were created in different agriculture farms/stations/commodity programs scattered in different agroecological regions of the country. The Institute of Agriculture and Animal Science (IAAS), Department of Botany and Institute of Science and Technology of Tribhuwan University, Royal Nepal Academy of Science and Technology, and the Royal Botanical Garden also carry out some research on crop diseases.

The plant pathological technologies generated by the above organizations are disseminated to farmers through the regional and district-level offices of the Department of Agriculture.

Human Resources and Physical Facilities

Lack of trained personnel is the major constraint to increased and improved plant nematology research in Nepal. To the extent of my knowledge, at present there is only one scientist who has specialized in plant nematology, at NARC. However, he is involved mainly with fungal diseases and administration. There is no nematology laboratory in any of the organizations under NARC. There is one nematologist at the Institute of Agriculture and Animal Science, who carries out some research on plant nematology, but he lacks a well-equipped laboratory.

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Status of Nematode Problems and Research on Pulses in Bangladesh

Ashraf Uddin Ahmed[1]

Introduction

Major pulses grown in Bangladesh are grass pea *(Lathyrus sativa),* lentil *(Lens culinaris),* chickpea *(Cicer arietinum),* mungbean *(Phaseolus aureus),* and blackgram *(Phaseolus mungo).* Among these chickpea is the third major pulse after grass pea and lentil, occupying 10% of total area cultivated to pulses. Only 1% of the area is cultivated to pigeonpea *(Cajanus cajan).* Usually pigeonpea is cultivated as a very minor crop around the homestead in Bangladesh. Pulse crops suffer from a number of diseases caused by fungi, bacteria, nematode, virus, and mycoplasma.

Plant Parasitic Nematodes Occurring in Bangladesh

According to Bakr (1994), chickpea suffers from 13 diseases and pigeonpea suffer from 11 diseases. Among these diseases, nematode is one of the most important causal agents. Nematodes that have been identified and associated with pulse crops in Bangladesh are presented in Table 1.

Control Measures

There are a number of management approaches, either chemical, cultural, physical or biological that could be utilized for nematodes on pulses in Bangladesh, but so far have not been. However, literature has revealed the following possible measures that could be taken to control nematode pests of pulses.

1. Pulses Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur 1701, Bangladesh.

Ashraf Uddin Ahmed. 1997. Status of nematode problems and research on pulses in Bangladesh. Pages 61-65 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Table 1. Nematodes associated with pulse crops in Bangladesh.

Host plant	Nematodes
Chickpea (*Cicer arietinum*)	*Meloidogyne incognita, M. javanica, Tylencorhynchus* sp
Cowpea (*Vigna unguiculata*)	*Helicotylenchus indicus, M. incognita, M. javanica*
Grass pea (*Lathyrus sativus*)	*Helicotylenchus indicus, M. incognita, M. javanica, Xiphinema index*
Lentil (*Lens culinaris*)	*Hoplolaimus* sp, M. *incognita, M. javanica, Pratylenchus penetrans*
Mungbean (*Phaseolus aureus*)	*Aphelenchoides* sp, *Helicotylenchus indicus, M. incognita, M. javanica, Xiphinema* sp
Blackgram (*Phaseolus mungo*)	*Aphelenchoides* sp, M. *incognita, M. javanica, Xiphinema index*
Pea (*Pisum sativum*)	*Helicotylenchus dihystera, M. incognita, Pratylenchus* sp
Pigeonpea (*Cajanus cajan*)	*Helicotylenchus indicus, Tylenchus* sp

Source: Mian 1986

Chemical control. Nematode diseases can be controlled chemically. Both fumigants and non-fumigant nematicides are effective to control the nematode pests of pulse crops.

Crop rotation of non-host plants. *Meloidogyne incognita,* a common and serious nematode pest of most of the pulses can be effectively controlled by growing non-host crops for 2 consecutive years followed by a host crop. Many other nematode species can also be controlled by crop rotation of non-host plants, in addition to *Meloidogyne.*

Sanitary practices. This includes cleaning of machinery, keeping land fallow, avoiding of use of contaminated seed, nursery stock, and containers, etc.

Flooding. Flooding of the land for a period of several months results in the death of the nematodes and thus frees the land from the pathogen.

Predators. It has been found that many nematophagous species of fungi are effective means of destroying the nematodes.

Antagonistic plants. Trap crops, resistant varieties, and antagonistic plants that release nematicidal root exudates may be considered as useful or potential for biological control of nematode pests.

Host plant resistance. Varietal resistance is, perhaps, the best measure against nematode pests. Although much work has been done in this area, this type of management approach is currently not being utilized for pulse crops in Bangladesh.

Future Research Needs

Survey of Nematodes Pests of Pulse Crops

In Bangladesh, emphasis has been placed on fungal diseases of different pulse crops. Nematode-induced diseases have received little attention. In this context, a survey is urgently needed to determine the prevailing nematode pests and their incidence on pulse crops, with special emphasis on chickpea and pigeonpea, which will enable formulation of possible management practices that will allow better yields.

Screening of Pulse Genotypes for Resistance Against Major Nematode Pests

Cultivation of resistant varieties is the best approach to control diseases. The literature indicates that a number of varieties/lines have been found resistant/ tolerant to different nematodes on chickpea, mungbean, blackgram, pigeonpea, and cowpea. But in Bangladesh there has been very little work on resistance screening. So, screening against important nematode pests will be helpful to select/detect the resistant varieties from exotic and local sources that are available.

Search for Low Cost Management Approaches

Bangladesh is a developing country. The development of low cost management approaches is a high priority for this country. A review of the literature has illus-trated that nematode pests such as the root-knot nematode can be controlled by growing resistant cultivars, by using chemicals, by manipulating cultural practices, and by adopting biological control on different pulse crops. In Bangladesh, work on different management approaches against nematode diseases of pulses has been conducted, but most of these studies were

preliminary and inconclusive. Studies will be initiated to select different management practices and to finally formulate integrated disease management practices to combat nematode diseases.

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Appendix 1. Personnel engaged in Hematology research in Bangladesh.

Name	Organization
Md Shahidul Haque	Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur
	Institute of Postgraduate Studies in Agriculture (IPSA), Salna, Gazipur
Main Uddin Ahmed	Bangladesh Agricultural University (BAU), Mymensingh
Md Abdul Latif	Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur
Md Iqbal Hussain	Bangladesh Sugarcane Research Institute (BSRI), Ishurdi
Md Arman Haider	Bangladesh Jute Research Institute (BJRI), Dhaka

Status of Nematode Problems and Research in Pakistan

S Hussain and B A Malik[1]

Introduction

Pests and diseases are important constraints on crop production in most countries including Pakistan. When compared with insect pest and fungal diseases, very little attention has been given to nematode problems. Plant parasitic nematodes have been recognized as one of the most important constraints to crop production throughout the world. Nematodes alone or in combination with other microorganisms have been found to attack almost every part of the plant including the root, stem, leaves, fruits, and seeds. They cause damage on a great variety of pulses, cereals, vegetables, fruits, and ornamental and fiber crops.

In Pakistan, a number of institutes are involved in nematological research work, such as the National Nematological Research Centre (NNRC), University of Karachi; Pakistan Council for Scientific and Industrial Research (PCSIR), Applied Biology Division, Nematology Laboratory, Karachi; Sindh Agriculture University, Tandojam; Crops Diseases Research Institute (Pakistan Agricultural Research Council), Karachi and Islamabad; University of Agriculture, Faisalabad, and University of Peshawar. At present only 12 qualified persons are exclusively involved in different institutions to work on nematode problems in different parts of the country (Maqbool 1992).

Plant Parasitic Nematodes Occuring in Pakistan

Since the establishment of Pakistan in 1947, plant parasitic nematodes and the diseases caused by them have been recognized as one of the most serious problems in agricultural crops. During pre-partition, the diseases caused by nematodes received very little attention due to lack of trained personnel, appropriate laboratory facilities, and information on the magnitude of crop losses, as well as preoccupation by scientists with research on fungal diseases. In the early 1950s studies on nematodes were initiated in Pakistan by Sattar and Hafeez (1952), who reported *Anguina tritici* from D G Khan, Muzaffargarh,

1. Pulses Program, NARC, Pakistan Agricultural Research Council, Islamabad, Pakistan.

Hussain, S., and Malik, B.A. 1997. Status of nematode problems and research in Pakistan. Pages 66-73 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

and Jhang areas of Pakistan. Since then, work on nematodes has received some attention, mostly on soil analysis leading to the recording and identification of plant parasitic nematodes and compilation of systematic lists. Some systematic studies were initiated in former East Pakistan (now Bangladesh) from 1955 to 1963, on plant and soil nematodes. Timm (1956) and Timm and Ameeti (1960) listed important nematodes associated with commercial crops in former East Pakistan. Akhtar (1962) reported 59 different genera of nematodes collected from soil around roots of 10 different plants.

A list of 20 genera and 70 species of plant parasitic nematodes of Pakistan along with their hosts were reported in a technical document of the Food and Agriculture Organization of the United Nations by Kafi (1963). Similarly Kamal and Moghal (1968) published a list of plant parasitic nematodes of Sindh. Eleven genera of nematodes were reported from soil around the roots of citrus nursery plants (Ashraf 1969) and 15 genera of nematodes from the soil around cotton *(Gossypium herbaceum)* roots (Wahid 1972). Saeed and Ashrafi (1973) reported the occurrence of 10 plant parasitic nematodes in association with new host plants from Karachi and its adjoining areas. Anwar and Chaudhry (1973) and Anwar et al (1973a, b, c, d, e, f; 1975a, b, and 1976) reported several plant parasitic nematodes associated with sugarcane *(Saccharum officinarum),* eggplant *(Solanum melongena),* maize *(Zea mays),* sorghum *(Sorghum bicolor),* banana *(Musa* spp), rice *(Oryza sativa),* chilli *(Capsicum frutescens),* tomato *(Lycopersicon esculentum),* mango *(Mangifera indica),* and citrus *(Citrus* spp).

Extensive surveys of the nematode fauna associated with cereals, vegetables, fruits, and other crops in the Sindh, Punjab, Balochistan, and North West Frontier Province were carried out by National Nematological Research Centre stationed at University of Karachi (Maqbool 1988; Maqbool and Shahina 1988; Maqbool and Zarina 1988; Firoza et al 1990). As a result of these investigations the present identified nematode fauna of Pakistan consists of 232 species belonging to 60 genera, 36 subfamilies, 21 families, 9 super families, 3 suborders, and 3 orders. It includes 173 known nematode species, 59 new species, 60 known genera, and 2 subgenera recorded from Pakistan.

Important Nematode Species of Pakistan

There are a number of nematode species on which some studies have been made in Pakistan (Table 1). Ear Cockle of wheat *(Triticum aestivum),* one of the most important diseases, caused by *Anguina tritici,* was investigated in the early 1930s.

Control Measures

Several scientists have worked on different aspect for the control of nematodes. Khan et al (1987) and Maqbool and Hashmi (1986) tested crop rotation on selected plant parasitic nematodes species. Similarly, pre-plant application of

Table 1. A list of economically important nematodes in Pakistan, number of species, and hosts.

Nematode	Number of species	Host
Ditylenchus	4	Citrus, sugarcane, maize, wheat, onion, and potato
Heterodera	9	Citrus, sugarcane, maize, potato, soybean, and tobacco
Hirschmanniella	5	Rice and aquatic grasses
Meloidogyne,	4	Cereals, fruits, pulses, and many vegetables
Pratylenchus	13	Cereals, fruits, and vegetables
Rotylenchulus	3	Soybean, potato, citrus, cotton, banana, and okra
Trichodorus	1	Mulberry, maize, and citrus
Tylenchulus	1	Citrus
Xiphinema	6	Banana, grapes, citrus, and wheat

dichloropropane dichloropropene (DD) and ethylene dibromide (EDB) was used for cyst nematodes, and dibromochloropropene (DBCP) for root-knot and ring nematodes of sugarcane, reniform nematodes on papaya *(Carica papaya),* and stem nematode on rice (Brown 1962). Certain pesticides have been developed for the control of plant parasitic nematodes at PCSIR Laboratories, Karachi (Qamar et al 1985). Six aromatic fraction of petroleum were used against *Helicotylenchus* sp (Ashrafi et al 1970). Petkolin, a chlorinated petroleum product, considerably decreased populations of plant parasitic nematodes (Ahmed et al 1975). On tobacco plants different nematicides were used for the control of *Meloidogyne* sp (Mohibullah et al 1980). Aldicarb and carbofuran significantly reduced populations of *Quinisulcius solani* on potato *(Solanum tuberosum)* (Maqbool et al 1984), *Meloidogyne* sp, on cauliflower *(Brassica oleracea)* (Maqbool et al 1985), *Helicotylenchus digonicus, H. indicus, Paratrichodorus mirzae, Tylenchorhynchus annulatus, Pratylenchus zeae,* and *Xiphinema* spp on sugarcane (Maqbool and Hasmi 1987a), T. *semipenetrans* on citrus (Maqbool and Hashmi 1987b), *Heterodera zeae* on maize, and *H. avenae* on barley *(Hordeum vulgare)* (Shahina and Maqbool 1991).

In view of the high cost and environmental hazard, scientists are giving some attention to biological agents for the control of nematodes (Maqbool and Zaki 1990; Gowen and Ahmed 1990). *Pasteuria penetrans* and *Paecilomyces lilacinus have been used* as biocontrol agents for the control of nematodes on different crops (Shatzad and Ghaffar 1988; Shahzad et al 1990; Saifullah and

Gul 1991; Zaki and Maqbool 1991a and b). Similarly, certain plant extracts such as *Calotropis procera, Euphorbia caducifolia, Nerium oleander,* and *Azadirechta indica* also showed some inhibitory effect on root-knot juveniles (Maqbool et al 1987; Qamar et al 1989; Zureen and Khan 1984). Some other plant extracts, oil cakes, or dried powder also showed significant results against root-knot nematodes (Abid and Maqbool 1991; Gul et al 1991).

Conclusions

In Pakistan, a smaller number of nematologists are involved in the management of nematodes. However, most of the surveys and identifications have been conducted on cereals, fruits, and vegetables. Chickpea *(Cicer arietinum)* is a major pulse crop in Pakistan, cultivated on 70% of the total area sown to legumes, and is the only winter crop that is successfully grown in the vast sandy barren (rainfed) tracts of Thal in Punjab and northern areas of Pakistan. Despite this, very little attention has been given to researching nematode problems in chickpea. Only two species, *Melodogyne incognita* and *Melodogyne javanica,* have been reported on chickpea. Therefore, an extensive survey is needed to determine distribution of nematodes, their races, biology, host-parasite relationship, and yield losses of chickpea under different environmental conditions and locations.

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Status of Nematode Problems and Research in India

S S Ali[1]

Introduction

Agriculture forms one of the major sectors of the Indian economy; 70% of the population is dependent upon it for livelihood and it contributes over 40% of gross national production. Great strides have been made in agricultural production that have helped transform agriculture from a subsistence level into a commercial farming system. This new commercial farming system has brought a number of pests and diseases that are of national importance by their very nature. Polyphagous destructive nematode pests have become one of the biggest constraints to production of commercial crops. The science of nematology has developed only during the last 35 years in India. The importance of nematodes as a constraint to successful crop production was first realized with the prevalence of the cyst nematodes on wheat (*Triticum aestivum)* in 1958 and potato *(Solanum tuberosum)* in 1961. Since then, a number of nematode problems of national importance have emerged (Tables 1 and 2). Root-knot nematodes are prevalent in 90% of agricultural crops and are considered to be the number one problem. Cyst-forming nematodes are restricted to wheat, barley *(Hordeum vulgare),* rice *(Oryza sativa),* maize *(Zea mays),* sorghum *(Sorghum bicolor),* potato, and pulses. Reniform and lesion nematodes are associated with a wide range of agricultural crops. Burrowing, citrus, wheat gall, rice, and mushroom nematodes are major constraints on a regional basis. These nematode pests have assumed a highly damaging role on a variety of crops, comprising cereals, vegetables, fruits, pulses, oilseeds, fiber, plantation, spices, horticultural, medicinal, aromatic, ornamental, and forest crops. Based on surveys undertaken and research work done on different agricultural crops in India, nematode problems may be categorized in the following manner.

1. Indian Institute of Pulses Research, Kanpur, Uttar Pradesh 208 021, India.

Ali, S.S. 1997. Status of nematode problems and research in India. Pages 74-82 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Table 1. Estimated, yield losses due to nematodes in India.

Crop	Nematode	Annual loss (Rs)	State
Wheat	*Heterodera avenae*	40 x 10^6	Rajasthan
Wheat	*Anguina tritici*	75 x 10^6	Rajasthan
Barley	*H. avenae*	30 x 10^6	Rajasthan
Coffee	*Pratylenchus coffeae*	20 x 10^6	Karnataka and Kerala

Table 2. Avoidable yield losses due to nematodes in India.

Crop	Nematode	Yield loss (%)	Location
Root-knot Nematodes			
Vegetables			
Brinjal	*Meloidogyne incognita*	27-70	Uttar Pradesh, Himachal Pradesh, Haryana, Maharashtra, Rajasthan
Tomato	M. *incognita*	46-70	Himachal Pradesh, Rajasthan, Maharashtra
Okra	*M. incognita*	28-90	Uttar Pradesh, Haryana, Karnataka
Bittergourd	*M. incognita*	37	Maharashtra
Oilseeds			
Groundnut	M. *arenaria*	59	Gujarat
	M. javanica	38-54	
Sunflower	M. *incognita*	38-84	Rajasthan
Pulses			
Urdbean	*M. incognita*	23-49	Uttar Pradesh, Madhya Pradesh, Gujarat
	M. javanica	26	Uttar Pradesh

continued

***Table 2** continued*

Crop	Nematode	Yield loss (%)	Location
Mungbean	M. *incognita*	18-65	Uttar Pradesh, Madhya Pradesh, Assam, Orissa Maharashtra, Rajasthan, Gujarat
	M. javanica	23-49	Uttar Pradesh, Gujarat
Cowpea	*M. incognita*	31-71	Assam, Gujarat, Rajasthan, Karnataka
French bean	*M. incognita*	36-43	Karnataka, Madhya Pradesh
	M. *javanica*	30-40	Uttar Pradesh
Field pea	M. *javanica*	20-28	Uttar Pradesh
Chickpea	*M. javanica*	22-84	Uttar Pradesh, Haryana
	M. incognita	25-60	Haryana, Uttar Pradesh, Gujarat, Rajasthan, Maharashtra
	M. arenaria	30	Rajasthan
Lentil	*M. javanica*	16	Uttar Pradesh
Pigeonpea	*M. incognita*	25-31	Uttar Pradesh, Bihar, Gujarat, Maharashtra
Others			
Rice	*M. graminicola*	21-64	Orissa
FCV tobacco	*M. incognita*	59.4	Karnataka
Cardamon	*M. incognita*	32	Karnataka
Chillies	*M. incognita*	19-70	Rajasthan, Bihar, Maharashtra
Jute	M. *incognita*	81	Assam
Ginger	M. *incognita*	51-74	Himachal Pradesh, Kerala
Turmeric	*M. incognita*	31	Himachal Pradesh
Cyst Nematodes			
Wheat	*Heterodera avenae*	50-100	Rajasthan
Barley	H. *avenae*	87-100	Himachal Pradesh, Punjab, Haryana, Jammu & Kashmir, Rajasthan

continued

Table 2 continued

Crop	Nematode	Yield loss (%)	Location
Rice	*H. oryzicola*	21-42	Kerala
Pigeonpea	*H. cajani*	16-34	Uttar Pradesh, Bihar, Andhra Pradesh
Potato	*Globodera pallida*	99	Tamil Nadu
Reniform Nematodes			
Finger millet	*R. reniformis*	4-14	Karnataka
Cowpea	*R. reniformis*	8-13	Karnataka
Lentil	R. *reniformis*	15	Madhya Pradesh
Pigeonpea	*R. reniformis*	16-19	Uttar Pradesh, Madhya Pradesh
Chickpea	*R. reniformis*	11-18	Madhya Pradesh, Uttar Pradesh
Maize	*R. reniformis*	6	Andhra Pradesh
Parmal	*R. reniformis*	48	Uttar Pradesh
Lesion Nematodes			
Rice	*Pratylenchus indicus*	18-55	Orissa
Sugarcane	*Pratylenchus* spp	15-16	Tamil Nadu
Coffee	*P. coffeae*	5-75	Karnataka
Chickpea	*P. thornei*	25-30	Madhya Pradesh

Nematode Problems at the National Level

Root-knot Nematodes

Meloidogyne spp, causing root-knot disease, are the most serious nematode problems on agricultural crops in India. Root-knot nematodes are a constraint to production of various crops and are widely distributed in all zones of the country. Eleven species of root-knot nematode have been reported; out of these, *M. incognita* and *M. javanica* are found on the majority of agricultural crops in nearly every state, whereas *M. graminicola* is specific to rice and restricted to only a few states. *Meloidogyne arenaria* infects only a few selected crops such as chickpea *(Cicer arietinum)* and groundnut *(Arachis hypogaea).* With different

agroclimafic conditions and varied cropping patterns, three physiological races of *M. incognita* and two races of *M. javanica* are prevalent. Race 1 of *M. incognita* is dominant in all the agroclimatic zones irrespective of *h*ost. Race 2 is present only in Karnataka and race 3 is present in both Tamil Nadu and Karnataka. Altogether, 232 plant species have been reported as hosts for *M. incognita* and 144 for *M. javanica* in India. Investigations have been made on host-parasite relationships, distribution and prevalence, yield losses, host range, interactions, biology, chemical Control, and host resistance.

Cyst Nematodes

Cyst nematodes are an important problem on many agricultural crops throughout the country. Sixteen species of *Heterodera* and two species of *Globodera* have been recorded in India. Several species of cyst nematodes cause considerable damage on a wide range of crops such as wheat, rice, maize, sorghum, potato, pigeonpea, mungbean, urdbean *(Phaseolus mungo),* chickpea, cowpea, and sesame *(Sesamum indicum).* Research on cyst nematodes has gained momentum in the last decade. Country-wide surveys for cyst nematodes on pigeonpea, potato, wheat, sorghum, rice, and bean were carried out to determine the magnitude of the problems in India. Despite the national importance of this group of nematodes, there have been only few reports mentioning the enormity of yield losses they cause. However, host-parasite relationships, biology, host range, management options, screening, etc., have been investigated in depth.

Lesion Nematodes

Lesion nematode problems in India have been identified on vegetables, coffee *(Coffea arabica),* plantation, pulses, and oilseed crops. Lesion nematodes have gained importance due to their wide host range and their migratory nature, which keeps the host plant more vulnerable to other pathogens due to extensive necrosis. While lesion nematode problems in India have not been given the attention they deserve, investigations have been done on their pathogenicity, host range, and, to some extent, distribution of the various species. In India, the role of *P. coffeae* in the decline of coffee bushes has been thoroughly investigated. Out of 36 species that have been reported from India on a number of agricultural crops, P. *thornei* is a serious problem on chickpea in Madhya Pradesh, which is the largest chickpea producing State of India. *Pratylenchus indicus* attacks rice, tomato *(Lycopersicon esculentum),* pulses, ragi *(Eleusine coracana),* and wheat, while P. *zeae* attacks maize, sugarcane, sorghum, mungbean, and groundnut.

Reniform Nematode

Rotylenchulus reniformis is one of the major nematode problems in India, where two races (A and B) have been shown to exist Investigations on its biology, pathogenesis, interactions with other organisms, and population management have been carried out in selected crops.

Nematode Problems at the Regional Level

White Tip

This disease is due to the infection of *Aphelenchoides besseyi.* This nematode is prevalent in all rice tracts except in the drought-prone areas of Karnataka and Rajasthan. Yield losses are caused by improper filling of kernels (21-46%) and sterility (20%). Nematode management and cultivation of such resistant varieties as Ratna, TKM 5, IR 5, Yamuna, Vijaya, Sona, IR 30, and TKM 9 controls the White Tip disease problem.

Ufra Disease

Ditylenchus angustus is the casual organism of Ufra disease or *Dak Pora.* Losses due to disease range from 10% in West Bengal to 50%. in Uttar Pradesh. Management strategies for this nematode have been developed.

Hirschmanniella spp

The rice-root nematodes are also widely distributed in rice tracts and cause considerable yield losses in rice fields.

Citrus Decline

Tylenchulus semipenetrans is the causal organism of slow decline of citrus. Disease management of T. *semipenetrans* has resulted in a 44% increase in yield. *Poncirus trifoliata* has been found to be highly resistant to the nematode. Application of carbofuran or phorate at 6 kg a.i ha^{-1} to sweet orange trees has been found very promising.

Ear Cockle Disease

Anguina tritici is present in the wheat-growing tracts of India and nematode management is now being practiced.

Tundu Disease

Anguina tritici acts as a vector for *Corynebacterium tritici* and results in Tundu disease. Control measures for Tundu disease have been developed.

Burrowing Nematodes

Radopholus similis causes reduction in yield in coconut *(Cocos nucifera),* arecanut *(Areca catechu),* pepper *(Piper nigrum),* and banana *(Musa* spp). Two biotypes of *R. similis* have been reported from India. Nematode management strategies have been developed and a resistant cultivar in coconut is also available.

Mushroom Nematodes

Ditylenchus destructor, D. *myceliophagus, Aphelenchoides compositicola, A. sacchari, A. saprophilus,* and *A. asterocaudatus* affect mushroom yields in the country. *Aphelenchoides compositicola* has been responsible for yield losses of 26-30%, *A. sacchari* for about 100%, and D. *myceliophagus* for about 50-100%. A nematode management schedule has been developed to check the disease.

Stunt, Spiral, and Lance Nematodes

Helicotylenchus spp, *Tylenchorhynchus* spp, and *Hoplolamius* spp have been reported to cause considerable damage to the sugarcane industry in Tamil Nadu and Uttar Pradesh. The total crop losses due to nematodes have been estimated between 16% and 32% in the plant crop and up to 76% in ratoon crops. Stunt nematodes also attack maize and groundnut, causing considerable yield loss to both crops.

Host-plant Resistance

A number of cultivars and landraces of several crops have been screened against the four major nematodes prevalent in India to identify sources of resistance, some of which are given in Table 3. Attempts have been made to integrate methods for the management of nematodes in various crop species, and appreciable success has already been achieved for targeted nematode species. Cultural methods, crop rotation, organic amendments, organic fertilizers, and biological management tactics have given encouraging results. The present status of nematode research in India is due to the dedicated work done by over 400 nematologists during the last 25-30 years in this century.

Table 3. Crop cultivars/lines found resistant to nematodes in India.

Crop	Nematode	Resistant cultivars/lines
Root-knot Nematodes		
Potato	*M. incognita*	HC 294, A 2708,
Brinjal	*Meloidogyne* spp	Vijay, Black Beauty, Banaras Giant
Capsicum	*M. incognita*	G4, Mirch 1, Red Long, NP 46A CA(P) 63
Tobacco	*Meloidogyne* spp	Speight, G 28
Rice	*M. graminicola*	TKM 6, Basanti, IR5-47-2, Manoharshli, IR 5-47
Tomato	*M. incognita*	SL 120, Punjab NN-7, Hisar Lalit
Cowpea	*M. incognita*	Cowpea 1, C 152, P 1327, EC 107 163, RS 9
	M. javanica	HFC 12, HFC 311, C 28, RS 9
Pea	*M. incognita*	T44
	M. javanica	DMR 7, HFP 4, KEP 130, KFPD 46
Lentil	*M. incognita*	DPL 14
	M. javanica	DPL 14, PL 81-D, PL81-340
Urdbean	*M. javanica*	TPU 3, WBU 105
Mungbean	*M. javanica*	GM 85-2, ML 323
Chickpea	*M. javanica*	BG 369, BGM 481, GL 88341, GMS 815
	M. incognita	BGM 481, BGM 483, G 288341
Pigeonpea	*M. incognita*	Pusa 23, GAUT 87-2
	M. javanica	H 82-1, 86-1, IPH 732, TH 9, UG 218
Reniform Nematodes		
Tomato	*R. reniformis*	Punjab NR-7, Hisar Lalit
Chilli	*R. reniformis*	Pusa Jwala
Cowpea	*R. reniformis*	Cowpea 1

continued

Table 3 continued

Crop	Nematode	Resistant cultivars/lines
Pigeonpea	*R. reniformis*	ICP 12744, Basant, Prabhat, UPAS 120
lesion Nematodes		
Coffee	*Pratylenchus coffeae*	S 2267
Rice	*P. indicas*	TNAU 6464, CR 115, CR 5029, CR 216 CRM B, CRM 3241, CRHP 1
Chickpea	*P. thornei*	GNG 543, GF 88428, PKG 24
Cyst Nematodes		
Barley	*Heterodera avenae*	Rajkiran
Potato	*Globodera* sp	Kufri Swarna
Pigeonpea	*H. cajani*	H82-1, H86-1, IPH 732

IV. Nematology in India

Development of Phytonematology in India

S P Tiwari[1]

Phytonematology is an important crop protection science in India. The root-knot nematodes *(Meloidogyne* spp) were the first plant parasitic nematodes associated with horticultural crops in southern India. Later, a new nematode disease of rice known as Ufra disease was reported in Bengal in 1913. In 1936, another disease of rice was reported from the Central Provinces of India, caused by a foliar nematode *(Aphelenchoides* sp). Incidences of plant parasitic nematode on economically important crops of India continued to be frequently reported. Nematologists began to take a scientific approach to investigating the damage to plants caused by nematodes, and started work on different aspects of applied phytonematology. With the occurrence of *Globodera rostochiensis* in the Nilgiri Hills of Tamil Nadu, a separate section for nematology was established in the Department of Entomology, Coimbatore, in 1961. Later, in 1964, nematology was added to the Divisions of Entomology and Plant Pathology, separately, at the Indian Agricultural Research Institute (IARI), New Delhi, and the first international course on nematology was organized at IARI. Further rapid growth and development of nematology took place between 1964 and 1977.

Some highlights of the development of this science in India include:

1. The first postgraduate nematology course in Southeast Asia was held at Aligarh Muslim University, Aligarh, and IARI, New Delhi. Subsequently, seven batches of postgraduate students completed the course under the guidance of nematologists from India and abroad.

2. Pioneering work on nematode surveys and taxonomy was done by scientists in Aligarh, Hyderabad, and New Delhi.

3. In 1977, a research plan was put into action at 14 agricultural universities as the All India Coordinated Research Project of Plant Parasitic Nematodes and their Control. The work centered on applied aspects of the root-knot,

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reniform, cyst, root-lesion, and burrowing nematodes. The project was further strengthened in the sixth, seventh, and eighth 5-year plans. Multilocational trials and management of the pest locally associated with economically important crops were facilitated by addition of more nematology centers from different agricultural universities. From 1972 onward, separate departments of nematology were established at many agricultural universities throughout the country

4. Nematode identification services became available at IARI, New Delhi; Aligarh Muslim University, Aligarh; the Zoological Survey of India, Calcutta; and Haryana Agricultural University, Haryana. Various universities developed information on crop losses by *Aphelenchoides besseyi, Anguina tritici, Heterodera avenae, H. zeae, H. cajani, Hirschmanniella oryzae, Pratylenchus* spp, *Radopholus similis, Meloidogyne* spp, *Rotylenchulus reniformis, Tylenchulus semipenetrans,* etc.

5. MSc and PhD courses on plant parasitic nematodes began for the first time at IARI in 1969, The first All India Nematology Symposium was held at IARI; the Nematological Society of India was founded and publication of the Indian Journal of Nematology started at the Division of Nematology, IARI. Subsequently, scientists working on different aspects of nematology began to publish their research in national and international journals. Research by national scientists has eventually comprised over 20% of all work published on nematology.

6. The Indian Council of Agricultural Research (ICAR), Department of Science and Technology, Department of Biotechnology, and other government agencies have provided support for research on plant parasitic nematodes associated with food and fiber crops.

7. In 1985 a separate scientific panel for nematology was constituted by ICAR to provide a systematic approach for nematode control.

8. The presence of several highly pathogenic species of *Meloidogyne, Heterodera,* and other plant parasitic nematodes has been detected in India. In 1991, a new multi-center activity on integrated approaches to management of nematodes was initiated. Genotypes of economically important crops with resistance to nematodes have been identified and developed at the agricultural universities and ICAR institutes. New methods such as soil solarization (mulching with polythene tarps during the hot summer season) have shown promise for management of nematode-caused damage and enhanced crop production.

Status of Nematode Problems and Research in Karnataka

B M R Reddy[1]

Introduction

The University of Agricultural Sciences, Bangalore, started a nematology section in 1968 as part of the Department of Plant Pathology. Consequently, a research laboratory with basic facilities for work on plant nematology was set up. The university was selected as an advanced center in Plant Protection by the United Nations Development Program (UNDP) and the Indian Council of Agricultural Research (ICAR), to develop and strengthen postgraduate agricultural education and research in Plant Protection, including Plant Nematology. Since then, extensive research programs have been undertaken on all aspects of the root-knot nematodes, with the goal of developing this laboratory as a center of research on root-knot nematodes in India. Three scientists have received advanced training in nematology in the UK and USA. Two scientists have participated in the International Workshop on root-knot nematodes at North Carolina State University, Raleigh, USA. At present, four nematologists are working in the university; three are located at Bangalore and one at the Regional Research Station, Shimoga. Six additional staff members, specialized in nematology, are working in the university under different programs.

Status of Research

Surveys

Extensive surveys have revealed association of 15 genera of plant parasitic nematodes with fruit and plantation crops. Surveys in groundnut *(Arachis hypogaea)-growing* districts of Karnataka revealed that populations of

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Reddy, B.MR. 1997. Status of nematode problems and research in Karnataka. Pages 87-91 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Rotylenchulus reniformis and *Helicotylenchus multicinctus* were widespread. On sunflower *(Helianthus annuus), R. reniformis* and *Meloidogyne incognita* were predominant. *Radopholus similis* and H. *mutticinctus* were the major nematode pests of banana (Musa spp). On grapes (Vitis *vinifera),* H. *mutticinctus, R. reniformis, Tylenchorhynchus dubius,* and M, *incognita* were the major pests. *Heterodera cajani* was widespread in the northern districts of Karnataka (Table 1). In chickpea *(Cicer arietinum),* the association of *M. incognita* with *Fusarium oxysporum f.* sp *ciceri* was recorded in certain regions of Karnataka. *Heterodera gambiensis* on ragi *(Eleusine coracana)* is reported for the first time from India.

Pathogenicity and Yield Loss

Concomitant inoculation of T. *dubius* and *Helicotylenchus crenatus* on their population buildup in tomato *(Lycopersicon esculentum)* showed that T. *dubius* was the more vigorous of the two and reduced the population of *H. crenatus.*

Yield losses from root-knot nematodes were estimated at 39% in brinjal *(Solanum melongena),* 31% in tomato, 33% in okra *(Hibiscus escutentus),* and 16% in sunflower. *Heterodera cajani* was found to cause a yield loss of up to 49% in pigeonpea *(Cajanus cajan).*

Table 1. Major nematode problems in Karnataka.

Crop	Nematode
Rice	*Heterodera oryzicola* *Hirschmanniella oryzae*
Potato	*Meloidogyne incognita* *Rotylenchulus reniformis*
Groundnut	*Meloidogyne sp* *Tylenchorhynchus* sp *Pratylenchus* spp
Sugarcane	R. *reniformis* *Pratylenchus* sp *Meloidogyne sp*

continued

Table 1 continued

Crop	Nematode
Maize	*Heterodera zeae* *Pratylenchus* sp *Trichodorus* sp
Vegetables	*M. incognita* *R. reniformis*
Banana	*Radopholus similis* *Helkotylenchus multicinctus* *Meloidogyne incognita*
Coconut	*R. similis* *Pratylenchus* spp
Cardamom	M. *incognita* *Pratylenchus coffeae* *R. reniformis*
Oil palm	*R. similis* *Pratylenchus* sp
Crossandra	*M. incognita* *R. reniformis* *Pratylenchus* spp
Grape	M. *incognita* *Xiphinema* spp *R. reniformis*
Coffee	*M. incognita* *P. coffeae*
Pulses	*Heterodera cajani* *M. incognita* *R. reniformis*
Oilseed crops	*R. reniformis* *Meloidogyne* spp

Management

Physical. Soil solarization with clear polythene mulch resulted in maximum reduction of root-knot nematode populations with the least weed growth and highest cost-benefit ratio of 1:1.8.

Host-plant resistance. In the evaluation of resistance to the root-knot nematode, tomato genotypes PAU 1, 2, 3, 4, and 5, Punjab NR 7, SL 120, BT 1, NT 1, NT 3, NT 8, NT 12, Mangala, IAHS 882, and Karnataka hybrid and brinjal cultivars Maroo Marvel, Ghatikia White, and Gulla were resistant to *M. incognita.* All the popular brinjal cultivars in Karnataka are susceptible to *M. incognita* and *M. javanica.* The following genotypes were resistant to root-knot nematode: IC 17005 and LBNC 3 of winged bean *(Psophocarpus tetragonolobus);* JP-169-1 of pigeonpea; M 546, LM 515 B, ML 33, and DMG/LM 1050 of mung bean *(Phaseolus aureus);* HC 1, ICC 47, and BG 305 of chickpea; VSH 8 of sunflower; PK 948, PK 946, JS 340, JS 2, KB 32, and PBN 110 of soybean *(Glycine max);* Jayanthi, Pankaj, Supriya, and Vijaya of rice *(Oryza sativa);* and ICG 2763 and ICG 6689 of groundnut. Banana cultivar Yelakki Bale was tolerant to *R. similis.*

Use of chemicals. Root-knot nematode was effectively controlled by foliar sprays of DL-phenylalanine and L-cystine. Carbofuran at 2 kg a.i. ha^{-1} was effective against *M. incognita.* Bare root-dip treatment for 80 min in 500-1000 ppm of carbofuran or turbofos was effective against *R. reniformis* on brinjal. Nursery treatment with Sebuphos at 0.6 g a.i. m^{-2} was superior in tomato, while brinjal recorded the highest yield against root-knot nematode with Ethoprop at 0.6 g a.i. m^{-2}.

Use of botanicals. Application of neem leaves (3 kg m^{-2}) was effective in reducing the population density of *M. incognita* and enhancing the plant growth and yield of vegetable crops. The combination of solarization plus neem leaves had the highest cost-benefit ratio and was the most effective treatment in reducing the population of *M. incognita,* followed by the combination treatment of solarization with application of castor leaves.

A study was conducted to determine the effect of R. *similis, H. multicinctus,* and *M. incognita,* individually and in various combinations, on the growth, development, and health of banana plants. *Radopholus similis* caused much greater root damage than the other two nematodes. Among different treatments imposed on banana plants to control the nematode infection, integration of physical (paring and hot water), cultural (neem cake), and chemical (carbofuran) methods of control was most effective in improving the growth of banana plants and reducing the number of nematodes in the roots and soil.

The biological agents *Pasteuria penetrans* (a gram-positive bacterium) and *Glomus fasciculatum* (a mycorrhiza) were tested in the integrated management of R. *similis* on banana. The combined treatment integrating neemark (400 g $plant^{-1}$),

carbofuran (20 g plant^{-1}),mycorrhiza (500 chlamydospores plant^{-1}), and bacterium (with soil containing second-stage larvae of *M. incognita* infected with seven spores of *P. penetrans* attached to their cuticle) produced the best plant growth, followed by the combination treatment of neemark plus bacterium plus mycorrhiza. The nematode population in soil was lowest [1052 (250 cm^3 soil)$^{-1}$] in plants treated with neemark plus bacterium plus mycorrhiza compared to the inoculated control [2430 (250 cm^3 soil)$^{-1}$]. The lowest nematode population in roots [382 (10 g)$^{-1}$] was in plants treated with neemark plus carbofuran plus mycorrhiza. Root lesions were significantly reduced in plants treated with the combination of neemark plus carbofuran, compared to the inoculated control.

Use of bioagents. Different methods of soil application of spores of *Pasteuria penetrans* were tested. The maximum attachment of spores on nematode bodies was seen in the treatment where soil infested with *P. penetrans* was added to soil containing tomato plants inoculated with *M. incognita* larvae. Of the different isolates tested, the Kanakapura isolate of *P. penetrans* was virulent and readily attached to *M. incognita* and *R. similis* larvae, compared to four other isolates collected from different locations.

Preliminary investigations were made to evaluate the efficacy of *P. penetrans,* both individually and in combination with other methods, in the integrated management of *M. incognita* on banana under greenhouse conditions. The best treatment was neemark (2%) plus carbofuran (13.3 g m^{-2}) plus *P. penetrans* (with soil containing second-stage larvae of *M. incognita* with seven *P. penetrans* spores attached to their cuticle) plus *G. fasciculatum* containing an average of 1500 chlamydospores (50 g soil)$^{-1}$. Compared with the inoculated control, this treatment recorded maximum plant growth and yield (4.0 kg m^{-2} against 1.1 kg m^{-2} for the control), with a cost-benefit ratio of 1:3.7. The treatment of neemark plus carbofuran plus *P. penetrans* plus *G. fasciculatum* gave the greatest reduction of nematodes in soil [200 (250 cm^3 soil)$^{-1}$] compared to the control [976 (250 cm^3 soil)$^{-1}$], along with reduction in root galls, eggmasses, and root-knots.

Status of Nemtode Problems and Research in Gujarat

B A Patel[1]

Introduction

In Gujarat, stunt nematode (*Tylenchorhynchus* sp) was first observed in bidi tobacco fields in 1962. Survey work has since revealed that bidi tobacco suffers heavy damage from root-knot, stunt, and reniform nematodes. In the Saurashtra region and Kapadwanj area of Kheda District, groundnut *(Arachis hypogaea)* is affected by root-knot nematodes (*Meloidogyne arenaria* and *M. javanica).* Throughout the state, pigeonpea (*Cajanus cajan)* is attacked by *Heterodera cajani, Rotylenchulus reniformis,* and *Meloidogyne* spp; chickpea (*Cicer arietinum)* suffers from root-knot nematode; castor (*Ricinus communis)* by *R. reniformis;* banana (*Musa* spp) by *Meloidogyne* spp, *Radopholus similis, Helicotylenchus, Hoplolaimus,* and *Tylenchorhynchus* spp; sugarcane *(Saccharum officinarum)* by *Helicotylenchus, Hoplolaimus,* and *Tylenchorhynchus* spp; cotton *(Gossypium herbaceum)* by *R. reniformis* and *Meloidogyne;* wheat (*Triticum aestivum),* pearl millet (*Pennisetum glaucum),* and maize *(Zea mays)* by stunt nematodes; and vegetables by root-knot nematodes.

Yield Losses

Studies undertaken on yield losses in different crops due to nematodes revealed a 50% loss due to root-knot nematode in the production of tobacco *(Nicotiana tabacum)* transplants in nursery and cured leaf yield, and 21% in banana and 34% in pigeonpea. In groundnut, yield losses of 10-23% were due to *M. javanica,* while 13-59% was due to *M. arenaria,* which also caused losses of 38% in fennel (*Foeniculum vulgare)* and 39% in chickpea. In castor, *R. reniformis* caused losses of 20%.

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Patel, B.A. 1997. Status of nematode problems and research in Gujarat. Pages 92-94 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Resistant Genotypes

Resistant genotypes are an important component of nematode management. GujaratTobacco5,abiditobaccovariety,hasfieldtolerance*toM.incognitaand M. javanica.* Studies have shown than it gives 27% higher yield, with a 64% reduction in disease over a susceptible tobacco cultivar, Anand 119. The cultivar Gujarat Cowpea 1 *(Vigna unguiculata):* and tomato *(Lycopersicon esculentum)* genotype SL 120 are both resistant to root-knot nematodes.

Diseases

In the presence of root-knot nematodes, the severity of wilt disease increases in pigeonpea and chickpea. In cotton, both *R. reniformis* and *Meloidogyne* spp increased the severity of root-rot disease caused by *Macrophomina* sp. *Rotylenchulus reniformis* increased wilt and root rot in castor, and oil content was reduced. In groundnut, the intensity of pod rot caused by *Aspergillus flavus* and. *A. niger* is also increased in the presence of *M. arenaria.*

Integrated Management

Application of carbofuran (at 3 kg ha^{-1}) reduced root-knot disease in pigeonpea by 54% and increased grain yield by 16%. Neem and mustard cake are effective in managing root-knot disease in pigeonpea, groundnut, tobacco, banana, okra *(Hibiscus esculentus),* tomato, and bottlegourd (Cucurbitaceae). Phenamiphos and sebufos are very effective in managing nematode-induced diseases in chickpea, tobacco, tomato, and banana. Pressmud (at 15 t ha^{-1}) very effectively controlled root knot in okra. Root-knot nematode population was reduced by 72% and tobacco yield was increased by 28% when castor genotype Anand 39-1 was utilized as a component of 2-year crop rotations for a bidi tobacco-based farming system. *Crotalaria spectabilis* trapped only *M. incognita* but not *M.javanica.* Root-knot disease was reduced and tobacco yield increased in a crop rotation of bidi tobacco in first year, summer bajra and hybrid Cotton 4 in the second year with summer fallow, and tobacco again in the third year. Application of carbofuran (at 1.5 g $plant^{-1}$) at 6 and 7 months after sowing banana enhanced yield and reduced root-knot infestation.

Rabbing with paddy husk or bajra husk gave excellent control of root-knot nematodes in tomato and tobacco nursery crops. Periwinkle *(Catharanthus roseus)* trapped root-knot, and reniform nematodes.

Extensive work on nematode management through soil solarization has been done. Tarping with 100-gauge low-density polyethylene clear film for 15 d during hot summer reduced root-knot disease, damping-off, and weeds in tomato and tobacco nurseries and increased the number of healthy trans-plantable seedlings.

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Status of Nematode Problems and Research on Pulses in Uttar Pradesh

K Dwivedi[1]

Introduction

India is the world's largest producer of pulses. A variety of pulse crops are grown in different parts of the country, including chickpea *(Cicer arietinum),* pigeonpea *(Cajanus cajan),* green gram *(Phaseolus aureus),* black gram *(Phaseolus mungo),* horse gram *(Dolichos biflorus),* cowpea *(Vigna unguiculata),* field beans *(Phaseolus* spp), and grass pea *(Lathyrus sativus).* Chickpea and pigeonpea are the major pulse crops, and amount to 45% of total land under pulse production. But over the past several years, pulse production in India has remained static due to a number of biotic and abiotic factors, either individually or in association with other microoganisms, that adversely affect productivity. One of the major factors in low productivity is nematodes. Globally, plant parasitic nematodes are estimated to cause yield losses of up to 14% in chickpea and 13% in pigeonpea.

Pulse Production in Uttar Pradesh

In 1994-95, Uttar Pradesh recorded nearly 900 000 ha under chickpea cultivation, with an average production of about 890 kg ha^{-1}. Similarly; nearly 600 000 ha was cultivated to pigeonpea, with an average production of about 1800 kg ha^{-1}. Due to various biotic and abiotic factors, production varied by region. During 1994-95 the highest production of chickpea was recorded in the Lucknow region (1848 kg ha^{-1}), while the lowest production was in Jhansi region (688 kg ha^{-1}). Agra region recorded the highest pigeonpea production (1888 kg ha^{-1}) and Gorakhpur region recorded the lowest (528 kg ha^{-1}) (Anonymous 1995). Nematode diseases were almost certainly responsible for some of this variation.

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Dwivedi, K. Status of nematode problems and research on pulses in Uttar Pradesh. Pages 99-100 *in* Diagnosis of key nematode pests of chickpea and pigeonpea and their management: proceedings of a Regional Training Course, 25-30 Nov 1996, ICRISAT, Patancheru, India (Sharma, S.B., ed.). Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Losses by Nematodes

Surveys in different parts of the state have shown that root-knot *(Meloidogyne* spp), cyst *(Heterodera cajani),* reniform *(Rotylenchulus reniformis),* lance *(Hoplolaimus* sp), and stunt *(Tylenchorhynchus* sp) nematodes are all important problems on chickpea and pigeonpea. The direct losses caused by these nematodes, especially root-knot and cyst nematodes, are higher in comparison to other nematodes. Nematodes are also involved with indirect losses in association with other pathogens. They are known to act as predisposing agents for many fungal and bacterial diseases, particularly in the Budelkhand region of the state. Additional information is still needed to determine their association in other soilborne diseases.

Conclusion

So far, limited importance has been placed on the management of nematodes, while much attention has been devoted to the use of different nematicides. But as ecofriendly approaches become more popular to maintain a pollution-free environment, integrated pest management practices for control of nematodes on pulse crops are gaining priority, and the use of bioagents has recently become an important aspect in control of nematode diseases.

Little progress can be made until more data is acquired on the distribution of important nematode species and the losses they cause on a regional basis. Research on cropping patterns and systems is another important aspect that will help in devising nematode disease management programs. Only when these research results are acquired can we then go forward in this field and expect to gain the desired results.

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Appendix

(Sitting, from left to right:) Kusum Dwivedi, G D S N Chandrasena, Jill Lenné, Y L Nene, F R Bidinger, S C Anand, K G Davies, T C Vrain

(Standing, from left to right:) B Diwakar, M A Ansari, Faujdar Singh, K K Sharma, S S Ali, S B Sharma, S P Tiwari, N L Mhase, B A Patel, Paban B Karki, Ashraf Uddin Ahmed, Fazul Rahaman

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The semi-arid tropics (SAT) encompasses parts of 48 developing countries including most of India, parts of southeast Asia, a swathe across sub-Saharan Africa, much of southern and eastern Africa, and parts of Latin America. Many of these countries are among the poorest in the world. Approximately one-sixth of the world's population lives in the SAT, which is typified by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils.

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ISBN 92-9066-383-9 Order code CPE 115 362-97