Plant Resistance to Parasitic Nematodes

Edited by J.L. Starr, R. Cook and J. Bridge

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Edited by

J.L. Starr
Department of Plant Pathology and Microbiology
Texas A&M University
USA

R. Cook
Institute of Grassland and Environmental Research
Aberystwyth
UK

and

J. Bridge
CABI Bioscience
Egham
UK

CABI *Publishing*

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CABI Publishing
CAB International
Wallingford
Oxon OX10 8DE
UK

Tel: +44 (0)1491 832111
Fax: +44 (0)1491 833508
Email: cabi@cabi.org
Web site: www.cabi-publishing.org

CABI Publishing
10 E 40th Street
Suite 3203
New York, NY 10016
USA

Tel: +1 212 481 7018
Fax: +1 212 686 7993
Email: cabi-nao@cabi.org

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Contributors

Ivan F. Bendezu, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132, USA

John Bridge, CABI Bioscience UK Centre, Bakeham Lane, Egham, Surrey, TW20 9TY, UK

G. Caubel, INRA, 35653 Le Rheu Cedex, France

Roger Cook, Institute of Grassland and Environmental Research, Aberystwyth, Ceredigion SY23 3EB, Wales, UK

D. De Waele, Laboratory of Tropical Crop Improvement, Catholic University Leuven (K.U.Leuven), Kasteelpark Arenberg 13, 3001 Leuven, Belgium

A. Elsen, Laboratory of Tropical Crop Improvement, Catholic University Leuven (K.U.Leuven), Kasteelpark Arenberg 13, 3001 Leuven, Belgium

R.S. Hussey, Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274, USA

G.J.W. Janssen, Novartis Seeds AB, PO Box 302, S-261 23, Landskrona, Sweden

David T. Kaplan, US Department of Agriculture, Agriculture Research Service, 2120 Camden Road, Orlando, FL 32803, USA

C. Kwoseh, CABI Bioscience UK Centre, Bakeham Lane, Egham, Surrey, TW20 9TY, UK. Present address: c/o Crop Science Department, University of Science and Technology, Kumasi, Ghana

K.A. Mizen, Institute of Grassland and Environmental Research, Aberystwyth, Ceredigion SY23 3EB, Wales, UK

J. Mudge, Department of Plant Pathology, University of Minnesota, St Paul, MN 55108, USA
G.R. Noel, USDA ARS, Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA
R.A. Plowright, CABI Bioscience UK Centre, Bakeham Lane, Egham, Surrey, TW20 9TY, UK
Phillip A. Roberts, Department of Nematology, University of California, Riverside, CA 92521, USA
A.F. Robinson, Agricultural Research Service, United States Department of Agriculture, 2765 F&B Rd, College Station, TX 77845, USA
J.L. Starr, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132, USA
Soledad Verdejo-Lucas, Departamento de Protección Vegetal, Institut de Recerca i Tecnologia Agroalimentarias, Crta. de Cabrils s/n, 08348 Cabrils, Barcelona, Spain
N.D. Young, Department of Plant Pathology, University of Minnesota, St Paul, MN 55108, USA

Preface

A primary topic of discussion in the mid 1980s for the Host Resistance Committee of the Society of Nematologists was how could the committee stimulate greater efforts in the identification, characterization, development and eventual deployment of resistance to nematodes. The outcome of those discussions was the manual 'Methods for Evaluating Plant Species for Resistance to Plant-Parasitic Nematodes', which was published by the Society of Nematologists in 1990. Unfortunately, the Society lacked the advantages of a commercial publishing house for effective advertisement and distribution of the manual, and thus apparently it has had little impact. Since 1990 there has been little evidence to suggest that efforts to develop and deploy host resistance have increased to any significant degree. Yet those factors that make the use of resistance an important goal have not diminished, indeed they have increased. These pressures include increasing limitations on the use of nematicides, the absence of any new and widely available nematicides, the narrow profit margins for many agricultural systems, lack of grower interest in other management alternatives, and the limited list of effective alternatives, such that host resistance must be given a higher priority. Additionally, in many regions where subsistence agriculture predominates, resistance is among the few management tactics that can be deployed to increase both yield potential and yield stability with little or no additional cost to the producer. Thus to continue the efforts to stimulate greater interest in the practical aspects of host resistance, I approached CAB *International* about publishing another text on this topic. I was then able to enlist the aid of John Bridge and Roger Cook as

co-editors, and together we convinced several colleagues and friends to contribute chapters to the text. The present volume is much improved over my original effort. Although the organization of this volume is very similar to the first manual, we have added three new chapters (one on the yam nematode, *Scutellonema bradys*, one on marker-assisted selection, and the editors' reflections on the current status of the use of resistance). The other chapters, some with the same and some with new authors, have all been rewritten and provide greater detail on how to establish successful resistance screening programmes. Our immediate goal is to stimulate increased activity in the identification, characterization, development and deployment of resistance to important nematode species. We firmly believe that if these objectives are achieved, all agricultural production systems will benefit through increased yields, improved yield stability, and a reduction in the use of potentially hazardous nematicides.

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J.L. Starr, Roger Cook and John Bridge
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Resistance to Plant-parasitic Nematodes: History, Current Use and Future Potential

J.L. Starr[1], J. Bridge[2] and R. Cook[3]

[1]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132, USA; [2]Tropical Plant Nematology Advisor, CABI Bioscience UK Centre, Bakeham Lane, Egham, Surrey TW20 9TY, UK; [3]Institute of Grassland and Environmental Research, Aberystwyth, Ceredigion SY23 3EB, Wales, UK

Resistance of plants to pathogens is often defined as the ability of the plant to lessen, inhibit or overcome the attack by the pathogen (Wingard, 1953). Entomologists frequently use a broader definition, defining resistance as the amount of heritable characteristics of the plant that influences damage done by insect pests (Painter, 1951), with non-preference, antibiosis and tolerance as types of resistance. In plant nematology, the most widely used definition is that it is the ability of a plant to inhibit the reproduction of a nematode species relative to reproduction on a plant lacking such resistance (Cook and Evans, 1987; see Roberts, Chapter 2). Additionally, nematologists frequently separate host response to nematode parasitism from the ability of the plant to support nematode reproduction. Thus, a susceptible plant may be intolerant with a relatively large degree of growth suppression due to nematode parasitism or it may be tolerant with limited growth suppression due to parasitism (Cook and Evans, 1987). Likewise, a resistant host can be either tolerant or intolerant. There are several reports that document differences in tolerance of susceptible plant species (Hussey and Boerma, 1989; Cook *et al.*, 1997).

These differences among disciplines reflect differences in how resistance is expressed in the plant to pathogens, insects and nematodes, the methods used to measure resistance, and the nature of the interaction of the pest or pathogen with the host. Regardless, resistance is of importance for protection of yield potential and, in some cases, for management of pest or pathogen population densities. In nematology,

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our emphasis on nematode reproduction reflects the general lack of discrete symptoms upon which assessment of resistance is often based when dealing with microbial plant pathogens. Further, nematode reproduction can be measured with sufficient ease, accuracy and precision to be a practical alternative to measurement of disease (i.e. symptoms). Additionally, because plant damage caused by nematodes is so strongly influenced by initial population densities (Seinhorst, 1965) compared with diseases or insects for which the rate of increase is of primary importance to the final amount of crop damage, then the effect of resistance on nematode population densities becomes an important aspect of the use of resistance in crop management systems.

Numerous recent reviews are available that discuss the arguments for differing definitions of resistance (Cook and Evans, 1987; Trudgill, 1991), the genetic basis for resistance (Roberts *et al.*, 1998), mechanisms of resistance (Williamson and Hussey, 1996; Williamson, 1998), breeding for resistance (Young, 1998) and bioengineering resistance (Opperman and Conkling, 1998; Vrain, 1999). This text deals primarily with the practical measurement of resistance and tolerance in plants. Our goal is to stimulate greater interest and use of resistance for management of plant-parasitic nematodes.

Why Resistance?

There are hundreds of reports that document crop yield suppression due to parasitism by a variety of nematode species (see Luc *et al.*, 1990; Evans *et al.*, 1993), yet nematodes are still frequently overlooked as crop pests, or are considered to be pests of minor significance. Numerous factors contribute to the general lack of appreciation of nematodes as crop pests, including that nematode parasitism often suppresses crop yields without other obvious symptoms of damage. If there is a general lack of awareness of nematodes as crop pests, then it is not surprising that there has been relatively little concern (or support) for development of effective and economical nematode management systems. Agricultural producers are typically faced with a multitude of problems. Environmental concerns, especially the limited availability of water (or less frequently the overabundance of water), probably top the list of concerns, followed closely by soil fertility. Weeds and, for most crops, insects (arthropods) are usually considered by the producer to be the most important crop pests. In addition to factors that directly affect crop productivity, the producer must also consider economic issues related to labour, land and equipment costs, and the market value of the commodity. Those involved in subsistence agriculture have additional concerns and have limited access to information that can help them deal with their problems. Thus, even for those crops for

which nematodes are widely recognized as important constraints to productivity, it is small wonder that relatively little time, thought, or effort is devoted to management of nematodes. If resistance to nematodes was more widely available, crop productivity could be improved with little effort or direct cost to the producer.

Is resistance inherently better than the other approaches to management of nematodes (i.e. use of nematicides, crop rotation or biological control)? Not really, but neither are any of the other approaches universally superior to resistance. Traditional nematicides such as the fumigant 1,3-dichloropropene, the carbamates aldicarb and oxamyl, and the organophosphate fenamiphos, when applied correctly will increase crop yield if initial nematode population densities exceed damage thresholds (see Whitehead, 1998). However, there is no long-term suppression of nematode population densities with the use of nematicides. Additionally, the use of nematicides is frequently cost prohibitive, especially in subsistence agriculture. Environmental and human health concerns have resulted in increased restrictions on the use of these toxic materials such that no effective nematicides are legally available for many nematode–crop combinations. No new nematicide that has widespread use has been developed in the past 20 years. It currently requires approximately 10 years and tens of millions of US dollars to develop and bring to market any new pesticide, and the market niche for any nematicide is limited relative to the market for herbicides or insecticides. Nematicide sales account for less than 1% of pesticide sales in the USA, whereas herbicides and insecticides account for 60 and 21%, respectively, of total pesticides sales for agriculture (Ware, 1994). It is unlikely that any new nematicide based on currently available chemistry will be developed in the near future. Thus nematicides are likely to have a diminishing role in crop protection.

Crop rotations can also decrease the potential for substantial yield losses due to nematodes (Luc *et al.*, 1990; Whitehead, 1998) and provide at least short-term suppression of nematode population densities. The magnitude of these benefits is generally positively correlated with the number of cropping seasons between the planting of susceptible crops. But rotation systems are seldom adopted unless there are additional benefits to the producer beyond nematode management. Regardless of whether the producer is involved in intensive production agriculture or subsistence farming, many factors are involved in deciding which cropping system best meets the needs of that producer. Overall, profitability and yield stability are the primary concern of the producer. It is seldom that nematode management is the critical factor in adopting a specific cropping system. Because many nematode species are polyphagous with wide host ranges and many fields have polyspecific communities of plant-parasitic nematodes, development of cropping systems that meet all of the needs of the producer and

suppress nematode population densities is a formidable challenge. None the less, there are numerous examples of effective nematode management with crop rotation.

Biological control holds some promise for the future (see Evans *et al.*, 1993), but with current knowledge it is difficult to promote or establish a microflora or fauna in soils that effectively suppresses nematode population densities, especially in the relatively short period of time of a single growing season. Reliable and effective biological control systems are likely to be limited to specialized situations (e.g. intensely managed crop systems where the environment can be manipulated to promote biological activity) for the near future.

Resistance is an effective management tool that improves crop yield (Table 1.1) in the presence of nematode population densities that exceed the damage threshold. Because resistance to nematodes is usually developed by selection of plants with reduced rates of nematode reproduction, nematode population densities are typically lower following a resistant cultivar than a susceptible cultivar. However, this is not always the case if the crop has only partial resistance. Niblack *et al.* (1986) demonstrated that at moderate to high initial population densities, population densities of *Meloidogyne incognita* reach their maximum levels at about 90 days after planting on a susceptible soybean cultivar (presumably due to extensive damage to the host), whereas on partially resistant cultivars that were less damaged by the nematodes, the population densities were still increasing at 120 days after planting. Resistance not only complements crop rotation for

Table 1.1. Selected examples of the effect of resistance to plant-parasitic nematodes on crop yield in nematode-infested and non-infested fields.

			Yield	
Crop	Nematode species	Cultivar[e]	Infested	Non-infested
Soybean[a]	*Heterodera glycines*	S	2141 kg ha^{-1}	3170 kg ha^{-1}
		R	2908 kg ha^{-1}	3177 kg ha^{-1}
Soybean[a]	*Heterodera glycines*	S	2383 kg ha^{-1}	3810 kg ha^{-1}
		R	3177 kg ha^{-1}	3541 kg ha^{-1}
Groundnut[b]	*Meloidogyne arenaria*	S	914 kg ha^{-1}	4678 kg ha^{-1}
		R	3771 kg ha^{-1}	5155 kg ha^{-1}
Tobacco[c]	*M. incognita*	S	301 g per plot	504 g per plot
		R	407 g per plot	477 g per plot
Cotton[d]	*M. incognita*	S	530 kg ha^{-1}	–
		R	1100 kg ha^{-1}	–

[a]G.L. Tylka, Iowa State University, USA, personal communication; [b]Starr *et al.* (1998); [c] Barker *et al.* (1981); [d]Ogallo *et al.* (1999); [e]S, susceptible cultivar; R, resistant cultivar.

nematode management, but also improves the ease with which effective rotation systems can be developed. Ogallo *et al*. (1999) demonstrated that resistance to root-knot nematodes in cotton increased lint yields and yield stability in nematode-infested fields compared to susceptible cultivars (Fig. 1.1). Additionally, they demonstrated that yield of susceptible lima beans planted in infested fields was greater following two crops of resistant cotton than after two crops of susceptible cotton. The yield increase was attributed to suppression of population densities of *M. incognita* by the resistant cotton cultivar. Typically, the direct cost to the grower for the use of resistance is minimal, thus resistance fits all agricultural production systems. Finally, resistance is an ecologically sound approach to nematode management, especially relative to commonly used nematicides.

Although resistance to plant-parasitic nematodes is usually identified and characterized based on inhibition of nematode reproduction, our primary interest in resistance has to be yield. The benefit to subsequent susceptible crops from suppression of nematode population densities must be considered a supplemental benefit. It will be difficult to convince plant breeders to introgress resistance into cultivars if the primary benefit will be to another crop through suppression of nematode population densities. It is doubtful if growers would be willing to plant a resistant cultivar if there was no yield benefit to that

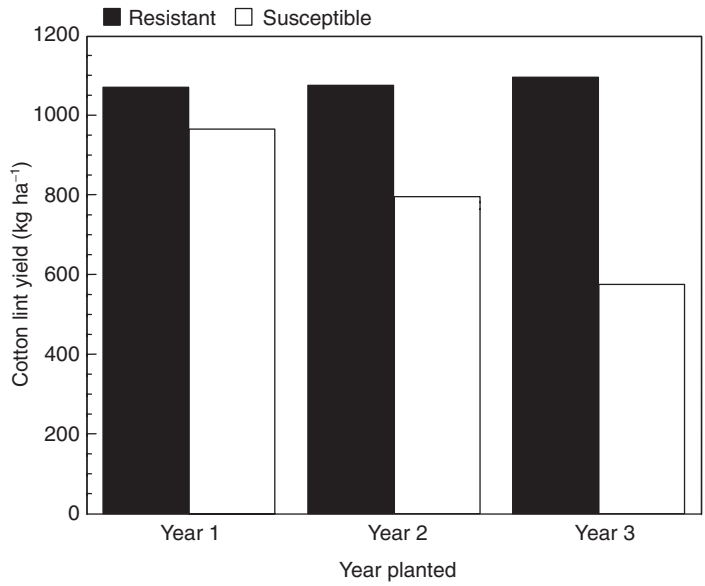

Fig. 1.1. Cotton lint yields for 3 consecutive years for a root-knot resistant cultivar compared to yields of a susceptible cultivar in a field infested with *Meloidogyne incognita* (from Ogallo *et al*., 1999).

crop. Thus, when working with host resistance yield must be the top priority.

Resistance is not a panacea that will solve all nematode management problems. No resistance to important nematode species (especially migratory ectoparasites such as *Belonolaimus* and *Hoplolaimus* spp.) is known for some crops or is present only in wild species or undeveloped genotypes, such that a major effort will be required to develop high-yielding crop genotypes with desirable levels of resistance. As with crop rotation and biological control systems, resistance is typically a highly specific trait and is expected to be effective against only a single nematode species or even a subspecific race or pathotype. It may take years of effort by traditional or transgenic methods to introgress new resistance genes into desirable crop genotypes. Additionally, after development of a resistant cultivar, that resistance may not be durable if the target nematode species has a high level of genetic variability (Young and Hartwig, 1992; Roberts, 1995; Kaloshian *et al.*, 1996). However, resistance can be made more durable by pyramiding of multiple resistance genes to reduce the probability of selection and by development of specific resistance deployment schemes that reduce the duration of selection pressure for development of virulent nematode populations.

There are few resistant cultivars relative to the amount of known resistant genotypes. A bibliography of resistance (Armstrong and Jensen, 1978) contains 1371 citations dealing with resistance in 119 crop species or genera. In the period 1995–2000, Nematological Abstracts contained about 300 abstracts annually that dealt with some aspect of resistance. Young (1998) reported that the Crop Science Society of America (CSSA) has registered 143 nematode-resistant cultivars or germplasm lines for 15 field crops. Additionally, in the texts by Luc *et al.* (1990) and Evans *et al.* (1993), resistant cultivars or the potential for their development from known resistant germplasm resources were discussed for nearly every crop. Nearly 90% of all reports involve *Meloidogyne*, *Globodera* or *Heterodera* species. This preponderance of effort on these genera reflects their overall importance as agricultural pests and the relative abundance of resistance to species of these genera. Our goal is to stimulate and encourage greater effort in the identification and especially the use of these many sources of resistance.

History of Resistance to Nematodes

Among the first reports of resistance to nematodes was that of Webber and Orton (1902), who described the resistance of a cowpea variety 'Iron' to root-knot nematodes based on reduced galling in field plots. Additionally, their report cites reports by Zimmerman (1897)

of observations of resistance to root knot in coffee, and Wilfarth (1900) on selection of sugar beets with resistance to nematodes. Ware (1936) reported that Orton made selections in 1905 from the cotton line 'Jackson Limbless' that had good resistance to Fusarium wilt and noted that it was 'somewhat resistant to root-knot but had little else to recommend it.' Moore (1960) cites Nilsson-Ehle (1920) as being the first to study heritability of nematode resistance and identified resistance to *Heterodera schachtii* (*sic*) in barley as being due to a single dominant gene. The lack of knowledge or appreciation of the importance of proper identification of the nematode population hampered these early efforts to identify and characterize resistance in host species.

Barrons (1939) was one of the first to study the mechanisms of resistance to nematodes, working with root-knot nematodes on cowpea. He distinguished resistance from tolerance and noted that resistance was not due to inhibition of root penetration. Barrons speculated that resistance might be due to chemical inhibitors in the roots and that these inhibitors 'may counteract or neutralize the giant cell inducing effect of salivary secretions of the nematode.'

A major achievement in resistance to nematodes was the introgression of the *Mi* gene for resistance to *M. incognita*, *M. arenaria* and *M. javanica* from *Lycopersicon peruvianum* into *L. esculentum* (Smith, 1944). Even though it was several decades before root-knot resistant tomato cultivars were widely grown commercially, resistance conditioned by the *Mi* gene has been a valuable research model and has added greatly to the understanding of resistance (Williamson, 1998). With the cloning and determination of DNA sequences of *Mi* (Milligan *et al.*, 1998) and the $Hs1^{pro\text{-}1}$ gene for resistance to *H. schachtii* (Cali *et al.*, 1997), it is likely the progress in the understanding of at least these two types of resistance will accelerate.

Resistance to *M. incognita* in tobacco was first reported in the early part of the 20th century (Clayton *et al.*, 1958), but resistant tobacco cultivars were not widely grown until the 1970s. Resistance to the potato cyst nematode (*Globodera rostochiensis*) was reported in 1954 by Ellenby and to the soybean cyst nematode (*Heterodera glycine*s) in 1957 by Ross and Brim. In the case of these two cyst nematodes, resistance was adopted fairly rapidly with resistant varieties derived from those discoveries being widely grown by the 1970s.

Examples of Current Use of Resistance

Today, resistance is widely and effectively used in some crops. In North Carolina (USA), 97% of the 84,000 ha tobacco crop is planted to cultivars resistant to *M. incognita* (T. Melton, North Carolina State University, USA, personal communication). Despite this high percentage

use of resistance, more than 70% of the crop is also treated with a nematicide. This reflects the fact that even after more than 25 years of use and an effective grower education programme, the producers of this high value crop are unwilling to put their complete trust in resistance. Factors that contribute to a lack of faith in resistance include the presence in some tobacco fields of *M. arenaria* and *M. javanica*, against which the resistance is not effective, and the promotional efforts of the nematicide industry. Because of the value of the crop, growers are willing to bear the additional cost of a nematicide for added protection from possible loss. Although the widespread use of this resistance resulted in an increased frequency of *M. arenaria* in North and South Carolina (Fortnum *et al.*, 1984; Schmitt and Barker, 1988), *M. incognita* remains the most frequently encountered species on tobacco and resistance is still effective in most fields.

Because the *Mi* gene for resistance to *M. incognita*, *M. javanica* and *M. arenaria* was linked to some horticulturally undesirable traits, it was not widely used in commercial tomato production in the USA until the 1980s. Currently, the majority of the tomatoes grown commercially in California carry this resistance (Williamson, University of California, Davis, USA, personal communication). Despite their apparent success in California, only recently have tomatoes with the *Mi* gene been widely grown in Florida. This recent use has been the result of the popularity of the cultivar Sanibell, which carries the *Mi* gene, because of its superior horticultural traits and not because of its resistance to *Meloidogyne* spp. Indeed, virulence against the *Mi* gene can develop in Florida populations of *M. incognita* after as few as five plantings of Sanibell (Noling, 2000).

Resistance to *Heterodera glycines* in soybean and *Globodera pallida* and *G. rostochiensis* in potato are representative of cases where the effectiveness of resistance is compromised by virulence in the nematode populations. The race situation with respect to *H. glycines* remains unsettled, with 16 races currently recognized (Riggs and Schmitt, 1988). Numerous high-yielding soybean cultivars have resistance to races 1 and 3 of *H. glycines*, and a few cultivars have resistance to races 6 and 14. The cultivar Hartwig has the broadest base of resistance, being resistant to races 1–6, 8 and 14, but has relatively poor yield potential. Fortunately, of the 16 described races of *H. glycines*, eight are rarely encountered. Races 1 and 3 predominate in the northern portion of the USA, whereas races 2–6, 9 and 14 predominate in the southern USA. In North Carolina, approximately 48% of the 573,000 ha soybean crop was planted to cyst-resistant cultivars in 1998 (J. Dunphy, North Carolina State University, USA, personal communication), but 60% of the infestations are races against which resistance is not effective. The development and use of marker-assisted selection (see

Young and Mudge, Chapter 12) should increase the efficiency of working with multiple genes for resistance to *H. glycines*.

Similarly, multiple pathotypes of *G. pallida* and *G. rostochiensis* have been described, but remain somewhat controversial due to incomplete data on the genetics of resistance in the host and virulence in the nematodes (Trudgill, 1985). None the less, resistance to *G. rostochiensis* has been widely used. Currently in the Netherlands, about 55% of the ware potatoes (those grown for sale as food) and 99% of the starch potatoes are resistant to one or more pathotypes of the cyst nematodes (F. Gommers, Wageningen Agricultural University, The Netherlands, personal communication). In the UK, approximately 45% of the potato crop carries the *Ro1* resistance and is effective against most populations of *G. rostochiensis* in that country (K. Evans, IARC, Rothamsted, UK, personal communication). However, the frequency of *G. pallida* is increasing in the UK and only about 1.5% of the potato crop carries effective resistance to prevalent races of *G. pallida*. Resistance to *G. rostochiensis* effectively controlled potato cyst nematodes in the UK until the appearance of *G. pallida* in the late 1970s. *Globodera pallida* resistance genes *Pa2* and *Pa3* from *Solanum vernei*, were introduced into cultivars during the 1980s. Despite the problems with maintaining effective resistance deployment against such variable pathogens as cyst nematodes, resistance has been useful in alleviating crop losses. Fortunately, the limited host range of the potato and soybean cyst nematodes has made the use of crop rotations an effective complement to resistance.

In the early and mid 1990s, three cotton cultivars (Acala NemX, Stoneville LA887 and Paymaster 1560) with moderate to good levels of resistance to *M. incognita* were released. Despite their value in increasing cotton yields in nematode-infested fields and in reducing population densities of *M. incognita* (Ogallo *et al.*, 1997, 1999; Zhou, 1999), these cultivars accounted for less than 1% of all cotton planted in the USA in 1999 (Anon., 1999). Cotton remains a case where there is both a need and opportunity for greater use of resistance.

Recently, resistance to *M. arenaria* from wild *Arachis* species has been introgressed into the cultivated peanut *A. hypogaea* and the first resistant cultivar (cv. COAN) was released in 1999 (Simpson and Starr, 2001). Grower education programmes are in progress to demonstrate the value of the resistance to the growers. Efforts are ongoing to identify additional nematode-resistance genes present within the available *Arachis* spp. germplasm resources and to introgress nematode resistance into cultivars that also have resistance to tomato spotted wilt virus and Sclerotinia blight. Introgression of additional resistance genes will increase the durability of the resistance and promote yield stability.

Resistance to Nematodes in Tropical Agriculture

Although in theory, the nematode management methods, including use of resistant cultivars, that can be employed in the tropics and developing countries differ little from those used in temperate agriculture and developed countries, in practice there are often important differences. The facets of tropical agriculture that differ most fundamentally from the temperate regions and markedly affect the control of plant nematodes are the crops grown, the farming systems, and the wide range of different nematodes found. Commercial and plantation crops are a common feature of tropical agriculture but by far the largest proportion of cultivated land in most of the tropical countries is farmed by small-scale farmers (Luc *et al.*, 1990). Tropical farming systems, especially with small farms, are generally far more complex than those found in temperate, developed agriculture and there is a greater diversity of cropping practices (Bridge, 1987). This complexity and cropping diversity is an essential consideration in the introduction of nematode management methods including resistant crop cultivars.

A much greater diversity of nematode genera and species (and probably pathotypes) exists in the tropics than in temperate countries. Nematodes also generally have shorter life cycles and more generations per crop season at higher temperatures, putting the crops under much greater pest pressure. Another important feature in tropical agriculture is that often a number of concomitant species of the same or several different genera occur together and they may all be major pests of the crop grown, which is obviously very relevant to the introduction of resistant cultivars.

The nematodes of economic importance in tropical agriculture cover a very wide range of genera and many of these do not occur on temperate crops (Table 1.2); some have a limited geographical distribution and narrow host range, others have a worldwide tropical distribution and wide host range.

Nematode-resistant cultivars can be one of the most useful, economical and effective means of managing nematodes for both large commercial and for small-scale farmers in the tropics and developing countries. Their use can be the ideal solution to managing nematode pests particularly in farming systems with low inputs. However, nematode resistance is not available for many crop–nematode combinations. The absence of nematode-resistant breeding has been attributed to a number of reasons including it having a low priority in certain crops (Cook and Evans, 1987); food crops grown in tropical situations with a low commercial value generally have a low priority for nematode-resistant breeding.

Unfortunately, relatively few of the existing resistant cultivars are accessible to or used by the majority of farmers in the developing

countries. Most available resistant cultivars have been bred for temperate or commercial crops with comparatively few available for food and other tropical crops in the developing countries. This is particularly

Table 1.2. Selected examples of crops in the tropics with some important plant nematode pests.

Crops	Nematode pests
Tomato (*Lycopersicon esculentum*)	*Meloidogyne* spp.
Aubergine (*Solanum melongena*)	
Okra (*Hibiscus sabdariffa*)	
Cucumber (*Cucumis sativus*)	
Cowpea (*Vigna unguiculata*)	
Beans (*Vigna*, *Phaseolus*, *Psophocarpus*)	*Meloidogyne* spp.
Groundnut (*Arachis hypogaea*)	*Aphelenchoides arachidis*, *Aphasmatylenchus straturatus*, *Belonolaimus longicaudatus*
Pigeon pea (*Cajanus cajan*)	*Heterodera cajani*, *Meloidogyne* spp.
Sweet potato (*Ipomoea batatas*)	*Meloidogyne* spp., *Rotylenchulus reniformis*
Cassava (*Manihot esculenta*)	*Meloidogyne* spp.
Yams (*Dioscorea* spp.)	*Scutellonema bradys*, *Pratylenchus coffeae*, *Meloidogyne* spp.
Taro (*Colocasia esculenta*)	*Meloidogyne* spp., *Hirschmanniella miticausa*, *Pratylenchus coffeae*
Ginger (*Zingiber officinale*)	
Turmeric (*Curcuma domestica*)	*Meloidogyne* spp., *Radopholus similis*, *Pratylenchus coffeae*
Rice (*Oryza sativa*)	*Aphelenchoides besseyi*, *Ditylenchus angustus*, *Hirschmanniella* spp., *Heterodera sacchari*, *Meloidogyne graminicola*, *Paralongidorus* spp., *Pratylenchus zeae*
Maize (*Zea mays*)	*Pratylenchus zeae*, *Meloidogyne* spp.
Coffee (*Coffea* spp.)	*Meloidogyne africana*, *Meloidogyne coffeicola*, *Meloidogyne decalineata*, *Meloidogyne exigua*, *Meloidogyne incognita*, *Pratylenchus coffeae*
Tea (*Camellia sinensis*)	*Meloidogyne brevicauda*, *Pratylenchus loosi*, *Radopholus similis*
Bananas and plantains (*Musa* spp.)	*Helicotylenchus multicinctus*, *Pratylenchus coffeae*, *Pratylenchus goodeyi*, *Radopholus similis*
Coconut (*Cocos nucifera*)	*Rhadinaphelenchus cocophilus*, *Radopholus similis*
Black pepper (*Piper nigrum*)	*Meloidogyne* spp., *Radopholus similis*
Cotton (*Gossypium* spp.)	*Meloidogyne acronea*, *Meloidogyne incognita*, *Rotylenchulus reniformis*
Tobacco (*Nicotiana tabacum*)	*Meloidogyne* spp.
Sugarcane (*Saccharum* spp.)	*Heterodera sacchari*, *Pratylenchus* spp., *Meloidogyne* spp.
Pineapple (*Ananas comosus*)	*Meloidogyne javanica*, *Pratylenchus brachyurus*, *Rotylenchulus reniformis*
Papaya (*Carica papaya*)	*Meloidogyne* spp., *Rotylenchulus reniformis*
Pyrethrum (*Chrysanthemum cinerariaefolium*)	*Meloidogyne hapla*

disappointing as resistance is most useful for low value crops which cannot support the cost of expensive pest management inputs (Fassuliotis, 1979). Even when resistant cultivars are available to farmers in the tropics many other factors have to be taken into account before their introduction. There is the obvious marked contrast in what can be achieved by the big commercial producer compared to the small farmer. The resistant cultivars are not always acceptable to small-scale farmers for a number of reasons: (i) nematode-resistant cultivars may be far more susceptible to local endemic but previously innocuous pests and diseases; (ii) they may have unacceptably high input requirements; (iii) their quality may be poor in relation to local food preferences and the required cooking characteristics; (iv) their growing period and harvesting time may not accord with the region; or (v) their appearance and marketability may not be acceptable relative to locally grown cultivars. On the other hand, the lack of uptake can also simply be because the farmers, extension officers or advisors are unaware of the existence or value of nematode-resistant cultivars (Bridge, 1996). None of these difficulties are insurmountable and resistant cultivars remain a very important potential component of a solution to many nematode problems of tropical agriculture especially for the low-input, small-scale farmers when used in combination with cultural techniques and traditionally grown crops.

Tolerance, where a plant suffers little injury even when heavily infected in natural conditions, can be of considerable value to nematode management (Cook and Evans, 1987) and this applies especially to resource-poor small-scale farmers. Tolerance appears to have arisen in traditional agriculture by farmer selection over many generations in fields infested with the nematodes. In different tropical countries many locally grown cultivars appear to have reverted to wild-type characteristics, such as the small-fruited tomatoes in West Africa, and these, for example, often show a high degree of tolerance to root-knot nematodes (Bridge, 1996).

Where there is genetic diversity in crops, normal selection by farmers for good growing traits will also select for tolerance or resistance to nematodes even though the farmers have no perception of nematodes as pests. Constant pressure from nematodes and other pests will ensure that the tolerance or resistance that arises will be automatically selected as the farmer selects for more recognizable and desirable characteristics such as higher yields and improved taste (Page and Bridge, 1993). An example is in Papua New Guinea where *Meloidogyne* is an important pest of sweet potato, particularly when the crop is grown continuously, but is not perceived to be a problem by the farmers. However, it is very likely that some subsistence farmers have managed to control *M. incognita* on sweet potato by rotations and by the careful selection of sweet potato cultivars which are resistant or

tolerant to the nematode. Some farmers were found to grow particular cultivars of sweet potato only after bush fallow and save others for use in a continuous cropping situation (Bridge and Page, 1982). It is possible for the farmers to make this selection because sweet potato clones resistant or highly resistant to *M. incognita* have been found more frequently in Papua New Guinea and neighbouring islands than in any other country (Shiga and Takemata, 1981). Therefore, where the root-knot nematode problem was more acute in areas of greatest land pressure the recommended solution was the active selection of nematode tolerance or resistance from the many different locally grown cultivars (Bridge and Page, 1982, 1984).

Resistance to tropical nematodes does exist in a number of crops including vegetables, food legumes, maize, tobacco, sugarcane, sweet potato, soybean, grape, citrus, cotton and lucerne. *Meloidogyne* species are the major nematode pests of tropical vegetables and some resistance to the widely occurring species of root knot has been found in green peppers, aubergine (eggplant), beans (*Phaseolus vulgaris*) and tomato. Tomato has the most cultivars with resistance to *Meloidogyne* and it is these cultivars that are, or can be, most used by farmers in the tropics. However, no vegetable cultivar has resistance to all the main species of the genus, normally only to one species, and resistance-breaking races have been found in *M. incognita*, *M. javanica* and *M. arenaria*. Because of these different root-knot species and races occurring naturally in tropical soils, it is recommended that any possible new introductions are tested first in the local soils (Roberts *et al*., 1986; Netscher and Sikora, 1990).

Tobacco is also seriously damaged by *M. incognita* but many cultivars have resistance to a number of races and can be grown in any part of the world which has a problem with these races (Shepherd and Barker, 1990). The benefits derived by growers using resistant tobacco 'NC 95' when it was introduced are described as spectacular (Fassuliotis, 1979). The main nematode pest of citrus is *Tylenchulus semipenetrans*, which now occurs worldwide in the tropics and subtropics having been spread on infected seedlings. Control of *T. semipenetrans* populations relies largely on the use of resistant rootstocks with the resistance being derived from *Poncirus trifoliata* (Cook and Evans, 1987; Duncan and Cohn, 1990; see Verdejo-Lucas and Kaplan, Chapter 9).

On food crops in the tropics some of the nematodes can pose serious threats to the farmers and can often be the most difficult to control, especially in low-input agriculture. In these situations, resistant cultivars can in many cases be the answer, although these crops are not normally a priority for the breeder. Possible exceptions that have interested breeders are found in rice. New genotypes with resistance to *Ditylenchus angustus*, the cause of ufra disease on

deepwater and lowland rice (Rahman, 1994), have been identified and could prove very important to rice farmers in southeast Asia where the nematode occurs. Studies have also identified the African rice, *Oryza glaberrima*, to be resistant to pest species of *Meloidogyne* and *Heterodera*. A recent breakthrough in breeding has been the interspecific hybridization between *O. glaberrima* and *O. sativa*. These interspecific hybrids have excellent agronomical traits and have greatly improved the possibility of selection of nematode resistance in improved varieties. Plowright *et al*. (1999) demonstrated resistance in interspecific progeny to *Heterodera sacchari* and *Meloidogyne graminicola* but not to *Pratylenchus zeae*. Their conclusions were that nematode resistance in rice cultivars with valuable agronomic traits represented by these *O. glaberrima–O. sativa* interspecific hybrids can be of enormous value to the sustainable management and preventative control of some of the major nematode pests in rice and represents a highly practical means of nematode management in smallholder, subsistence agriculture.

In commercial bananas, resistance to nematodes has so far proved elusive (see De Waele and Elsen, Chapter 8). There is, as yet, no widely grown clone of a commercial dessert banana resistant to the major nematodes despite years of searching (Gowen and Quénéhervé, 1990), nor is there such a clone of food banana or plantain (Ortiz, 2000). One commercial hybrid, 'FHIA-01', appeared to have partial resistance to *Radopholus similis* but even this has now been disproved (Stoffelen *et al*., 2000). Relatively few real attempts have been made at incorporating resistance in *Musa* against the major nematodes because of the difficulties of working on such a genetically complex plant and the cost of developing a breeding programme (Pinochet, 1992). In commercial dessert bananas, there are a limited number of land-races with an extremely narrow genetic base and, as a result, the system is highly vulnerable to pests and diseases (Ortiz *et al*., 1995). This is not necessarily the case for the all-important bananas and plantains grown as food crops by small-scale and subsistence farmers in West, Central and East Africa, which require processing before consumption as a carbohydrate food or beverage. These crops have a much greater diversity and the possibilities of finding resistant clones are considerably enhanced (Bridge, 2000). Also the chances of breeding for nematode resistance in hybrids acceptable to farmers and consumers are greater, partly because cultivar type is not restricted by the pressure and the high quality demands of the export trade (Gowen, 1994; Ortiz *et al*., 1995). In West and Central Africa, 116 plantain cultivars have been identified (Swennen, 1990). Karamura and Karamura (1994) have listed 145 cultivars of the cooking type East African Highland bananas (AAA-EA) of the Lujugira-Mutika subgroup and 88 beer cultivars of the same subgroup from Uganda. In comparison to the commercial

bananas, this provides an enormous resource for selecting or breeding new cultivars resistant to *R. similis*, *Pratylenchus goodeyi* or *P. coffeae*.

In spite of all the difficulties, the possibilities of finding resistance in bananas and plantains holds out one of the best means of controlling the nematodes for small-scale farmers in Africa. However, experiences in other parts of the tropical world have shown that where resistance in bananas exists it is not a universal resistance against all nematodes; those showing resistance to *R. similis* can be highly susceptible to *P. coffeae* (Pinochet and Rowe, 1978; Stoffelen *et al.*, 2000). Both of these nematodes can occur together in tropical soils, which adds to difficulties of selecting for resistance. More positively, it is considered that there are now good prospects for developing banana and plantain cultivars with resistance to nematodes that would probably have characteristics outside the narrow requirements of the commercial banana export trade but would be suitable for non-export and subsistence farmers in the tropics (Gowen, 1994). Also the prospects of genetically engineered nematode-resistant banana cultivars are now within reach (De Waele *et al.*, 1994). Unfortunately the research on this latter aspect will almost certainly focus on commercial, export crops with a possible trickle-down to the crops of the small-scale farmer in Africa and elsewhere at some future time.

The active selection of tolerance in bananas to nematodes or its recognition has generally received little attention. It could play a very important part in nematode management with small-scale farmers as variability in levels of nematode root populations in *Musa* are possibly associated with degrees of tolerance to the nematodes (Sarah, 1988; Gowen, 1993, 1994; Price, 1994). Tolerance, not resistance, to *R. similis* and other nematodes is also rated as one of the ideotype requirements for a commercially acceptable banana hybrid by the breeders (Ortiz *et al.*, 1995).

In tropical agriculture, particularly with small-scale farmers, tolerance and resistance can play a key role in the reduction of crop yield losses caused by nematodes. The introduction or selection of new cultivars resistant to the range of nematode pests present is desirable if all local or regional factors are considered. Introduction of such cultivars should not be at the expense of the traditional resistant cultivars or the traditional farmer selection processes that have produced them.

The Future

Host resistance is a management tactic that has much potential and needs to be more effectively utilized. Many of the problems associated

with resistance can be overcome or minimized with additional research, breeding effort and effective grower education programmes.

Much of the available germplasm resources remains to be characterized for resistance to nematodes and additional germplasm remains to be collected for many crop species. The screening of a large germplasm collection is tedious. Holbrook *et al.* (1999) advocate the use of core collections for more effectively screening germplasm. Even after resistance phenotypes have been identified, further research will be needed to determine the number of genes for resistance that have been identified. For example, Robinson and Percival (1997) recently identified accessions of *Gossypium hirsutum* from the Yucatán peninsula of Mexico with *M. incognita* resistance phenotypically similar to that of Clevewilt 6 and Wild Mexico Jack Jones, which are the sources of much resistance currently in use. The question remains, do these accessions represent unique resistance genes or are they identical to genes already in use? As DNA-based markers linked to resistance loci become more readily available, they can be used to determine if the resistance phenotypes are due to unique genes more rapidly than with traditional genetic analysis.

Research has only begun to explore the possibilities for engineered resistance and as yet no crop cultivar with engineered resistance to a nematode is available for growers. Fenoll *et al.* (1997) list numerous possibilities for engineered resistance, including anti-nematode genes, antifeedants and plantibodies. Many researchers are confident that such sources of resistance will become valuable additions to our arsenal in the near future. It is expected that engineered resistance will help overcome fertility barriers that limit use of some native sources of resistance and will provide sources of resistance to nematodes for which no resistance is currently known. A major question, and goal, will be whether engineered resistance can be more durable than many currently available resistance genes, especially with respect to *Globodera* and *Heterodera* spp. Based on present knowledge, however, we must assume that engineered resistance may be no different from native resistance with respect to durability. Indeed, technologies are available that will allow either engineered resistance genes or cloned natural genes to be more readily transferred into a wider range of crop cultivars or even species. This would very much increase the selection pressure for virulence within nematode populations and the need for development of soundly based strategies for management of nematode resistance genes.

Regardless of the source of resistance, it will be little more than a research tool if we do not form effective linkages with plant breeders to move the resistance into appropriate crop genotypes with the highest yield potentials and other important agronomic and horticultural

characteristics. It is the nematologists' responsibility to convince public and private sector plant breeders that introgression of resistance to nematodes into the elite crop germplasm lines or cultivars will be beneficial. We need to work with them to identify appropriate sources of resistance and in the development of effective screening systems that will permit timely introgression of that resistance. During this effort, we must recognize and accept that resistance will not be the top priority of the breeder, rather they will argue that improving yield potential must receive the top priority. One often-used argument against it is that resistance frequently comes at the expense of yield. Yet there are no data that prove that yield must be sacrificed to achieve resistance. As has been recently demonstrated with cotton (Ogallo *et al*., 1999), groundnut (peanut) (Church *et al*., 2000) and soybean (see Table 1.1), the linkage between lower yield potential and resistance can be broken and resistant genotypes with yield potentials equal to those of the best yielding susceptible genotypes are possible. Similarly, the use of the *Mi* gene in tomato was initially limited by linkages to undesirable horticultural traits (Williamson, 1998) but this negative linkage has been broken and tomato cultivars carrying the *Mi* gene are now widely grown commercially in California. Modern breeding technologies, notably marker-assisted selection (see Young and Mudge, Chapter 12) should be used to select for resistance and to minimize linkage drag of undesirable characteristics.

Lastly, once high-yielding cultivars with improved levels of resistance to nematodes are developed, it is necessary that effective grower (and crop consultant) education programmes be implemented. Resistance may lack durability due to variability in the nematode population, or some yield loss may be incurred at high initial nematode population densities if only partial resistance is available. Thus, it is essential that the resistance be deployed in a responsible manner to enhance durability or along with other nematode management tactics to achieve optimal benefits with respect to yield. We must work in cooperation with extension specialists from a variety of disciplines to develop effective education programmes.

The identification, development, and deployment of resistance requires a long-term and extensive effort. In one of the few studies of the economic benefits of resistance, Brady and Duffy (1982) documented that US$1 million to develop one cultivar with resistance to *H. glycines* resulted in benefits of US$400 million. Host resistance will not be the solution to all problems caused by plant-parasitic nematodes, but resistance could and should play a bigger role in many nematode management systems. The era of nematicides is ending and we must develop alternative management systems. The use of host resistance must be one of these alternatives.

References

Anon. (1999) *Cotton USA Buyers Guide*. National Cotton Council of America, Memphis, Tennessee, 45 pp.

Armstrong, J.M. and Jensen, H.J. (1978) *Indexed Bibliography of Nematode Resistance in Plants*. Bulletin 639, Agriculture Experiment Station, Oregon State University, Oregon, 96 pp.

Barker, K.R., Todd, F.A., Shane, W.W. and Nelson, L.A. (1981) Interrelationships of *Meloidogyne* species with fluecured tobacco. *Journal of Nematology* 13, 67–79.

Barrons, K.C. (1939) Studies of the nature of root-knot resistance. *Journal of Agricultural Research* 58, 263–271.

Brady, E.B. and Duffy, M. (1982) *The Value of Plant Resistance to Soybean Cyst Nematodes: a Case Study of Forrest Soybean*. US Department of Agriculture, Natural Resources Economics Division, Washington, DC.

Bridge, J. (1987) Control strategies in subsistence agriculture. In: Brown, R.H. and Kerry, K. (eds) *Principles and Practice of Nematode Control in Crops*. Academic Press, New York, pp. 389–420.

Bridge, J. (1996) Nematode management in sustainable and subsistence agriculture. *Annual Review of Phytopathology* 34, 201–225.

Bridge, J. (2000) Nematodes of bananas and plantains in Africa: research trends and management strategies relating to the small-scale farmer. In: Karamura, E. and Vuylsteke, D. (eds) *Proceedings of the First International Conference on Banana and Plantain for Africa*. ISHS, Acta Horticulturae, Vol. 540, Leuven, Belgium, pp. 391–405.

Bridge, J. and Page, S.L.J. (1982) Plant nematodes of Papua New Guinea: their importance as crop pests. *Report of a Plant Nematode Survey in Papua New Guinea*. CAB Commonwealth Institute of Parasitology, St Albans, UK, 91pp.

Bridge, J. and Page, S.L.J. (1984) Plant nematode pests of crops in Papua New Guinea. *Journal of Plant Protection in the Tropics* 1, 99–109.

Cai, D., Kleine, M., Kifle, S., Harloff, H.J., Sandal, N.N., Marcker, K.A, Klein-Lankhorst, R.M., Salentijn, E.M.J., Lange, W., Stiekema, W.J., Wyss, U., Grundler, F.M.W. and Jung, C. (1997) Positional cloning of a gene for nematode resistance in sugarbeet. *Science* 275, 832–834.

Clayton, E.E., Graham, T.W., Todd, F.A, Gates, J.G. and Clark, F.A. (1958) Resistance to the root-knot disease of tobacco. *Tobacco Science* 2, 53–63.

Church, G.T., Simpson, C.E., Burow, M.D., Paterson, A.H. and Starr, J.L. (2000) Use of RFLP markers for identification of individuals homozygous for resistance to *Meloidogyne arenaria* in peanut. *Nematology* 2, 575–580.

Cook, C.G., Robinson, A.F. and Nampken, L.N. (1997) Tolerance to *Rotylenchulus reniformis* and resistance to *Meloidogyne incognita* in high-yielding breeding lines of upland cotton. *Journal of Nematology* 29, 322–328.

Cook, R. and Evans, K. (1987) Resistance and tolerance. In: Brown, R.H. and Kerry, B.R. (eds) *Principles and Practice of Nematode Control in Crops*. Academic Press, New York, pp. 179–231.

De Waele, D., Sagi, L. and Swennen, R. (1994) Prospects to engineer nematode resistance in banana. In: Valmayor, R.V., Davide, R.G., Stanton, J.M., Treverrow, N.L. and Roa, V.N. (eds) *Banana Nematodes and Weevil Borers in Asia and the Pacific: Proceedings of a Conference-workshop on Nematodes and Weevil Borers Affecting Bananas in Asia and the Pacific*, Serdang, Selangor, Malaysia, 18–22 April, 1994. INIBAP/ASPNET, pp. 204–216.

Duncan, L.W. and Cohn, E. (1990) Nematode parasites of citrus. In: Luc, M., Sikora, R.A. and Bridge, J. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*.

CAB International, Wallingford, UK, pp. 321–346.

Ellenby, C. (1954) Tuber forming species and varieties of the genus *Solanum* tested for resistance to the potato root eelworm *Heterodera rostochiensis* Wollenweber. *Euphytica* 3, 195–202

Evans, K., Trudgill, D.L. and Webster, J.M. (eds) (1993) *Plant Parasitic Nematodes in Temperate Agriculture*. CAB International, Wallingford, UK, 648 pp.

Fassuliotis, G. (1979) Plant breeding for root-knot nematode resistance. In: Lambert, F. and Taylor, C.E. (eds) *Root-knot Nematodes* (Meloidogyne *species*) *Systematics, Biology and Control*. Academic Press, New York, pp. 425–453.

Fenoll, C., Grundler, F.M.W. and Ohl, S.A. (eds) (1997) *Cellular and Molecular Aspects of Plant Nematode Interactions*. Kluwer Academic Publishers, Dordrecht, The Netherlands, 286 pp.

Fortnum, B.A., Krausz, J.P. and Conrad, N.G. (1984) Increasing incidence of *Meloidogyne arenaria* in flue-cured tobacco in South Carolina. *Plant Disease* 68, 244–245.

Gowen, S.R. (1993) Possible approaches for developing nematode resistance in bananas and plantains. In: Ganry, J. (ed.) *Breeding Banana and Plantain for Resistance to Diseases and Pests*. CIRAD/INIBAP, Montpellier, France, pp. 123–128.

Gowen, S.R. (1994) A review of control strategies for banana pests as part of the research priorities at NRI and associated institutes in the UK. In: Valmayor, R.V., Davide, R.G., Stanton, J.M., Treverrow, N.L. and Roa, V.N. (eds) *Banana Nematodes and Weevil Borers in Asia and the Pacific: Proceedings of a Conference-workshop on Nematodes and Weevil Borers Affecting Bananas in Asia and the Pacific*. Serdang, Selangor, Malaysia, 18–22 April, 1994. INIBAP/ASPNET, pp. 147–158.

Gowen, S. and Quénéhervé, P. (1990) Nematode parasites of bananas, plantains and abaca. In: Luc, M., Sikora, R.A. and Bridge, J. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, UK, pp. 431–460.

Holbrook, C.C., Timper, P. and Xue, H.Q. (1999) Evaluation of the core collection approach for identifying resistance to *Meloidogyne arenaria* in peanut. *Proceedings American Peanut Research and Education Society* 31, 69 (Abstract).

Hussey, R.S. and Boerma, H.R. (1989) Tolerance in maturity groups V–VII soybean cultivars to *Heterodera glycines*. *Supplement to Journal of Nematology* 21, 686–692.

Kaloshian, I., Williamson, V.M., Miyo, G., Lawn, D.A. and Westerdahl, B.B. (1996) 'Resistance-breaking' nematodes identified in California tomatoes. *California Agriculture* 50, 18–19.

Karamura, D.A. and Karamura, E.B. (1994) *A Provisional Checklist of Banana Cultivars in Uganda*. National Agricultural Research Organisation, Uganda/INIBAP, Montpellier, France, 28 pp.

Luc, M., Bridge, J. and Sikora, R.A. (1990) Reflections on nematology in subtropical and tropical agriculture. In: Luc, M., Bridge, J. and Sikora, R.A. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, UK, pp. xi–xvii.

Milligan, S.B., Bodeau, J., Yaghoobi, J., Kaloshian, I., Zabel, P. and Williamson, V.M. (1998) The root-knot resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine rich repeat family of plant genes. *Plant Cell* 10, 1307–1319.

Moore, E.L. (1960) Some problems and progress in the breeding and selection of plants for nematode resistance. In: Sasser, J.N. and Jenkins, W.R. (eds) *Nematology: Fundamentals and Recent Advances with Emphasis on Plant Parasitic and Soil Forms*. The University of North Carolina Press, Chapel Hill, North Carolina, pp. 454–460.

Netscher, C. and Sikora, R.A. (1990) Nematode parasites of vegetables. In: Luc, M., Sikora, R.A. and Bridge, J. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, UK, pp. 237–283.

Niblack, T.L., Hussey, R.S. and Boerma, H.R. (1986) Effects of environments, *Meloidogyne incognita* inoculum levels, and *Glycine max* genotype on root-knot nematode–soybean interactions in field microplots. *Journal of Nematology* 18, 338–346.

Nilsson-Ehle, H. (1920) Über resistenz gegen *Heterodera schachtii* bei gewissen Gersten-Sorten, ihre Vererbungsweise und bedeutung für die Praxis. *Hereditas* 1, 1–34.

Noling, J. (2000) Effects of continuous culture of a resistant tomato cultivar on *Meloidogyne incognita* soil population density and pathogenicity. *Journal of Nematology* 32, 452 (Abstract).

Ogallo, J.L., Goodell, P.B., Eckert, J.W. and Roberts, P.A. (1997) Evaluation of NemX, a new cultivar of cotton with high resistance to *Meloidogyne incognita*. *Journal of Nematology* 29, 531–537.

Ogallo, J.L., Goodell, P.B., Eckert, J.W. and Roberts, P.A. (1999) Management of root-knot nematodes with resistant cotton cv. NemX. *Crop Science* 39, 418–421.

Opperman, C.H. and Conkling, M.A. (1998) Bioengineering resistance to plant-parasitic nematodes. In: Pederson, G.A., Windham, G.L. and Barker, K.R. (eds) *Plant–Nematode Interactions*. Agronomy Society of America, Madison, Wisconsin, pp. 239–250.

Ortiz, R. (2000) Understanding the *Musa* genome: an update. In: Karamura, E. and Vuylsteke, D. (eds) *Proceedings of the First International Conference on Banana and Plantain for Africa*. ISHS, *Acta Horticulturae*, Vol. 540, Leuven, Belgium, pp. 157–163.

Ortiz, R., Ferris, R.S.B. and Vuylsteke, D.R. (1995) Banana and plantain breeding. In: Gowen, S. (ed.) *Bananas and Plantains*. Chapman and Hall, pp. 110–146.

Page, S.L.J. and Bridge, J. (1993) Plant nematodes and sustainability in tropical agriculture. *Experimental Agriculture* 29, 139–154.

Painter, R.H. (1951) *Insect Resistance in Plants*. Macmillan, New York, 520 pp.

Pinochet, J. (1992) Breeding bananas for resistance against lesion forming nematodes. In: Gommers, F.J. and Maas, P.W.Th. (eds) *Nematology from Molecule to Ecosystem. Proceedings Second International Nematology Congress*, 11–17 August, 1990, Veldhoven, The Netherlands. European Society of Nematologists, pp. 157–169.

Pinochet, J. and Rowe, P.R. (1978) Reaction of two banana cultivars to three different nematodes. *Plant Disease Reporter* 62, 727–729.

Plowright, R.A., Coyne, D.L., Nash, P. and Jones, M.P. (1999) Resistance to the rice nematodes *Heterodera sacchari, Meloidogyne graminicola* and *M. incognita* in *Oryza glaberrima* and *O. glaberrima* × *O. sativa* interspecific hybrids. *Nematology* 1, 745–751.

Price, N.S. (1994) Field trial evaluation of nematode susceptibility within *Musa*. *Fundamental and Applied Nematology* 17, 391–396.

Rahman, M.L. (1994) New ufra-resistant rice lines. *International Rice Research Newsletter* 19, 16.

Riggs, R.D. and Schmitt, D.P. (1988) Complete characterization of the race scheme for *Heterodera glycines*. *Journal of Nematology* 20, 392–395.

Roberts, P.A. (1995) Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. *Annual Review of Phytopathology* 33, 199–221.

Roberts, P.A., May, D. and Matthews, W.C. (1986) Root-knot resistance in processing tomatoes. *California Agriculture* 40, 24–26.

Roberts, P.A., Matthews, W.C. and Veremis, J.C. (1998) Genetic mechanisms of host-plant resistance to nematodes. In: Pederson, G.A., Windham, G.L. and Barker, K.R. (eds) *Plant–Nematode Interactions*.

Agronomy Society of America, Madison, Wisconsin, pp. 209–238.

Robinson, A.F. and Percival, A.E. (1997) Resistance to *Meloidogyne incognita* race 3 and *Rotylenchulus reniformis* in wild accessions of *Gossypium hirsutum* and *G. barbadense* from Mexico. *Supplement to Journal of Nematology* 29, 746–755.

Ross, J.P. and Brim, C.A. (1957) Resistance of soybeans to the soybean cyst nematode as determined by a double row method. *Plant Disease Reporter* 41, 923–924.

Sarah, J.L. (1988) Perspectives d'avenir dans la lutte contre les nématodes parasites des bananiers et des plantains. *Nematodes and the Borer Weevil in Bananas. Present Status of Research and Outlook. Proceedings of a Workshop Held in Bujumbura, Burundi, 7–11 December 1987*. International Network for the Improvement of Bananas and Plantain, Montpellier, France, pp. 75–79.

Schmitt, D.P. and Barker, K.R. (1988) Incidence of plant-parasitic nematodes in the coastal plains of North Carolina. *Plant Disease* 72, 107–110.

Seinhorst, J.W. (1965) The relation between nematode density and damage to plants. *Nematologica* 11, 137–154.

Shepherd, J.A. and Barker, K.R. (1990) Nematode parasites of tobacco. In: Luc, M., Sikora, R.A. and Bridge, J. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, UK, pp. 493–517.

Shiga, T. and Takemata, T. (1981) Distribution of sweet potato clones with resistance to root-knot nematodes in the Pacific Islands. In: *Proceedings of the Third Research Planning Conference on Root-knot Nematodes*, Jakarta, Indonesia, pp. 64–68.

Simpson, C.E. and Starr, J.L. (2001) Registration of 'COAN' peanut. *Crop Science* 41, 918.

Smith, P.G. (1944) Embryo culture of a tomato species hybrid. *Proceedings of the American Society of Horticultural Science* 44, 413–416.

Starr, J.L., Simpson, C.E. and Lee, T.A., Jr (1998) Yield of peanut genotypes resistant to root-knot nematodes. *Peanut Science* 25, 119–124.

Stoffelen, R., Verlinden, R., Pinochet, J., Swennen, R. and De Waele, D. (2000) Screening of fusarium wilt resistant bananas to root-lesion nematodes. *InfoMusa* 9, 6–8.

Swennen, R. (1990) *Plantain Cultivation Under West African Conditions. A Reference Manual.* International Institute of Tropical Agriculture, Ibadan, Nigeria, 24 pp.

Trudgill, D.L. (1985) Potato cyst nematodes: a critical review of the current pathotype scheme. *EPPO Bulletin* 15, 273–279.

Trudgill, D.L. (1991) Resistance and tolerance of plant parasitic nematodes in plants. *Annual Review of Phytopathology* 29, 167–192.

Vrain, T.C. (1999) Engineering natural and synthetic resistance for nematode management. *Journal of Nematology* 31, 424–436.

Ware, G.W. (1994) *The Pesticide Book*. Thompson Publications, Fresno, California, 384 pp.

Ware, J.O. (1936) Plant breeding and the cotton industry. In: *Yearbook of Agriculture*. US Department of Agriculture, Washington, DC, p. 657.

Webber, H.J. and Orton, W.A. (1902) Some diseases of cowpea. II. A cowpea resistant to root knot (*Heterodera radicicola*). Bulletin no. 17, US Department of Agriculture, Bureau of Plant Industry, Washington, DC.

Whitehead, A.G. (1998) *Plant Nematode Control*. CAB International, Wallingford, UK, 384 pp.

Wilfarth, H. (1900) Ein neuer gesichtspunkt zur bekämpfung der nematoden. *Zeitschrift des Vereins des Deutschen Zucker-Industrie*, Lieferung 529, 195–204.

Williamson, V.M. (1998) Root-knot resistance genes in tomato and their potential for future use. *Annual Review of Phytopathology* 36, 277–293.

Williamson, V.M. and Hussey, R.S. (1996) Nematode pathogenesis and

resistance in plants. *Plant Cell* 8, 1735–1745.

Wingard, S.A. (1953) The nature of resistance to disease. In: *The Yearbook of Agriculture*. US Department of Agriculture, Washington, DC, pp. 165–173.

Young, L.D. (1998) Breeding for nematode resistance. In: Pederson, G.A., Windham, G.L. and Barker, K.R. (eds) *Plant and Nematode Interactions*. American Society of Agronomy, Madison, Wisconsin, pp. 187–207.

Young, L.D. and Hartwig, E.E. (1992) Problems and strategies associated with long-term use of nematode resistant cultivars. *Journal of Nematology* 24, 228–233.

Zimmerman, A. (1897) *Het groepsgewijs afsterven der koffie heesters in gesloten plantsoenem*. Taysmannia, 23pp.

Zhou, E. (1999) Resistance to *Meloidogyne incognita* in cotton. PhD dissertation, Texas A&M University, College Station, Texas, USA.

Concepts and Consequences of Resistance

2

P.A. Roberts

Department of Nematology, University of California, Riverside, CA 92521, USA

This publication is designed to guide agricultural scientists in the use of screening procedures needed to identify and quantify nematode resistance and phenotypes in breeding materials. The adoption of sound screening procedures in breeding is necessary for advancement and release of improved crop cultivars and rootstocks for nematode management programmes. The following chapters outline the various components of resistance and tolerance screening for major phytoparasitic nematode genera and species. Protocols are described for maintenance and preparation of inoculum; plant inoculation and soil infestation; evaluation of nematode reproduction and host response; and various glasshouse, microplot, and field screening and testing procedures. Reference is also made to the genetics of resistance where known and to variations in pathogenicity and virulence that are often encountered among populations of the same nematode species.

Several considerations are important in determining the objectives of breeding for nematode resistance and tolerance traits, and in choosing a selection and screening plan to achieve those objectives. The breeder must identify resistance and tolerance traits as a starting point, either directly through screening or from the previous screening work of others. The value of the traits for crop improvement must be defined as much as possible to gauge whether a significant added benefit will justify the considerable investment in breeding. The potential value added to the crop from a resistant or tolerant cultivar or rootstock is determined by several factors. The target nematode pathogens must be defined on the basis of their distribution in crop production areas,

 Plant Resistance to Parasitic Nematodes (eds J.L. Starr, R. Cook and J. Bridge)

the amount of yield loss they cause and the availability of viable safe alternative control tactics, i.e. a definition of market potential. The expression of the resistance or tolerance must be known preferably under field conditions, to determine the extent to which nematode multiplication is suppressed and crop yield loss is prevented. The nematode variability for response to the resistance or tolerance must be assessed at the species and population levels. Thus, the breadth of utility must be defined and the likelihood that resistance-breaking virulent nematode infestations exist or will develop should be considered. Furthermore, any unique attributes of the resistance or tolerance, such as expression at high soil temperatures, should be considered. The breeder will be aided greatly by knowledge of the inheritance of the resistance and tolerance trait and will be concerned about ability to introgress and advance the traits without linkages to undesirable traits. A few selected examples are provided to illustrate the principles that underpin a successful breeding effort for nematode resistance and tolerance.

Terminology

Definitions of important terms used here are provided to clarify meaning, particularly because certain terms applied to nematology are not quite the same as their meanings in classical plant pathology. These terms have been defined and described by several authorities in reviews, and the reader is referred to those references for additional descriptions (Roberts, 1982; Cook and Evans, 1987; Cook, 1991; Trudgill, 1991; Shaner *et al.*, 1992; Davis *et al.*, 2000).

A figurative explanation of common terms is given in Fig. 2.1. Most plants are immune or non-host to most nematodes. They do not allow nematode attack, often blocking initial root invasion and thereby preventing nematode development and reproduction, nor are they damaged by nematodes. For example, root-knot nematodes (*Meloidogyne* spp.) completely avoided roots of Royal Blenheim apricot; thus, the tree is immune to root-knot infection.

Resistance is used to describe the ability of a plant to suppress development or reproduction of the nematode. It can range from *low* to *moderate* (*partial* or *intermediate*) resistance, to *high* resistance. A completely or highly resistant plant allows no nematode reproduction, or only trace amounts. Partially or moderately resistant plants allow some intermediate amounts of reproduction. *Susceptibility* is used as the opposite of resistance; thus a *susceptible* plant allows normal nematode development to take place, and the expression of any associated disease. The nematode population axis in Fig. 2.1 depicts these major categories. The term resistance is also used to describe the capacity to

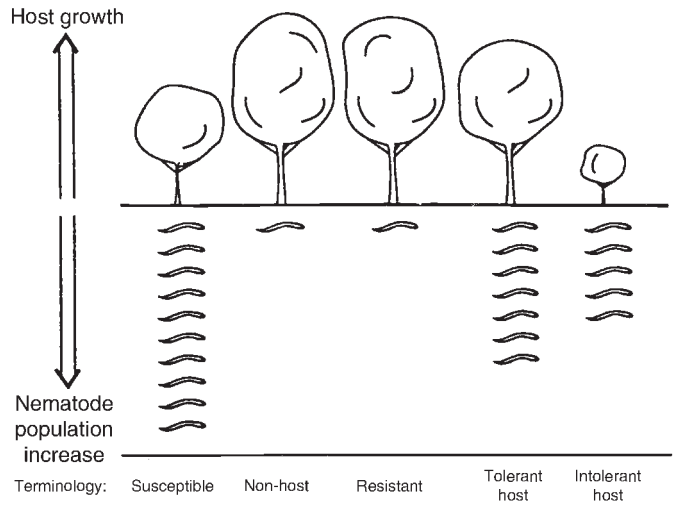

Fig. 2.1. Diagrammatic representation of terms describing plant growth response to nematodes and nematode reproduction on plants (from McKenry and Roberts, 1985).

suppress the disease, especially root-knot (Sasser *et al.*, 1984), and for plant disease in general.

Tolerance and its opposite, *intolerance*, are used to describe the ability of the plant to withstand nematode infection; *intolerant* plants are injured and grow less well or even die when infected. Resistant plants are generally more tolerant than similar plants lacking resistance, and the majority of susceptible plants are injured to some extent by most nematodes. However, resistance and tolerance are not always coupled and have been shown to be under separate genetic control in some plant–nematode interactions (Evans and Haydock, 1990; Trudgill, 1991). The concept of tolerance to nematodes is sometimes used in a broader sense to describe general plant responses to infections (Barker, 1993). A helpful discussion of concepts of tolerance is given by Wallace (1987).

Resistance as it relates to the mode of inheritance can be *monogenic* (single gene), *oligogenic* (a few genes) or *polygenic* (many genes). Resistance genes can be further defined according to the amount of the phenotypic effect they express, being either *major genes* (large effects) or *minor genes* (small effects) for phenotypic expression. Other descriptions of resistance follow Vanderplank's (1978) classification of *vertical* resistance (race-specific or *qualitative*, differentiating intraspecific variants – races, pathotypes or biotypes – of the pathogen) and horizontal resistance (*race-non-specific* or *quantitative*, effective against all variants of the pathogen). Vertical resistance is usually

controlled by one to as many as three genes and is identified with the gene-for-gene type of plant–pathogen interaction. Horizontal resistance is usually polygenically inherited as several minor genes, often with additive effects that confer a quantitative level of resistance. In general, quantitative resistance tends to be more durable or less circumvented due to selection pressure operating on the nematode parasite population. *Preinfectional* and *postinfectional* resistance reactions are referred to, respectively, as those that occur independent of infection (e.g. an impenetrable root surface) and those that occur in response to nematode infection within the root (e.g. failure to form and maintain a feeding site) (Roberts *et al.*, 1998).

In the nematode, genes for virulence are present that match resistance genes in the host plant. *Virulence* is defined according to the ability of a nematode or other pathogen to reproduce on a host plant that possesses one or more resistance genes. *Virulent* nematodes are able to reproduce, whereas *avirulent* nematodes are unable to reproduce in the presence of specific resistance gene(s). An important aspect of virulence is that populations of nematodes comprise a mixture of virulent and avirulent individuals. The frequency of each can range from one to zero. The frequency of virulent individuals will determine the potential for selection of virulence in the presence of resistant host plants. In plant pathology, the genes encoding this trait are typically called *avirulence* or *Avr* genes. Nematologists sometimes refer to avirulence genes as *genes for parasitism* or *parasitism* genes. A recent in-depth review (Davis *et al.*, 2000) of nematode genes related to parasitism and virulence has helped to better define appropriate use of these terms.

Different terms have been used to categorize the types or forms of physiological variation based on host response that are encountered within a nematode species. Terms used to categorize these differences are somewhat confusing because of a largely indiscriminate use of them for different nematode groups: *race* or *host-race* has been used for categorizing variations within soybean cyst nematode (*Heterodera glycines*); *pathotype* has been used for potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) and for the cereal cyst nematode (*Heterodera avenae*); and *biotype* for variations within the stem and bulb nematode (*Ditylenchus dipsaci*). A common, but not universal, interpretation of these terms has been that races of nematode species are separated by differential reactions on hosts of widely different plant species (e.g. races of root-knot differentiated on pepper, tobacco, cotton, groundnut and tomato), whereas pathotypes are differentiated by genes for resistance in different cultivars and breeding lines of the same or related plant species (e.g. *Globodera* spp. on potato).

Triantaphyllou (1987) offered the term biotype as a biological unit consisting of 'a group of genetically closely related individuals sharing a common biological feature or phenotypic trait,' in relation to parasitic

ability on given differential hosts. Field populations may consist of individuals of different biotypes, and combinations of biotypes comprising field populations could be designated as races. Thus, a field population could represent a race with one, two, three or more biotypes and with different proportions of each. An individual nematode may be assigned to more than one biotype, depending on the array of genes for avirulence that it possesses in relation to the genetic constitution of the host differentials used to classify the biotypes (Triantaphyllou, 1987). Roberts (1995) adapted elements of this biotype concept to provide a comprehensive framework for categorizing variants within species of root-knot nematodes (*Meloidogyne* spp.) defined by reaction to resistance genes in different host plants, and some application of this scheme has been made (Van der Beek *et al.*, 1999).

Benefits of Resistance

Host plant resistance has been prioritized over chemical, biological, cultural, and regulatory control components as a major goal for pest management (Barker *et al.*, 1994). Several advantages and benefits can be achieved by breeding crop plants resistant to injurious parasitic nematodes for production on infested land. Resistant crops provide an effective and economical method for managing nematodes in both high- and low-value cropping systems. Assuming the resistance is coupled with tolerance to nematode infection, the resistant crop is 'self-protected' and should yield well on infested land. Furthermore, resistant crops in annual cropping systems can reduce or suppress nematode population densities in soils to levels that are non-damaging to subsequent crops, thereby enabling shorter and more manageable rotations. Additional important benefits of resistant crops are their environmental compatibility, that they do not require specialized applications, and apart from preference based on agronomic or horticultural desirability, usually they do not require an additional cost input or deficit. An exception to this is the higher seed cost of, for example, resistant hybrid tomato cultivars compared with that of susceptible open pollinated cultivars. In developing countries and in low-cash crop systems, plant resistance is probably the only viable long-term solution to nematode problems. Resistance and tolerance are also amenable to integration with other management tactics, an important consideration for promoting resistance durability and when resistance or tolerance is not expressed at high levels (Roberts, 1993).

Several reviews address the current availability and/or use of resistant cultivars and rootstocks for nematode management (Sasser and Kirby, 1979; Sidhu and Webster, 1981; Roberts, 1982; Cook and Evans, 1987; Trudgill, 1991; Roberts, 1992; Roberts *et al.*, 1998; Young,

1998). Considerable success has been achieved in several programmes for identifying and evaluating resistance sources, incorporating them into commercially acceptable crop selections, and implementing them in management programmes. However, relative to the very large potential of genetic resources of nematode resistance or tolerance, only a few crop and nematode combinations utilizing plant resistance or tolerance have been developed to the point of commercial acceptance and success. Technical advances in marker-assisted breeding (see Young and Mudge, Chapter 12), resistance gene cloning and plant transformations, and in bioengineering novel types of resistance to nematodes will undoubtedly expedite development of nematode resistant crops (Milligan *et al.*, 1998; Opperman and Conkling, 1998; Boiteux *et al.*, 2000). New technologies will enable more efficient genetic transfer across conventionally difficult biological barriers, thereby broadening the prospects for major contributions to world food and fibre production through crop resistance and tolerance to nematodes.

Plant resistance has been found and developed mainly to the highly specialized parasitic nematodes such as *Globodera*, *Heterodera*, *Meloidogyne*, *Rotylenchulus*, *Tylenchulus* and *Ditylenchus*; these nematodes (except *Ditylenchus*) have a sedentary endoparasitic relationship with their host. Resistance may be effective against nematode species of different genera, against more than one species from the same genus, against a single species, or against certain within-species variants (Roberts, 1992). Resistance to less-specialized parasitic groups such as the migratory endoparasitic genera *Aphelenchoides* and *Pratylenchus* has been developed in only a few cases, and also to a few ectoparasitic nematodes, for example, to *Xiphinema* in grapevines (Meredith *et al.*, 1982; Harris, 1983). This pattern of resistance reflects the co-evolutionary forces between host and parasite; the more highly specialized relationships having resulted in specific genes for resistance and parasitism as genetic advantage was sought (Roberts, 1982; Stone, 1985). The root-browsing ectoparasitic nematodes, with less specific feeding requirements, apparently have not been a strong selection force for resistance in plant hosts in most interactions, although general tolerance traits could be useful in breeding programmes.

Nematode resistance traits in plants have come from wild plant species or their derived breeding lines. This important source of resistance genes continues to hold considerable potential for identification of additional genes. For example, focused efforts to identify additional root-knot nematode resistance genes in tomato beyond the original *Mi* gene have revealed the presence of at least eight additional genes in the tomato relative *Lycopersicon peruvianum* L., and more are likely to be characterized (Roberts *et al.*, 1998; Veremis *et al.*, 1999). However, they present a challenge in breeding work because of problems of incompatibility, particularly among the more divergent taxa or genotypes, and

the association of resistance with various undesirable traits. Embryo rescue and somatic hybridization techniques may facilitate otherwise difficult gene transfers, as will plant transformation with cloned resistance genes (Milligan *et al.*, 1998). Mutants induced by irradiation may express increased levels of resistance to nematodes, e.g. in potato, although their stability must be assessed (Tellhelm and Stelter, 1984). Plant regeneration from organs, tissues and cells can facilitate selection of somaclonal variants with desirable resistance traits arising from single nuclear changes. These tissue-culture induced genetic variations and their potential as sources of resistance to diseases and nematodes were reviewed by Litz (1986). However, this approach has not proved to be successful so far. Recently, the potential for bioengineering novel forms of resistance based on molecular approaches has gained considerable attention and holds much promise, particularly as technologies advance to enable a more streamlined approach, and some progress has been made in developing novel root-knot nematode resistance (Opperman and Conkling, 1998).

Most programmes for breeding cultivars and rootstocks resistant to nematodes have utilized simply inherited major gene resistance. Generally, this type of resistance is easier to identify and to incorporate in short backcrossing or pedigree programmes using conventional breeding techniques, compared to the polygenically controlled quantitative resistance traits that require extensive intercrossings and recrossing selections in a recurrent selection programme. Also, most breeders backcross the desired resistance to commercial lines and do not use the numbered or older genotypes in their programmes. Breeding for tolerance may be similarly confounded because of a polygenic background in most cases. These issues have been discussed in several reviews (Simmonds, 1985; Young, 1998). The successes and problems associated with breeding potatoes for resistance to the potato cyst nematodes using monogenic (i.e. *Solanum tuberosum* ssp. *andigena*) and polygenic (i.e. *S. tuberosum* ssp. *andigena* and *S. vernei*) resistance sources exemplify this issue (Phillips and Trudgill, 1983; Jones, 1985). The trend toward breeding with potentially non-durable resistance sources is perhaps not likely to change through new opportunities to transform heterologous susceptible crops with cloned resistance genes such as *Mi* for root-knot resistance, because single major gene resistance provides the most direct route to developing resistant genotypes by these methods.

Tolerance and Yield

The impact of resistance and tolerance traits on crop yield is summarized in Fig. 2.2. The general relationship of relative yield to initial

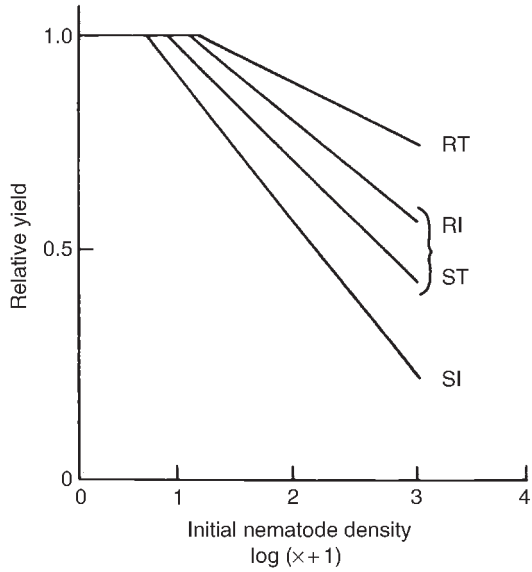

Fig. 2.2. Hypothetical damage functions (relationship between yield and initial nematode density) for crop cultivars possessing different nematode resistance and tolerance traits. RT = resistant, tolerant; RI = resistant, intolerant; ST = susceptible, tolerant; SI = susceptible, intolerant (from Roberts, 1982).

nematode density described by Seinhorst (1965) is commonly called the nematode damage function. For susceptible, intolerant crops, this relationship is linear except at very low or very high population densities. Both the position and the slope of the curve will be governed by the relative tolerance of the particular cultivar. The incorporation of resistance and tolerance will tend to: (i) shift the curve to the right, indicating a higher initial population density required to cause detectable yield reduction, and (ii) reduce the slope, because less damage per nematode results in better yields when exposed to high population densities. Knowledge of these damage function curves is desirable for successful nematode management planning based on nematode sampling and assay procedures (Duncan and Noling, 1998).

It is often difficult to select tolerance to nematodes because its accurate assessment requires comparative plant growth measurements on candidate plants challenged with nematode infestations under field conditions. A comparison in the later stages of the programme of the candidate breeding material with standard cultivar or rootstock, such as the currently preferred genotype, in both the presence and absence of nematodes is helpful. This protocol is time consuming and labour intensive relative to the quite rapid resistance evaluation procedures using assays of nematode reproduction in glasshouse or laboratory

screenings or where molecular or isozyme markers for resistance can be used (Williamson *et al.*, 1994). Some attempts have been made to correlate readily assayed markers with tolerance; for example, calcium ion concentration in potato tissues was found to be correlated with tolerance to potato cyst nematodes but was not considered a wholly reliable marker (Evans and Franco, 1979). Recent advances in the molecular analysis of quantitative trait loci (QTLs) hold promise for application to nematode tolerance selection (see Young and Mudge, Chapter 12). The incorporation of tolerance to nematodes into crop plants is most desirable and may be crucial for sustaining yield in crops where resistance is unavailable. Acala-type cotton cultivars bred on *M. incognita*-infested land have incorporated some level of tolerance to root-knot nematodes although they are still susceptible. These cultivars have enabled the growing of cotton on low to moderate infestations without the need for preplant fumigations (Roberts and Goodell, 1997). Tolerance combined with resistance is more desirable than tolerance alone because the large, healthy root systems of tolerant, susceptible plants allow nematode populations to increase. This population increase then creates problems for subsequent susceptible or intolerant crops. However, in well-managed cropping systems where strategies to control nematodes are integrated or combined, tolerant crops can be very important.

Resistance and Nematode Populations

In annual cropping systems, where one to several crops per year may be grown on the same field, nematode problems can be managed by including crops with different levels of resistance and (or) tolerance, either singly or in combination. Susceptible crops allow large increases in nematode populations from even low initial densities, although the rate of population increase declines at higher initial densities. This relationship reflects the density-dependent effect of increased competition for feeding sites and food reserves at high initial densities, and is compounded on intolerant plants by the presence of smaller root systems due to nematode injury (Ferris, 1985; McSorley, 1998). Due to these interacting factors, quite different initial densities of nematodes can produce the same final population density. The impact of tolerance on nematode multiplication rates is a trend toward greater population increase at higher initial densities because of the larger, healthier root systems of tolerant plants.

The effect of resistance on nematode multiplication is determined by the extent to which the resistance trait restricts the ability of the nematode to reproduce on the plant. As described under terminology, resistance may have minor, moderate or large effects, and these expression levels will largely determine the multiplication rate. The

relationships between initial and final *M. incognita* population densities from field experiments in the San Joaquin Valley, California are shown in Fig. 2.3 for resistant 'NemX' cotton and susceptible 'Maxxa' cotton (Ogallo *et al.*, 1999). Some reproduction occurred on the resistant genotype, and at very low initial nematode population densities, a multiplication rate (or reproductive factor, defined as final density, P_f, over initial density, P_i, or P_f/P_i ratio) of > 1 was found. However, the trend was that the multiplication rates for *M. incognita* were significantly lower on the resistant compared to on the susceptible genotype. This difference occurred over a wide range of initial population densities, from those at or near the detectable level to those well in excess of the damage threshold. The *Mi* gene in tomato is another good example of highly expressed resistance that prevents all but trace amounts of root-knot nematode reproduction, resulting in final population densities consistently much lower than initial densities (P_f/P_i ratio < 1) (Roberts and May, 1986).

Several factors can influence these seasonal population dynamics of nematodes on resistant plants. The level of resistance gene expression may be modified in the plant according to genetic constitution, environmental effects and virulence status of the nematode population. In quantitative, polygenic resistance, the numbers of genes and their additive effects will determine the level of resistance expression (Jones, 1985). Some major resistance genes have been shown to be incompletely dominant under certain conditions. For example, the resistance in common bean to root-knot nematode conferred by gene *Me2*

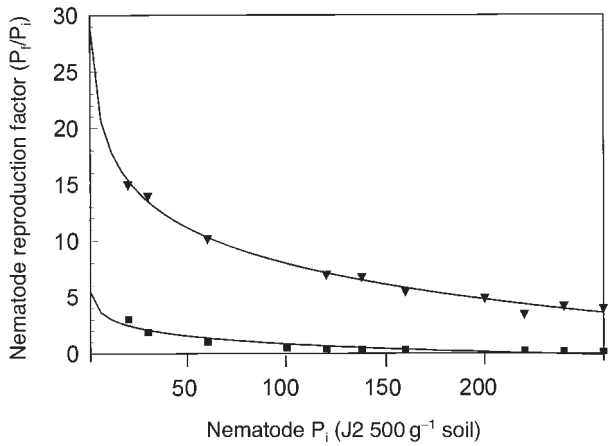

Fig. 2.3. Varying initial densities (P_i) of *M. incognita* and nematode reproduction factors (P_f/P_i) on plots planted to resistant NemX (■) and susceptible Maxxa (▼) cotton in rotations. The curves represent a logarithmic function (from Ogallo *et al.*, 1999).

(Omwega and Roberts, 1992) was found to be completely dominant at 26°C but showed an allelic dosage response of incomplete dominance at 28°C (Fig. 2.4). The parent plants homozygous for *Me2* were completely resistant at 28°C, whereas the F_1 plants heterozygous for *Me2* expressed an intermediate level of resistance. The resistance to root-knot nematodes identified recently in carrot also has a tendency toward incomplete dominance in the heterozygous condition (Simon *et al.*, 2000) although heterozygous resistance is still quite effective in preventing significant galling and forking of the carrot tap-root. Even the *Mi* gene in tomato, long recognized as a completely dominant resistance gene able to suppress root-knot nematode reproduction, has been shown to have some gene dosage response in the presence of nematode isolates that express moderate levels of virulence to *Mi* (Tzortzakakis *et al.*, 1998). The implications of incomplete gene expression are important in breeding programmes where the choice of producing hybrid versus fixed resistant cultivars must be made.

Temperature effects on resistance gene expression may not only influence expression of incomplete dominance but, at high soil temperatures, several nematode resistance genes show a loss of expression, rendering plants susceptible and allowing high nematode

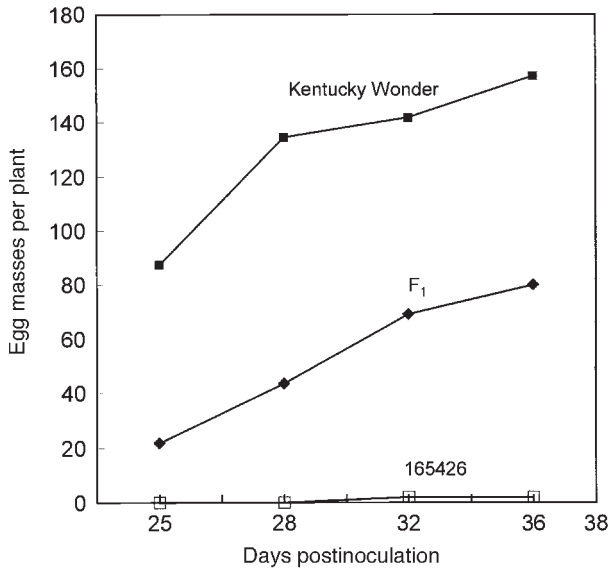

Fig. 2.4. Egg mass production of *M. incognita* on common bean genotypes Kentucky Wonder (KW), 165426 and their F_1, showing incomplete dominance at 28°C of gene *Me2* that is in the homozygous recessive, susceptible, homozygous dominant, resistant and heterozygous condition in the three genotypes, respectively (from Omwega and Roberts, 1992).

multiplication rates (Roberts *et al.*, 1998). A further complication is the greater number of nematode generations that are completed under warm growing conditions. The *Mi* gene in tomato is a classic example of resistance gene sensitivity to temperature, with almost complete loss of expression at or above 28–30°C. Breeding programmes will benefit from knowing the limitations of the resistance in response to temperature for the traits being used. In the case of tomato resistance to root-knot nematodes, temperature sensitivity of *Mi* stimulated the search for additional root-knot resistance genes. Several additional genes for resistance identified in *L. peruvianum* have been found to be heat-stable, with resistance expressed at temperatures ≥ 34°C (Roberts *et al.*, 1998; Veremis and Roberts, 2000). Thus, an important breeding objective in tomato is to develop cultivars with heat-stable root-knot nematode resistance for use in production areas that encounter high seasonal temperatures, such as Florida, USA, and the southern Mediterranean countries.

A major benefit can be gained for the protection of subsequent crops in a rotation by growing resistant cash or cover crops that suppress nematode multiplication. In Californian fields heavily infested with root-knot nematodes, highly resistant tomatoes with *Mi* typically are followed by susceptible cotton, lima bean or other crops without measurable yield loss, thereby avoiding the need to protect the second crop with nematicides. A highly resistant crop can provide at least two years of nematode control benefit. The benefits in rotation derived from growing resistant cv. NemX cotton are illustrated in Fig. 2.5. The suppression of the *M. incognita* multiplication rate on NemX, shown in Fig. 2.3, translates into significant protection for a following crop of susceptible lima bean (Fig. 2.5) or susceptible cotton (not shown) (Ogallo *et al.*, 1999). In Fig. 2.5a, the reduction in nematode population density achieved following resistant NemX is contrasted with the much higher residual populations following susceptible cv. Maxxa. The effects of growing these resistant and susceptible cotton cultivars for one or two years is also illustrated. In Fig. 2.5b, the yield of susceptible lima bean grown on these cotton plots demonstrates the protection from nematode damage gained by 1 or 2 years of preceding resistant cotton.

There is increased interest in the development and application of non-cash crops in production systems to reduce nematode infestation levels. Among these are cover crops and trap crops, used for the purpose of reducing nematode population densities in soil before planting the primary cash crop. The challenges of using these approaches in the framework of the overall farming operation and its market constraints must be considered (Noe, 1998). However, the principle of using cover

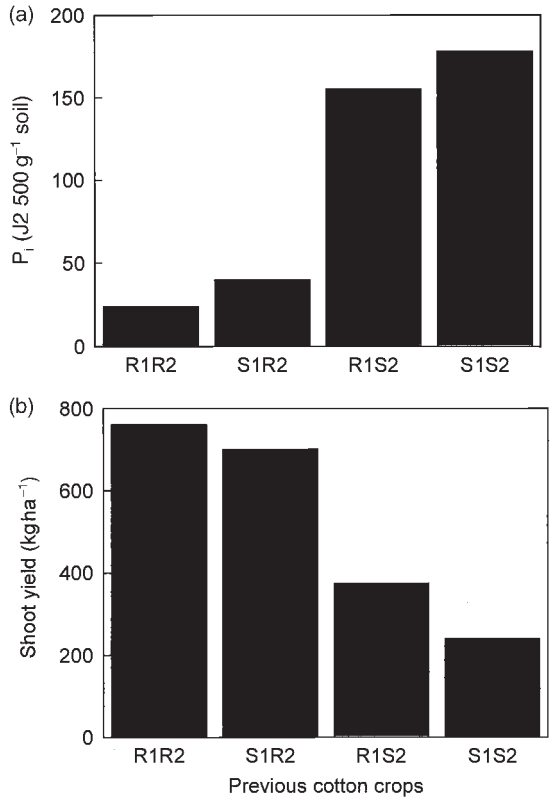

Fig. 2.5. (a) Initial (preplant) population densities (P_i) of *M. incognita* and (b) total shoot yields of lima bean planted in 1996 after resistant NemX (R) and (or) susceptible Maxxa (S) cotton were planted in 1994 (1) and 1995 (2) (from Ogallo *et al.*, 1999).

or trap crops is simple. In either case, nematodes are challenged with a resistant planting that stimulates them to become active and invade roots, but they are unable to reproduce. Plant breeding to develop improved trap and cover crops is a worthwhile goal in light of the increasing demand to find alternatives to traditional chemical control tactics. In our programme, efforts are being directed toward developing cowpea cover crop cultivars with broad-based root-knot nematode resistance, combined with multiple pest and disease resistance, heat tolerance, and plant architecture and biomass production that will benefit soil health and vegetation management.

Unlike the previously mentioned examples of resistance, in which the individual plants of the crop are similarly resistant, some crops

such as lucerne (alfalfa) are out-crossing, insect-pollinated crops with a high degree of heterozygosity. Thus, in cultivars selected for stem nematode (*Ditylenchus dipsaci*) and root-knot nematode (*M. hapla*, *M. incognita*) resistance, plant populations are mixtures of susceptible and resistant plants, with proportions of resistant plants ranging from 20% up to 98% (Lundin, 1969; Peaden *et al.*, 1976; Cook and Evans, 1987). In these heterogeneous populations, the improvement of stand and yield through resistance is mainly a result of creating a higher proportion of undamaged plants that survive longer, and this requires a different breeding approach. The impact of mixed resistant and susceptible stand on nematode population dynamics will reflect the proportion of resistant plants in the stand.

In perennial vine, tree fruit and nut crops (e.g. citrus, grapes, *Prunus* spp., walnut), successful development of cultivars and rootstocks with resistance and tolerance to several nematode groups has been achieved (Cook and Evans, 1987; Nyczepir and Becker, 1998). For these crops improved yield and longevity are the primary objectives of incorporating nematode resistance and tolerance, and the majority of forms of resistance in these crops also confer the required tolerance to infection to meet these objectives (Nyczepir and Becker, 1998).

Resistance Durability and Nematode Virulence

The reliance on single major gene resistance in plant breeding has been remarkably successful, despite concerns about its durability because of the potential for selecting virulent nematode populations. The development of resistance-breaking nematode populations has occurred in some instances (Roberts *et al.*, 1998), and this will continue to be a challenge as agriculture relies increasingly on host plant resistance for nematode management. A few brief examples follow to illustrate some of the challenges created by introducing resistant cultivars and rootstocks.

Problems may be encountered at the genus and species levels because specific resistance may change the relative impact of nematodes in a polyspecific community. For example, fruit tree cultivars or rootstocks resistant to *Meloidogyne* spp. that protect the crop from root-knot are susceptible to species from different genera (*Pratylenchus*, *Xiphinema*, *Helicotylenchus* and *Criconemella*) (Nyczepir and Becker, 1998). The reduction of root-knot in the rhizosphere soil may favour one or more of the other parasitic nematodes that could develop to damaging population levels. Resistance to only one of two or more closely related injurious nematode species that coexist in field populations may result in a competitive advantage to the species that is not restrained by the resistance. For example,

growing potatoes possessing gene *H1* for resistance to one potato cyst nematode species, *Globodera rostochiensis*, but not to a second species, *G. pallida*, has selected *G. pallida* populations, resulting in an increase in the incidence of *G. pallida* injury to potato (Cook and Evans, 1987).

Selection may occur of intraspecific variant nematode forms (races, pathotypes, biotypes) already present in field populations or through mutation, recombination or other genetic processes. The pattern emerging from long-term use of resistance and experimentation is that some nematode populations already are heterogeneous for virulence factors and resistance-breaking types can be selected quite quickly on resistant plants, whereas other populations lack virulent individuals and selection does not occur (Roberts *et al.*, 1998). That virulent individuals already exist in relatively high frequencies in field populations, even in those without exposure to resistant plantings, implies that the advantages of maintaining the virulence condition outweigh any costs to fitness. Individuals may have an array of genes for virulence in different combinations and in different sources of resistance, such as with *H. glycines* and soybean cultivars (Riggs and Schmitt, 1988). Reports of *Mi*-gene virulent populations of *Meloidogyne* spp. in tomato production areas (Kaloshian *et al.*, 1996; Tzortzakakis *et al.*, 1998) have stimulated the identification of additional genes able to resist these virulent biotypes, and efforts are underway to transfer these new genes into tomato cultivars (Roberts *et al.*, 1998). A similar situation exists in cowpea breeding for dry bean production, where recent efforts have focused on breeding cultivars with a broad-based form of root-knot resistance using two genes (Ehlers *et al.*, 2000).

Summary

In summary, breeding for resistance and tolerance to plant-parasitic nematodes has important, demonstrated potential for managing nematode pest problems throughout the world. The scheme presented in Fig. 2.6 attempts to capture the primary components and information requirements for breeding crops with nematode resistance and tolerance as discussed here. Considerations indicated in the scheme are based on the need to have appropriate methods for the evaluation of resistance and tolerance to nematodes in plants. There is no question that a considerable plant genetic resource of useful traits is available for breeding programmes, together with the potential for designing novel forms of resistance through bioengineering. This publication is aimed at stimulating successful nematode resistance and tolerance breeding programmes to tap these resources.

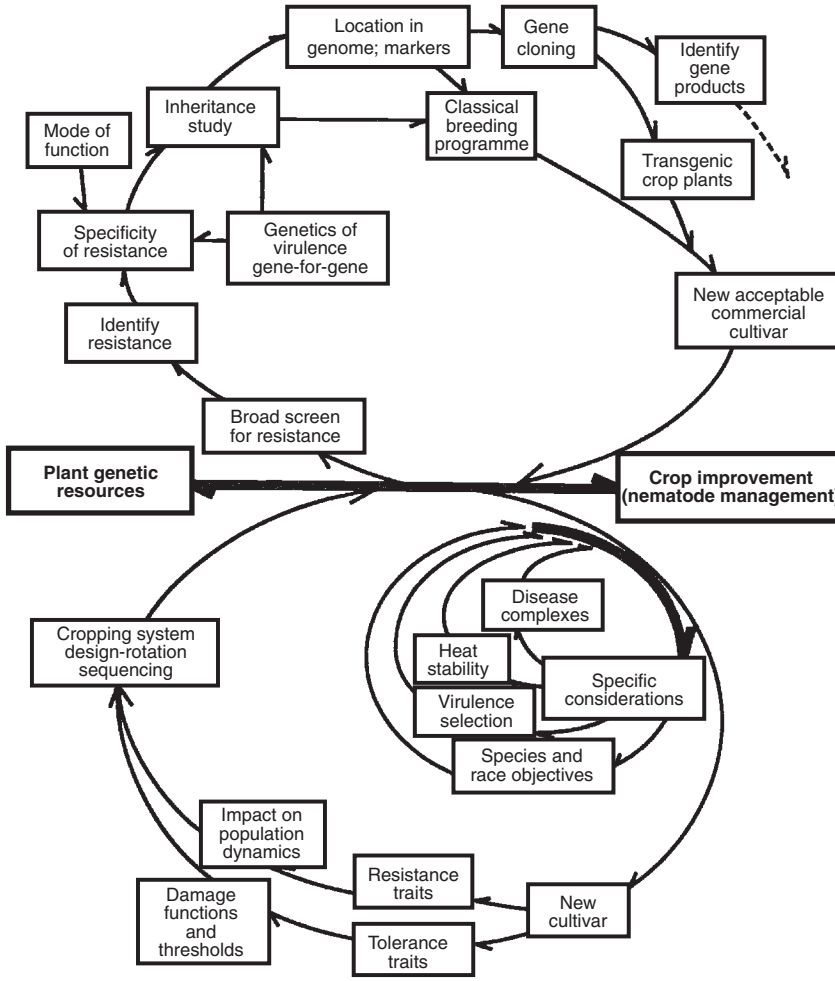

Fig. 2.6. Schematic presentation of the utilization of plant genetic resources in the development of nematode resistant or tolerant cultivars for crop improvement, emphasizing nematological research components and information requirements (from Roberts, 1992).

References

Barker, K.R. (1993) Resistance/tolerance and related concepts/terminology in plant nematology. *Plant Disease* 77, 111–113.

Barker, K.R., Hussey, R.S., Krusberg, L.R., Bird, G.W., Dunn, R.A., Ferris, H., Ferris, V.R., Freckman, D.W., Gabriel, C.J., Grewal, P.S., MacGuidwin, A.E., Riddle, D.L., Roberts, P.A. and Schmitt, D.P. (1994) Plant and soil nematodes: societal impact and focus for the future. *Journal of Nematology* 26, 127–137.

Boiteux, L.S., Belter, J.G., Roberts, P.A. and Simon, P.W. (2000) RAPD linkage map of the genomic region

encompassing the root-knot nematode (*Meloidogyne javanica*) resistance locus in carrot. *Theoretical and Applied Genetics* 100, 439–446.

Cook, R. (1991) Resistance in plants to cyst and root-knot nematodes. *Agricultural Zoology Reviews* 4, 213–240.

Cook, R. and Evans, K. (1987) Resistance and tolerance. In: Brown, R.H. and Kerry, B.R. (eds) *Principles and Practice of Nematode Control in Crops*. Academic Press, Sydney, pp. 179–231.

Davis, E.L., Hussey, R.S., Baum, T.J., Bakker, J., Schots, A., Rosso, M.N. and Abad, P. (2000) Nematode parasitism genes. *Annual Review of Phytopathology* 38, 365–396.

Duncan, L.W. and Noling, J.W. (1998) Agricultural sustainability and nematode integrated pest management. In: Barker, K.R., Pederson, G.A. and Windham, G.L. (eds) *Plant–Nematode Interactions*. American Society of Agronomy, Madison, Wisconsin, pp. 251–287.

Ehlers, J.D., Matthews, W.C., Hall, A.B. and Roberts, P.A. (2000) Inheritance of a broad-based form of root-knot nematode resistance in cowpea. *Crop Sciences* 40, 611–618.

Evans, K. and Franco, J. (1979) Tolerance to cyst-nematode attack in commercial potato cultivars and some possible mechanisms for its operation. *Nematologica* 25, 153–162.

Evans, K. and Haydock, P.P.J. (1990) A review of tolerance by potato plants of cyst-nematode attack, with consideration of what factors may confer tolerance and methods of assaying and improving it in crops. *Annals of Applied Biology* 117, 703–740.

Ferris, H. (1985) Density-dependent nematode seasonal multiplication rates and overwinter survivorship: a critical point model. *Journal of Nematology* 17, 93–100.

Harris, A.R. (1983) Resistance of some *Vitis* rootstocks to *Xiphinema index*. *Journal of Nematology* 15, 405–409.

Jones, F.G.W. (1985) Modeling multigenic resistance to potato cyst nematodes. *OEPP/EPPO Bulletin* 15, 155–166.

Kaloshian, I., Williamson, V.M., Miyao, G., Lawn, D.A. and Westerdahl, B.B. (1996) 'Resistance-breaking' nematodes identified in California tomatoes. *California Agriculture* 50, 18–19.

Litz, R.E. (1986) Germplasm modification and its potential for finding new sources of resistance to diseases. *Journal of Nematology* 18, 18–22.

Lundin, P. (1969) Breeding of lucerne for resistance to stem nematode and Verticillium wilt. *Sveriges Utsadesforenings Tidskrift* 79 (Suppl.), 133–139.

McKenry, M.V. and Roberts, P.A. (1985) *Phytonematology Study Guide*. Publication 4405, University of California Press, Oakland.

McSorley, R. (1998) Population dynamics. In: Barker, K.R., Pederson, G.A. and Windham, G.L. (eds) *Plant–Nematode Interactions*. American Society of Agronomy, Madison, Wisconsin, pp. 109–133.

Meredith, C.P., Lider, L.A., Raski, D.J. and Ferrari, N.R. (1982) Inheritance of tolerance to *Xiphinema index* in *Vitis* species. *American Journal of Enology and Viticulture* 33, 154–158.

Milligan, S.B., Bodeau, J., Yaghoobi, J., Kaloshian, I. and Williamson, V.M. (1998) The root-knot nematode resistance gene *Mi* from tomato is a member of the leucine-zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* 10, 1307–1319.

Noe, J.P. (1998) Crop- and nematode-management systems. In: Barker, K.R., Pederson, G.A. and Windham, G.L. (eds) *Plant–Nematode Interactions*. American Society of Agronomy, Madison, Wisconsin, pp. 159–171.

Nyczepir, A.P. and Becker, J.O. (1998) Fruit and citrus trees. In: Barker, K.R., Pederson, G.A. and Windham, G.L. (eds) *Plant–Nematode Interactions*. American Society of Agronomy, Madison, Wisconsin, pp. 637–684.

Ogallo, J.L., Goodell, P.B., Eckert, J. and Roberts, P.A. (1999) Management of root-knot nematodes with resistant cotton cv. NemX. *Crop Science* 39, 418–421.

Omwega, C.O. and Roberts P.A. (1992) Inheritance of resistance to

Meloidogyne spp. in common bean and the genetic basis of its sensitivity to temperature. *Theoretical and Applied Genetics* 83, 720–726.

Opperman, C.H. and Conkling, M.A. (1998) Bioengineering resistance to plant-parasitic nematodes. In: Barker, K.R., Pederson, G.A. and Windham, G.L. (eds) *Plant–Nematode Interactions*. American Society of Agronomy, Madison, Wisconsin, pp. 239–250

Peaden, R.N., Hunt, O.J., Fulkner, L.R., Griffin, G.D., Jensen, H.J. and Stanford, E.H. (1976) Registration of a multiple-pest resistant alfalfa germplasm. *Crop Science* 16, 125–126.

Phillips, M.S. and Trudgill, D.L. (1983) Variations in the ability of *Globodera pallida* to produce females on potato clones bred from *Solanum vernei* or *S. tuberosum* ssp. *andigena* CP 2802. *Nematologica* 29, 217–226.

Riggs, R.D. and Schmitt, D.P. (1988) Complete characterization of the race scheme for *Heterodera glycines*. *Journal of Nematology* 20, 392–395.

Roberts, P.A. (1982) Plant resistance in nematode pest management. *Journal of Nematology* 14, 24–33.

Roberts, P.A. (1992) Current status of the availability, development, and use of host plant resistance to nematodes. *Journal of Nematology* 24, 213–227.

Roberts, P.A. (1993) The future of nematology: integration of new and improved management strategies. *Journal of Nematology* 25, 383–394.

Roberts, P.A. (1995) Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. *Annual Review of Phytopathology* 33, 199–221.

Roberts, P.A. and Goodell, P.B. (1997) Nematodes. In: *Integrated Pest Management for Cotton in the Western Region of the United States*, 2nd edn. University of California Publ. No. 3305, pp. 91–96.

Roberts, P.A. and May, D.M. (1986) *Meloidogyne incognita* resistance characteristics in tomato genotypes developed for processing. *Journal of Nematology* 18, 353–359.

Roberts, P.A., Matthews, W.C. and Veremis, J.C. (1998) Genetic mechanisms of host plant resistance to nematodes. In: Barker, K.R., Pederson, G.A. and Windham, G.L. (eds) *Plant–Nematode Interactions*. American Society of Agronomy, Madison, Wisconsin, pp. 209–238.

Sasser, J.N. and Kirby, M.F. (1979) *Crop Cultivars Resistant to Root-knot Nematodes, Meloidogyne Species, with Information on Seed Sources*. North Carolina State University, Raleigh, North Carolina.

Sasser, J.N., Carter, C.C. and Hartman, K.M. (1984) *Standardization of Host Suitability Studies and Reporting of Resistance to Root-knot Nematodes*. North Carolina State University, Raleigh, and United States Agency for International Development.

Seinhorst, J.W. (1965) The relationship between nematode density and damage to plants. *Nematologica* 11, 137–154.

Shaner, G., Lacy, G.H., Stromberg, E.L., Barker, K.R. and Pirone, T.P. (1992) Nomenclature and concepts of pathogenicity and virulence. *Annual Review of Phytopathology* 30, 47–66.

Sidhu, G.S. and Webster, J.M. (1981) The genetics of plant–nematode parasitic systems. *The Botanical Review* 47, 387–419.

Simmonds, N.W. (1985) A plant breeder's perspective of durable resistance. *FAO Plant Protection Bulletin* 33, 13–17.

Simon, P.W., Matthews, W.C. and Roberts, P.A. (2000) Evidence for a simply inherited dominant resistance to *Meloidogyne javanica* in carrot. *Theoretical and Applied Genetics* 100, 735–742.

Stone, A.R. (1985) Co-evolution of potato cyst nematodes and their hosts: implications for pathotypes and resistance. *OEPP/EPPO Bulletin* 15, 131–137.

Tellhelm, E. and Stelter, H. (1984) Mutability of the character nematode resistance in potato. *Archiv fur Zuchtungsforschung* 14, 423–426.

Triantaphyllou, A.C. (1987) Genetics of nematode parasitism on plants. In:

Veech, J.A. and Dickson, D.W. (eds) *Vistas on Nematology*. Society of Nematologists, Hyattsville, Maryland, pp. 354–363.

Trudgill, D.L. (1991) Resistance to and tolerance of plant parasitic nematodes in plants. *Annual Review of Phytopathology* 29, 167–192.

Tzortzakakis, E.A., Trudgill, D.L. and Phillips, M. (1998) Evidence for a dosage effect of *Mi* gene on partially virulent isolates of *Meloidogyne javanica*. *Journal of Nematology* 30, 76–80.

Van der Beek, J.G., Maas, P.W.T., Janssen, G.J.W., Zijlstra, C. and van Silfhout, C.H. (1999) A pathotype system to describe intraspecific variation in pathogenicity of *Meloidogyne chitwood*. *Journal of Nematology* 31(4), 386–392.

Vanderplank, J.E. (1978) *Genetic and Molecular Basis of Plant Pathogenesis*. Springer Verlag, Berlin.

Veremis, J.C. and Roberts, P.A. (2000) Diversity of heat-stable resistance to *Meloidogyne* in Maranon races of *Lycopersicon peruvianum* complex. *Euphytica* 111, 9–16.

Veremis, J.C., van Heusden, A.W. and Roberts, P.A. (1999) Mapping a novel heat-stable resistance to *Meloidogyne* in *Lycopersicon peruvianum*. *Theoretical and Applied Genetics* 98, 274–280.

Wallace, H.R. (1987) A perception of tolerance. *Nematologica* 33, 419–432.

Williamson, V.M., Ho, J.Y., Wu, F.F., Miller, N. and Kaloshian, I. (1994) A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato. *Theoretical and Applied Genetics* 87, 757–763.

Young, L.D. (1998) Breeding for nematode resistance and tolerance. In: Barker, K.R., Pederson, G.A. and Windham, G.L. (eds) *Plant–Nematode Interactions*. American Society of Agronomy, Madison, Wisconsin, pp. 187–207.

3

Root-knot Nematodes: *Meloidogyne* Species

R.S. Hussey[1] and G.J.W. Janssen[2]

[1]Department of Plant Pathology, University of Georgia, Athens, GA 30602–7274, USA; [2]Novartis Seeds AB, PO Box 302, S-261 23, Landskrona, Sweden

Root-knot nematodes are the most economically important group of plant-parasitic nematodes worldwide, attacking nearly every crop grown (Sasser and Freckman, 1987). Their worldwide distribution, extensive host ranges, and interaction with other plant pathogens in disease complexes rank them among the major plant pathogens affecting the world food supply (Sasser, 1980). Four species, *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, account for 95% of all root-knot nematode infestations in agricultural land with *M. incognita* the most economically important species. These highly successful pathogens cause an estimated average crop loss of 5% worldwide and are one of the major obstacles to production of adequate supplies of food in many developing nations.

The sedentary endoparasitic root-knot nematodes are among nature's most successful parasites. These biotrophic parasites, which attack more than 2000 plant species, have evolved highly specialized and complex feeding relationships with their hosts. A successful host–parasite relationship requires root-knot nematodes to elaborately modify several root cells into feeding cells to obtain nourishment necessary for their development and reproduction (Hussey, 1985). Infective second-stage juveniles migrate in the soil and are attracted to root tips where they penetrate behind the root cap. The juveniles migrate intercellularly in the cortical tissue to the region of the root where the vascular cylinder is differentiating. The juveniles inject secretory proteins produced in their oesophageal gland cells through a stylet into five to seven undifferentiated procambial cells to transform

 Plant Resistance to Parasitic Nematodes (eds J.L. Starr, R. Cook and J. Bridge)

these root cells into very specialized feeding cells called giant-cells, which become the permanent feeding site for the parasite throughout its life cycle (Hussey *et al.*, 1994). Giant-cell formation is one of the most complex responses elicited in plant tissue by any parasite. Cells parasitized by juveniles become multinucleate by undergoing repeated karyokinesis uncoupled from cytokinesis. Each host cell fed upon enlarges dramatically; the large central vacuole is replaced by small vacuoles, the cytoplasm increases in volume and density, and the cell wall is remodelled to form elaborate ingrowths. The wall ingrowths are sites of influx of assimilates into the giant-cell to meet the nutrient demands of the feeding nematode (Hussey and Grundler, 1998). The metabolically active giant-cells are induced and maintained in susceptible hosts only by the feeding activities of the nematode. The juvenile develops into a globose adult female whose eggs are deposited in a gelatinous matrix on the surface of a galled root. This intimate relationship between the root-knot nematode and host is controlled by genetic systems of both organisms and has resulted in the evolution of resistance genes in many crop species (Sidhu and Webster, 1981). The sensitivity (tolerance) of hosts to root-knot nematode parasitism is not well documented (Hussey and Boerma, 1992) and therefore is not discussed in this chapter.

Sources of Resistance

Even though resistance to root-knot nematodes is available in several crop species, new sources of resistance are needed for some of these species to improve the level of root-knot resistance and genetic material has still not been identified for resistance in many other crop species. The transfer of resistance into an acceptable commercial cultivar is greatly simplified if resistant germplasm can be found in adapted cultivars or in advanced breeding lines or populations. Fehr (1987) recommended searching for resistance for a crop species among germplasm in the following order: (i) commercial cultivars of self-pollinators, inbred parents of hybrid cultivars, or parents of synthetic cultivars; (ii) elite breeding lines that may soon become cultivars; (iii) acceptable breeding lines with superiority for one or a few characters (i.e. germplasm lines or obsolete cultivars); and (iv) plant introductions of the cultivated species.

If a systematic search within the crop species is unsuccessful or levels of root-knot resistance identified are inadequate, germplasm accessions of wild relatives of the crop species should be screened (Boerma and Hussey, 1992). Wild relatives are usually difficult to hybridize with the crop species and will normally contribute many unacceptable characteristics together with nematode resistance to the

resulting progeny. A classic example of the use of wild relatives is the incorporation of root-knot resistance into cultivated tomato, *Lycopersicon esculentum*, from its wild relative, *L. peruvianum*. Embryo culture was required to produce the initial hybrid (Smith, 1944) and repeated backcrosses to the cultivated tomato were used to recover the desired quality and productivity traits.

General Considerations for Screening for Root-knot Resistance

Identification of root-knot nematode species

Root-knot nematode cultures can be established from a single egg mass or from a field population. Establishing cultures from a single egg mass ensures the culturing of a single species but reduces the genetic variability that might be present if a culture was started from a field population (Roberts and Thomason, 1989). Once a culture is established, the identity of the species needs to be confirmed. Accurate identification of root-knot nematode species and detection of mixed species in glasshouse cultures are problematic. Four methods are used for species identification and/or checking purity of stock cultures: (i) isozyme phenotypes of adult female nematodes; (ii) the North Carolina differential host test; (iii) the morphology of perineal patterns of adult female nematodes; and (iv) molecular diagnostics. The most reliable and widely used method for species identification is examining the isozyme phenotype of individual adult females using polyacrylamide gel electrophoresis (Esbenshade and Triantaphyllou, 1985, 1986). Isozyme patterns within species are consistent for isolates from different parts of the world, making species identification with this sensitive technique very reliable. Esterase (Fig. 3.1) and/or malate dehydrogenase patterns from single adult females are diagnostic for most common root-knot nematode species, and a combination of the two patterns is especially recommended when analysing unknown field isolates. The automated electrophoresis system, PhastSystem™ by Amersham Pharmacia Biotech (Piscataway, New Jersey, USA), has greatly simplified electrophoretic analysis and made isozyme phenotyping of single adult females a practical application (Esbenshade and Triantaphyllou, 1990). The differential host test is only used for the four common root-knot nematode species: *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla* (Hartman and Sasser, 1985). The differential hosts (cotton, *M. incognita*-resistant tobacco, pepper, watermelon, groundnut and tomato) have a predictable response to these four species and the host races of *M. incognita* and *M. arenaria* that can be used to assist in species identification and detecting mixed species in

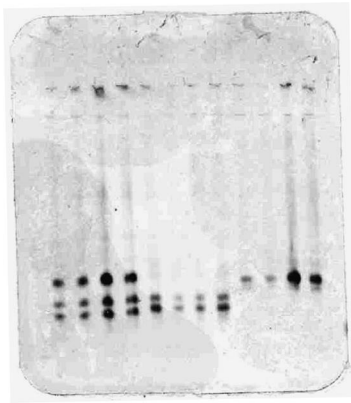

Fig. 3.1. Esterase patterns from single adult females of (lanes left to right, 1–4) *Meloidogyne javanica*, (lanes 5–8) *M. arenaria* and (lanes 9–12) *M. incognita*.

stock cultures. Guidelines for conducting a differential host test are described by Hartman and Sasser (1985). The results of a differential host test need to be confirmed by microscopic examination of perineal patterns of adult female nematodes (Taylor and Sasser, 1978; Eisenback and Triantaphyllou, 1991). The perineal pattern, which comprises cuticular striations in the posterior region of adult female nematodes, is the best diagnostic character of adult female nematodes that can be used to assist in species identification. However, this morphological character is variable within species and requires careful interpretation for accurate identification (Taylor *et al.*, 1955). Preparation of perineal patterns is described by Hartman and Sasser (1985).

Molecular techniques for identification of *Meloidogyne* species using DNA are being developed using polymerase chain reaction (PCR) technology, which allows analysis on a single nematode. For *M. chitwoodi*, *M. fallax* and *M. hapla*, PCR-based identification methods have been developed which distinguish these nematode species on ribosomal DNA (Zijlstra *et al.*, 1995; Castagnone-Sereno *et al.*, 1999). The four major *Meloidogyne* species can be identified by amplification and digestion of mitochondrial DNA (Powers and Harris, 1993). Refinement of the molecular techniques will undoubtedly expand their use in root-knot nematode identification in the future.

The host differential test is the best method for detecting mixed isolates since several thousand eggs are used to inoculate the plants and provides the best odds for detecting contaminating species. Since only 10–20 adult females are usually used for the perineal pattern or isozyme phenotype analysis, the opportunity to detect contaminating species is limited. When selecting adult female nematodes for the latter two procedures, females should be collected from different areas of the infected root system to increase the probability of detecting contaminating species. Species-specific cultures are started by isolating adult females with egg masses. Only egg masses from which the females are

positively identified to species by isozyme analysis are used to inoculate individual plants to initiate the cultures.

Rearing and preparation of inoculum

Selection of isolates

The selection of root-knot nematode isolates for use as inocula is a critical step in a screening programme. Utilization of an aggressive root-knot nematode isolate will allow detection of plant genotypes possessing the highest level of resistance. In addition, using a mixture of isolates of the same species from widely separated geographical regions as inocula is a way to capture the genetic diversity in a root-knot nematode species for screening purposes. Using a mixture of aggressive isolates will reduce variability among tests and, more importantly, facilitate identification of breeding lines with broad resistance that should have utility over a wide geographic area (Hussey and Boerma, 1981).

Maintaining pure stock cultures

Maintaining pure stock cultures is critical for the success of any screening programme. Several strategies may be used to limit the opportunities for glasshouse stock cultures to become contaminated with other isolates. Different coloured pots should be used for each species and/or isolate. This prevents contamination from inoculum that might be carried over in pots even though the pots are thoroughly cleaned. Benches constructed of wire mesh and splash guards between isolates should be used to help avoid contamination. Any unnecessary contact with pots and soil through handling and watering should be avoided. For example, spray nozzles are easily contaminated with soil that can be spread to other pots. Every time the stock cultures are subcultured, detailed records of source of inoculum and date subcultured should be kept. If a culture becomes contaminated with another isolate, the contamination can then be traced back to its source and provide information to determine if any screening tests need to be repeated because of mixed inoculum. The purity of the stock cultures must be monitored regularly by checking for mixed species every 6 months using one of the procedures discussed above. In addition, key hosts to help detect contamination can be inoculated when stock cultures are subcultured. For example, *M. incognita*-resistant tobacco can be inoculated when subculturing *M. incognita*. If galls develop on the resistant tobacco, the stock culture has probably become contaminated with another species. A standard crop rotation is also helpful to maintain clean and aggressive isolates. For isolates of *M. chitwoodi*

and *M. fallax*, a rotation on potato, tomato, and wheat is applicable, whereas for *M. hapla* wheat is replaced by lucerne.

Maintenance of the virulence and aggressiveness of root-knot nematode isolates is also important and can be accomplished by culturing the root-knot nematode species or host race on a host that maintains selection pressure on the isolate. However, for maximum egg production for inoculum, root-knot nematodes are cultured on a very susceptible host, such as tomato or aubergine. Therefore, it may be necessary to cycle some isolates through a host that would exert some selection pressure on the isolate. For example, soybean is not a good host for *M. incognita*. After culturing aggressive soybean isolates of *M. incognita* on tomato for a period of time, the stock culture is cycled through susceptible soybean to maintain the aggressiveness in the isolate used to screen soybean genotypes.

Inoculum considerations

Stock cultures of root-knot nematode isolates are maintained on hosts in the glasshouse. Second-stage juveniles, egg masses, or egg suspensions can be used as inoculum. However, the many advantages of using a suspension of eggs collected with sodium hypochlorite (the sodium hypochlorite dissolves the gelatinous matrix to free the eggs) has made this the most widely used type of inoculum for root-knot nematodes (Hussey and Barker, 1973). The advantages of collecting eggs by this procedure include: (i) it is a simple and rapid procedure for collecting large quantities of inoculum; (ii) the inoculum is easily standardized for reproducible inoculations; (iii) the inoculum can be distributed uniformly around root systems; (iv) eggs are surface sterilized; and (v) the inoculum is not adversely affected by handling in this manner. Second-stage juveniles or egg masses can also be used for inoculum. The use of juveniles as inoculum gives a very reliable estimate of timing and level of infection and may be preferred in more detailed studies of resistance. However, juveniles are more sensitive to handling than eggs and lose infectivity more rapidly with storage. Egg masses, in addition to being difficult to collect, do not allow easy standardization of inoculum, the inoculum cannot be dispersed in the soil, and they may harbour pathogenic microorganisms that would be introduced into the soil.

Collecting root-knot nematode eggs for inoculum

1. Wash soil from galled roots of stock culture plant harvested around 45–50 days (longer in winter months) after inoculation when egg production peaks; however, the timing may vary depending on glasshouse temperatures. Old plants with deteriorated roots are not a good source of inoculum.

2. Cut off roots and wash well. The cleaner the roots, the easier it will be to collect the eggs by sieving. For large root systems only process half at a time.
3. Prepare a solution of 0.525% sodium hypochlorite (NaOCl) (equivalent to 0.23% Cl). Higher concentrations of NaOCl will reduce egg viability (Hussey and Barker, 1973).
4. Place washed roots in a 1 litre container, add 200 ml of the 0.525% NaOCl solution and seal top. Vigorously shake container manually for 3.5 min. Do not expose the eggs to the NaOCl solution for any longer than 4 min. Overexposure of eggs to NaOCl will reduce egg viability.
5. After shaking for 3.5 min, quickly pass the NaOCl solution through a 200-mesh (75 μm-pore) sieve nested in a 500-mesh (25 μm-pore) sieve. After pouring out the NaOCl solution, fill the container with the roots with water and set it aside. After the NaOCl solution has passed through the sieves, remove the 200-mesh sieve and thoroughly rinse the eggs on the 500-mesh sieve with a stream of water to remove residual NaOCl. Finally, rinse eggs from the 500-mesh sieve into a 2 litre beaker or similar vessel containing water.
6. Rinse the roots in the first container at least twice with water to remove additional eggs which are collected by sieving. When planning to collect eggs of more than one species or isolate, thoroughly soak all equipment in hot (> 50°C) water for 15 min between collections.
7. Determine the concentration of eggs by removing three 1-ml samples while stirring the egg suspension, counting the eggs in each sample under a stereomicroscope, and using the average to represent the number of eggs per ml. Finally, adjust the volume of water to dilute eggs to 1000 per ml for inoculation.

Inoculating plants

The number of root-knot nematode eggs to use for inoculum will depend on the size of the container the plants are grown in, the suitability of the plant species as a host for the nematode, environmental conditions, and possibly other factors. For these reasons, it is necessary to conduct preliminary tests to determine the optimum inoculum concentration for each root-knot nematode–host combination (Hussey and Boerma, 1981). The concentration of inoculum for eggs collected with 0.525% NaOCl is usually based on an expected hatch of 20–25% although a higher percentage of eggs can hatch. Hatch following egg collection using NaOCl is highest with older egg masses which have a high proportion of embryonated eggs (Ehwaeti *et al.*, 1998). For juveniles an inoculum density of 1 to 2 juveniles cm^{-3} of soil is a good starting point.

For small tests, eggs can be dispensed easily and accurately by using a calibrated bottle-top dispenser (10-ml volume) attached to a 2-l

bottle, or for large tests a digital dispensing pump is more practical (Fig. 3.2). The stock inoculum should be continuously stirred to keep eggs in suspension or, in the case of inoculating large studies using the digital dispensing pump, the eggs are kept suspended with a magnetic stirrer or air pump.

Eggs can be added to a depression in the soil at the time of transplanting seedlings or planting seeds, or for plants started from seed, eggs can be added to 2 or 3 depressions in the soil around the stem base of young seedlings. Eggs can be distributed in the soil, some during watering of plants. Inoculation with juveniles is delayed for a couple of weeks after seeding or transplanting until more roots are available for infection. Excessive water must be avoided for the first few days after inoculating with eggs or juveniles.

Evaluating genotypes for root-knot resistance

Resistance describes the ability of a plant to suppress root-knot nematode development and/or reproduction (see Roberts, Chapter 2). Conversely, a susceptible plant allows root-knot nematodes to reproduce freely. In practice, resistance is a relative concept, derived through genotype comparisons, and frequently includes an indication of levels of resistance within a continuum of host–nematode interactions. A highly resistant genotype supports little root-knot nematode

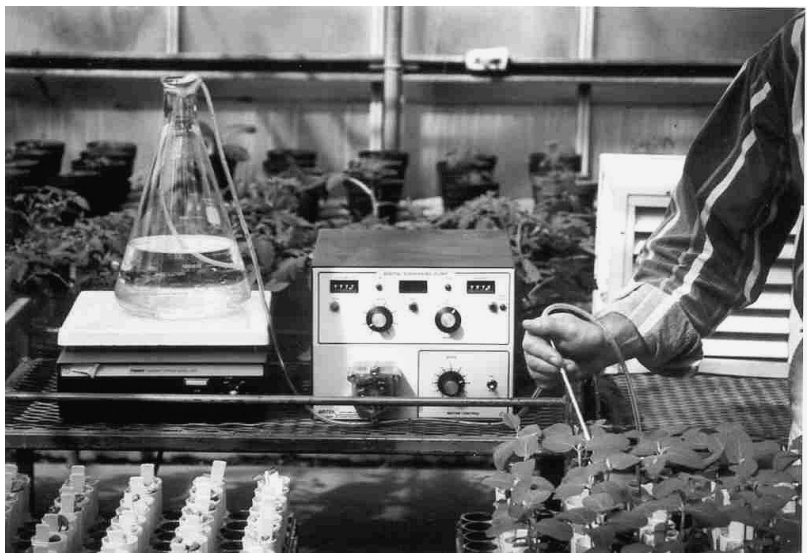

Fig. 3.2. Digital dispensing pump used to inoculate plants with root-knot nematode eggs that are kept in suspension in a 2 litre flask with a magnetic stirrer.

reproduction (< 10% of the susceptible genotype), whereas a partially resistant genotype supports an intermediate level of reproduction relative to a susceptible genotype.

Genotypes can be evaluated for root-knot resistance based on the degree of galling, egg mass number, or total number of eggs collected from the root system. However, for some crops root galling is not a completely satisfactory indicator of root-knot nematode resistance (suppressed reproduction) and usually a preliminary test should be conducted to determine if a strong correlation exists between galling and nematode reproduction (Hussey and Boerma, 1981). For galling and egg mass evaluations, an index, usually 0–5, is developed based on the internal susceptible and resistant standard genotypes included in each test (Fig. 3.3).

For advanced breeding lines it is useful to obtain quantitative data on egg number which will give a better indication of root-knot nematode resistance than either gall or egg mass numbers (Luzzi *et al.*, 1987). The procedure outlined above for obtaining egg inoculum is also used to collect eggs for determining the number of eggs per plant or per gram of root. However, for collecting eggs for this purpose a 1.05% concentration of NaOCl is used to maximize egg recovery. The higher NaOCl concentration can be used for this purpose since egg viability is not a concern. Fresh root weights should be measured before collecting eggs so that the egg data can be expressed on a per gram root basis. Egg data can be used to develop an index of resistance (total number of eggs per plant ÷ number of eggs on standard susceptible plant) for comparing genotypes.

Some eggs may be deposited within the root tissue with certain *Meloidogyne* species and host combinations, making egg recovery problematic. In these cases, eggs may be extracted by macerating the roots in 1.05% NaOCl in a blender before collecting the eggs by sieving (Veremis and Roberts, 1996b). Since some plant tissue will be retained with the eggs during sieving, eggs in a subsample can be stained with acid fuschin for easier recognition when counted under a stereomicroscope. A subsample of the collected eggs is transferred to a small beaker containing 30 ml of water, mixed with two drops of stain (3.5 g acid fuchsin, 250 ml lactic acid and 750 ml water), and heated to a boil (Byrd *et al.*, 1972). When rating reproduction by counting eggs, the length of the growth period and ambient temperature become critical. The plants should usually be harvested 40–45 days after inoculation if grown within a temperature range of 25–30°C. Therefore, periodically harvest one replication of the susceptible standard genotype in the test to monitor egg mass development and determine when to terminate the test. Well-developed egg masses are large and can be easily observed (Colour Plate 1) after staining the root system with an aqueous solution of Phloxine B (0.15 g l^{-1} water) (Dickson and Strubble, 1965). The root

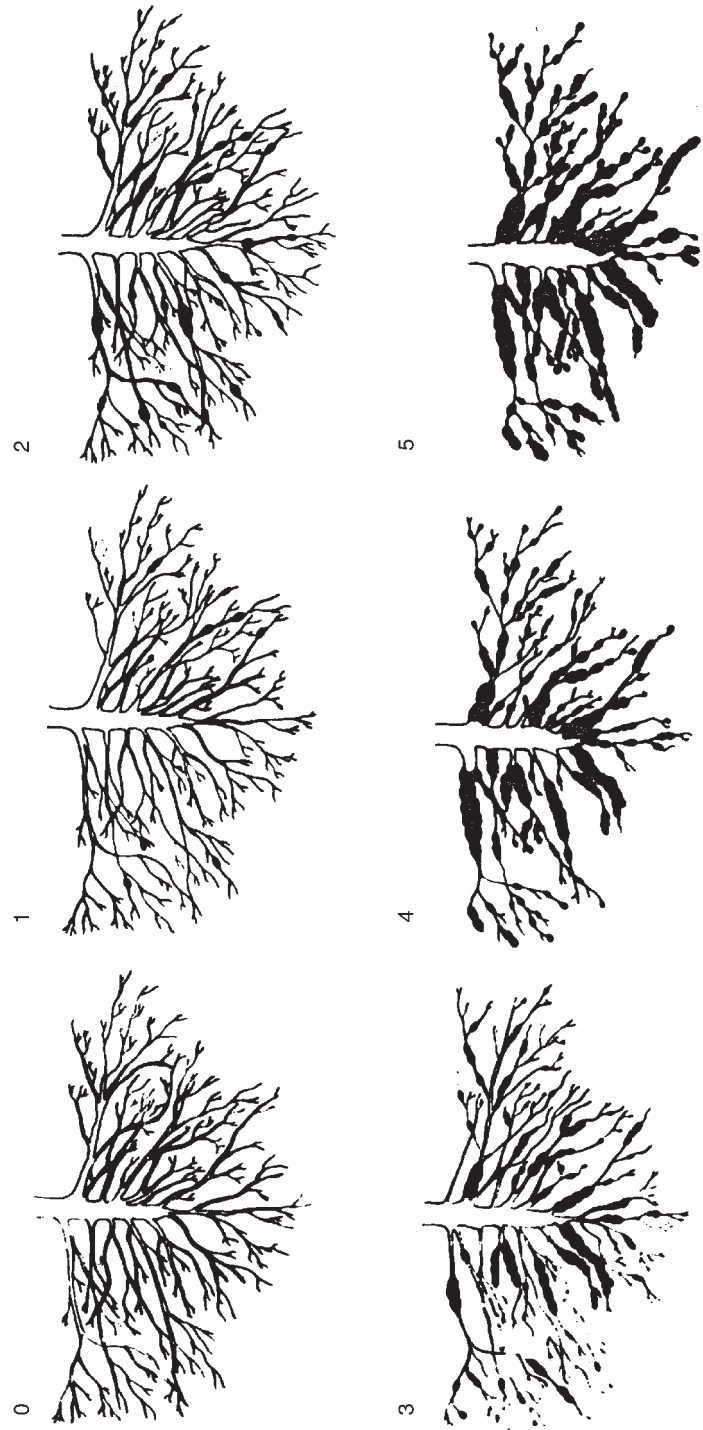

Fig. 3.3. Schematic of a root-knot nematode gall rating system where 0 = no galling, 1 = trace infection with a few small galls, 2 = < 25% roots galled, 3 = 25–50%, 4 = 51–75%, and 5 = > 75% of roots galled. (Drawing courtesy of K.R. Barker.)

system is rinsed in water to remove residual stain and reveal the egg masses stained pink (Colour Plate 1). Mature unstained egg masses are light brown in colour.

Screening Protocols

Requirements for screening

The screening protocol used to identify root-knot nematode resistant breeding lines should be capable of readily and reliably evaluating thousands of genotypes encountered in a breeding programme (Boerma and Hussey, 1992). This requirement is best fulfilled in a glasshouse environment that permits tests to be conducted throughout the year. Although breeding lines are commonly evaluated in naturally infested fields, the non-uniformity of root-knot nematode infestations in fields, seasonal restrictions, and polyspecific nematode communities are disadvantages to field screening or evaluation. Although naturally root-knot nematode-infested soil can be utilized in glasshouse tests, non-uniformity of inoculum and the potential introduction of contaminating organisms (including other nematode species) make glasshouse-cultured root-knot nematodes the preferred inocula source. Additional benefits of using glasshouse cultures as the source of inocula include standardization of inoculum levels, uniform distribution of inoculum, evaluation of resistance in localities where a specific root-knot nematode species or host race are not indigenous, and the elimination of seasonal restrictions when evaluating genotypes (Hussey and Boerma, 1981). Although 15 cm diameter pots can be used for screening genotypes in the glasshouse, their size limits the number of genotypes that can be screened at one time and allows large root systems to develop on plants. High-throughput screening is facilitated if plants have small root systems to examine for galls or for collecting eggs. Polystyrene Todd Planter Flats (Model 150–5) (Speedling, Inc., Sun City, Florida, USA), which contain 128 70-cm^3 inverted pyramid-shaped root cells (11.25 cm long) opened at the bottom, and the Ray Leach Single Cell Cone-tainer™ System (Model LD-UV SC-10) (Stuewe & Sons, Inc., Corvallis, Oregon, USA), which uses 150 cm^3 plastic tubes 20.6 cm long with open bottoms, are excellent containers for screening large numbers of genotypes for root-knot resistance (Fig. 3.4). Roots growing through the bottoms of the Todd Flats or the Cone-tainers™ are ‘air-pruned’ thus limiting the size of the root systems for easy processing. The greater depth in the Cone-tainers™ allows for more roots to be exposed to the inoculum, which can enhance the infection rate (Fig. 3.4).

Fig. 3.4. White polystyrene Todd Planter Flats and Ray Leach Single Cell Cone-tainer™ System used to screen plants for root-knot resistance.

Environmental conditions, light intensity and temperature, can vary in a glasshouse and significantly influence the reactions of test plants to root-knot nematodes. Cold temperatures will slow nematode development and retard galling, whereas high soil temperatures may alter resistant responses. Therefore, it is extremely important to include root-knot susceptible and resistant genotypes as internal standards in each test to help normalize variations in test conditions (Hussey and Boerma, 1981). The response on the susceptible standard can be monitored to determine when the test should be terminated, preferably at maximum gall or egg mass development on the susceptible standard. Since resistance is a relative concept that is derived through genotype comparison, the internal standard genotypes are required for developing a rating scale for each test. Furthermore, inclusion of a standard resistant genotype will facilitate the identification of genotypes with superior levels of root-knot resistance (with a higher level of resistance than is present in the standard resistant genotype). Finally, for thorough testing of resistance, breeding lines identified with superior root-knot resistance in the glasshouse tests need to be evaluated in root-knot nematode-infested fields in several environments.

Laboratory and growth chamber

Although laboratory assays for identifying resistant genotypes exist, these assays are usually labour intensive and limit the number of genotypes that can be readily evaluated. Root explant culture has been

suggested as an alternative method for screening for plant resistance to root-knot nematodes (Haroon *et al.*, 1993). Although screening for resistance with tissue culture techniques would not require the space of glasshouse tests, this method is not very practical for screening the large number of genotypes encountered in a breeding programme. However, another approach to screening germplasm for root-knot resistance involves growing plants in transparent growth pouches (Fassuliotis and Corley, 1967; Omwega *et al.*, 1988). This method permits the assessment of root-knot nematode reproduction in a non-destructive manner, enabling resistant plants to be propagated following their identification. The plants are grown in the transparent pouches in a growth chamber under a controlled environment and egg masses are stained with erioglaucine dye for ease of counting.

Glasshouse screening protocols

University of Georgia soybean screening protocol

This soybean improvement programme has evolved to being able to screen over 13,000 genotypes for root-knot nematode resistance on a year-round basis in the glasshouse. Three seeds of each genotype are planted in Cone-tainers™ (Model LD-UV SC-10) filled with fumigated sandy loam soil to within 5 cm of the top and then covered with 2.5 cm of fumigated sand. Ten Cone-tainers™ each of a susceptible and a resistant standard cultivar are included in each test. Forty-nine Cone-tainers™ are placed in a RL-98 tray, filling every other row of the tray. The trays (45) are placed on a greenhouse bench under supplemental light provided by 400 W metal halide lamps and under an automatic irrigation system. At 7–10 days after planting, the seedlings are thinned to one seedling per Cone-tainer™ and inoculated with 3000 root-knot nematode eggs collected with 0.5% NaOCl. Each seedling is inoculated with a 3–5 ml (depending on egg concentration) egg suspension added to a 2–3 cm depression in the soil around the base of the seedling stem using a digital dispensing pump (Fig. 3.2). The plants are manually lightly watered for 1–2 days after inoculation before turning on the automatic irrigation system.

At 30 days after inoculation, roots of two of the standard susceptible and resistant cultivars are examined for galls to assess whether to begin evaluating the entire test. For evaluation, the shoots are excised and the root systems removed from the Cone-tainers™ and washed free of soil. If there is a need to grow superior resistant genotypes for crossing or seed collection, the shoots are not removed and the seedlings can be transplanted into large pots after scoring the roots for gall development. The number of galls on the susceptible and resistant standard cultivars are used to develop a gall index of 1 (< 10 galls per

plant) to 5 (> 90 galls per plant) for evaluating the genotypes. Each year progeny of new crosses are sequentially screened through three rounds. Recurrent screening with two replications for root-knot resistance is used in the early segregating generations of a cross. After two rounds to eliminate the susceptible genotypes, the advanced breeding lines are retested for root-knot resistance using three replications and field evaluated for yield and agronomic performance.

CPRO-DLO, Wageningen, The Netherlands, potato screening protocol

The resistance programme in potato against *M. chitwoodi*, *M. fallax* and *M. hapla* has mainly focused on screening large numbers of seedlings from wild potato accessions on a year-round basis in the glasshouse (Janssen *et al.*, 1996). Since these *Meloidogyne* species cause relatively small to no galls at all on potato, analysis is based on counting egg masses. To obtain an accurate timing and level of infection, juveniles are used as inoculum. Seeds are sown in germination soil (potting soil/silver sand mixture) and transplanted after 1 week into a square of $4 \times 4 \times 15$ cm plastic tubes (240 cm^3) which are filled with moist silver sand and slow release NPK fertilizer (Osmocote; Scott-Sierra Horticultural Products Co., Marysville, Ohio, USA). Tubes are put in containers and placed on benches in a temperature controlled glasshouse (22 ± 2°C). Each container also has susceptible and resistant standard genotypes. When plants show strong growth, mostly after 2–3 weeks, seedlings are inoculated with 400 freshly hatched juveniles in a 1 ml suspension around the base of the seedlings using an automatic syringe. Since plants and nematodes are very sensitive to too much watering, special attention is needed in the first couple of weeks after inoculation to avoid excess moisture.

From 7 weeks after inoculation, roots are washed free from soil and egg masses are stained with the Phloxine B solution. Resistant seedlings are retested by using cuttings for direct retesting or by growing cuttings to produce tubers. Plants from tubers can be tested similarly by taking tuber pieces with an eye and planting them directly in the tubes. The use of square tubes has proved to be efficient in use of space, but ingrowth of stolons into neighbouring tubes has occasionally led to contamination.

Field

Breeding lines can also be screened in field plots, which have their advantages and disadvantages. Field screening, is probably best suited for the evaluation of advanced generation breeding lines and not for initial evaluations to identify potentially useful sources of resistance. The population density of the nematode will usually be uneven in the

field, but plant vigour and yield data can be obtained for the entries. Natural infestations of *Meloidogyne* spp. can be made more uniform by planting a susceptible crop for one to two seasons prior to establishing a screening test. Alternatively, for relatively small test sites (0.1–0.5 ha) it is possible to infest the soil. Two methods that have been used with success to infest a permanent site for screening cotton breeding lines (J.L. Starr, Texas A&M University, personal communication) include adding highly infested soil and infected root fragments from glasshouse cultures to the rows by hand and incorporating to a depth of about 15 cm with a rotary tiller immediately prior to planting. Alternatively, eggs can be collected from glasshouse cultures as described above, suspended in 0.1% agar, and applied to each row (before or after planting) with a liquid fertilizer applicator, again to a depth of about 15 cm. The 0.1% agar keeps the eggs suspended and eliminates the need for constant agitation during the application process. This application method is similar to injection of fumigants. In both of these methods approximately 4000 eggs were added per metre row, with about 1 litre of egg suspension added per metre row through the fertilizer applicator.

Hill planting, where several seeds are planted in a group rather than in rows, is an efficient field screening method. Hill spacing is varied according to plant growth habit. As with glasshouse tests, standard susceptible and resistant cultivars should be included as check entries. Entries are evaluated for root-knot resistance using a gall index (Fig. 3.3) and/or by comparison of final nematode population densities.

Because of variation in initial nematode population densities it is critical to have adequate controls and replications of each line being tested. Traditional experimental designs such as randomized complete block and latin square are well suited for field screening efforts. A lattice design, in which blocks are subdivided into mini-blocks, works well and allows within-block variation to be measured. A unique approach to the problem of variation in nematode population densities is to divide the number of lines being evaluated into groups of eight, plus a susceptible and resistant genotype (Kappelman and Bird, 1981). This group of ten genotypes is then replicated four to six times within the test. Thus if 32 lines were being evaluated, there would be four groups, each with its own internal standards. The groups are planted in randomized manner, typically using a randomized complete block experimental design. For data analysis, each group is evaluated independently. This approach ensures that in relatively large field tests, each test line is compared to appropriate controls that were grown in close physical proximity to the test lines. Thus the effects of substantial variation in initial nematode population densities or variation in soil type are minimized.

Marker-assisted selection

Molecular marker technologies are beginning to be integrated into root-knot nematode resistance breeding programmes. Marker-assisted selection eliminates the time-consuming propagation of nematodes for inoculum, permits analyses of young plant tissue, is non-destructive, and is more reliable and efficient than screening with nematodes since it is a more direct method of selecting the genes conditioning the resistant trait. In addition, marker-assisted selection is useful for the rapid and efficient introgression of resistance genes from wild or non-cultivated species into improved cultivars and for the pyramiding of resistance genes in cultivars to generate multiple and more durable resistances. In tomato the *Mi* gene for resistance to *M. incognita*, *M. javanica* and *M. arenaria* is tightly linked to an acid phosphatase-1 (*Aps-1*) locus and resistant genotypes are reliably identified by assaying for a variant allele of *Aps-1* as an isozyme marker (Rick and Fobes, 1974). This was probably the first use of marker-assisted selection for nematode resistance.

DNA polymorphism is being exploited for marker-assisted selection in breeding for nematode resistance. DNA markers – restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP) and simple sequences repeats (SSR) or microsatellite – allied to resistance genes are being used to enhance our understanding of root-knot resistance and in developing resistant cultivars in several crop species (Staub *et al.*, 1996). Indeed, use of the *Aps-1* isozyme marker for screening for *Mi* resistance in tomato is being replaced with a RAPD-derived sequence characterized amplified regions (SCAR) marker since the polymerase chain reaction (PCR) assay is easy and can be done with small amounts of plant tissue (Williamson *et al.*, 1994). Root-knot resistance in soybean is multigenic and quantitative and RFLP markers have been identified that are associated with quantitative trait loci (QTL) conferring resistance to *M. incognita*, *M. javanica* and *M. arenaria* (Tamulonis *et al.*, 1997a,b,c). SSRs are codominant framework markers that can be automated for high-throughput genotyping (Staub *et al.*, 1996). SSR markers linked to the two QTL for *M. incognita* resistance are being used to select for *M. incognita* resistance in soybean to accelerate the development of root-knot resistance cultivars (Li *et al.*, 2001). DNA markers tightly linked to root-knot resistance have also been identified in groundnut (Burow *et al.*, 1996; Garcia *et al.*, 1996; Church *et al.*, 2000), tobacco (Yi *et al.*, 1998), wheat (Barloy *et al.*, 2000), peach (Lu *et al.*, 1998) and potato (Brown *et al.*, 1996; van der Voort *et al.*, 1999). As molecular maps for more crop species are developed, additional DNA markers linked to root-knot resistance genes will be identified. Adaptation of molecular marker technologies for large-scale

screening for nematode resistance will depend on the relative cost and time required for each procedure. Clearly, as DNA marker technologies become automated for high-throughput genotyping at a reasonable cost, marker-assisted selection will have a significant impact in breeding for nematode resistance for crop improvement. Application of marker-assisted selection in breeding resistance to the soybean cyst nematode is discussed in Chapter 12.

Genetics of Root-knot Resistance

Soybean

Resistance to *M. incognita*, *M. javanica* and *M. arenaria* is present in numerous soybean cultivars. More cultivars are very resistant to *M. incognita* than to *M. javanica* and *M. arenaria* and several cultivars are resistant to two or even all three of the *Meloidogyne* species (Hussey *et al.*, 1991). Partial resistance to *M. incognita* in cultivar Forrest is conditioned by a single additive gene, *Rmi1* (Luzzi *et al.*, 1994a). Plant introductions with higher levels of resistance to the three *Meloidogyne* species than present in currently grown cultivars have been identified in the Southern Soybean Germplasm Collection (Luzzi *et al.*, 1987). The inheritance of root-knot resistance was studied in two plant introductions for each *Meloidogyne* species and was determined to be conditioned in an oligogenic fashion in each plant introduction (Luzzi *et al.*, 1994b, 1995a,b). Most of the plant introductions possess unique resistance genes which, if pyramided into soybean cultivars, might provide more durable resistance to root-knot nematodes.

Tomato

Probably the most used and investigated root-knot nematode resistance gene is the *Mi* gene in tomato. *Mi*, a single dominant gene located on chromosome 6 above the isozyme marker *Aps-1*, was introgressed from the wild tomato species *L. peruvianum* and is currently present in many modern tomato cultivars. The *Mi* gene is effective against *M. incognita*, *M. arenaria* and *M. javanica*, but the resistance breaks down at temperatures above 28°C. Moreover, resistance-breaking field isolates have been identified as a result of selection towards virulence as well as among field isolates without any exposure to the resistance gene (Roberts and Thomason, 1989). Both factors have stimulated a continued search for other root-knot resistance genes, which to date have been mostly found in the *L. peruvianum* complex. The novel *Mi* genes, described as *Mi-2* to *Mi-8*, express different spectra of effectivity

towards *Mi* gene virulent *Meloidogyne* spp. isolates, as well as heat sensitivity (Yaghoobi *et al.*, 1995; Veremis and Roberts, 1996a,b; Williamson, 1998).

The *Mi* gene has been cloned after intensive and long searches by several laboratories (Williamson *et al.*, 1998). This gene encodes a member of the plant resistance protein family characterized by the presence of a nucleotide binding site and a carboxyl-terminal leucine-rich repeat region (Williamson, 1998). It was also discovered that *Mi* also confers resistance to potato aphids (Rossi *et al.*, 1998). This is the first discovery of a plant gene that conditions resistance to two diverse pests and future investigations should provide valuable insights into the mechanism of recognition and the resistant response.

Potato

Most severe problems with root-knot nematodes in potato in Europe and the USA are associated with *M. chitwoodi*, *M. fallax* and/or *M. hapla*. Resistance to these nematode species appears to be absent in currently used potato cultivars, but sources of resistance have been identified in wild *Solanum* spp. (Brown *et al.*, 1994; Janssen *et al.*, 1996). The resistance in *S. bulbocastanum* is based on a single, dominant gene *Rmc1*, which is located on chromosome 11 (Brown *et al.*, 1996) and is effective against all three root-knot nematode species. A second single, dominant resistance gene *Rmc2* has been identified in *S. fendleri* and is only effective against *M. chitwoodi* and *M. fallax*. Other resistant *Solanum* species such as *S. hougasii*, *S. stoloniferum* and *S. chacoense* bear more resistance genes with various resistance levels and mode of inheritance (Janssen *et al.*, 1997a). The existence of isolates of *M. chitwoodi* and *M. hapla* virulent on several resistant sources has already been observed. In particular, there is an extensive number of *M. hapla* putative resistant sources and these vary in expression of resistance towards different isolates (Janssen *et al.*, 1997b; Van der Beek *et al.*, 1998).

Resistance to *M. incognita*, *M. javanica* and *M. arenaria* has been identified in wild *S. sparsipilum* and appears to be based on a few genes (Gomez *et al.*, 1983). The resistance identified against *M. chitwoodi*, *M. fallax* and *M. hapla* is not effective against the tropical nematode species.

Other crops

Resistance screenings have revealed the presence of root-knot nematode resistant sources for many crops and introgression of resistance

genes into cultivated forms or commercial cultivars has been successful or is in an advanced state. Besides the earlier described examples, other economically important crops with *Meloidogyne* resistance include lucerne, *Vicia* and *Phaseolus* bean, carrot, cotton (Colour Plate 2), cowpea, groundnut (Colour Plate 3), pepper, *Prunus* fruit tree, tobacco and wheat.

For plant breeding, simply and dominantly inherited resistance is strongly preferred, especially when the source of resistance is unimproved genotypes or related wild species and requires numerous backcrosses with cultivated types. Also, a recessive mode of inheritance complicates and hampers the backcross procedure considerably. As an example, resistance to *M. hapla* in carrot is found to be controlled by two recessive genes (Wang and Goldman, 1996). Since most carrot cultivars to date are based on hybrids, a backcross programme is required for several inbred lines with large progenies in every generation and test crosses to express the resistance for selection. A search for other sources of resistance is likely to give better efficacy.

A potential threat of using simply inherited resistance could be a smaller spectrum of effectivity and hence a higher risk of selecting virulent isolates of the nematode. So far, experiences with the monogenic inherited *Meloidogyne* resistance genes in *Prunus* fruit trees and tomato show that resistance remains useful and effective in large areas despite the occurrence of virulent isolates in some locations (Roberts, 1992). In comparison with monogenic fungal resistances against foliar fungi where this threat is more common, nematode resistances will in general last longer due to longer generation times and slower dispersal. Moreover, the loss of effectivity of nematode resistance is in most cases due to selection within the present field population rather than actual mutation processes. The spread and contamination of virulent isolates as well as other *Meloidogyn*e species therefore impose a higher risk for durable use of resistance than selection pressure for virulence.

Root-knot Nematode Virulence and Pathogenicity

Intraspecific variation

The intraspecific variation of *Meloidogyne* spp. can be expressed in the plant–nematode interaction on three levels; (non-)host status, aggressiveness and virulence. In this context, plant species are good, poor or non-host for a nematode species or group within the species. Host range differences leading to race deviations have been described for *M. incognita*, *M. arenaria* (Sasser, 1980) and *M. chitwoodi* (Mojtahedi *et al.*, 1988). For other species, e.g. *M. hapla* and *M. javanica*, host range differences have been noticed but they have not led to well-defined

subdivisions. Aggressiveness reflects the reproductive ability of nematodes on a susceptible good or poor host, whereas virulence is the ability to reproduce on a resistant host. The former trait is related to general fitness factors, where in the latter case there is an interaction of a virulence gene with resistance genes in the respective parasite and host. In reality, these levels have overlapping areas due to lacking knowledge on genetic background in both nematodes and plants, the likely presence of genotypic mixtures in (field) isolates, and inconsistency of literature.

Genetics of virulence

Most information on virulence in *Meloidogyne* spp. is known with regard to the *Mi* resistance gene in tomato. In the 1950s the occurrence of resistance-breaking isolates was noticed in *M. incognita*, *M. arenaria* and *M. javanica* and designated as 'B-races' (Riggs and Winstead, 1959). In addition to the development of virulence under selective conditions, naturally resistance-breaking field populations have been observed even when they were not previously exposed to resistant cultivars (Roberts and Thomason, 1989).

Selection experiments under laboratory conditions have shown that the proportion of virulent nematodes gradually increases after each successive generation on resistant tomato plants (Netscher, 1977). Since *M. incognita*, *M. arenaria* and *M. javanica* are obligatory mitotically parthenogenetic species, mechanisms other than genetic recombination must be responsible for the increasing virulence. Triantaphyllou (1987) proposed that the action of a high frequency of mutations in minor genes affects virulence. Castagnone-Sereno *et al.* (1994a) hypothesized a gene amplification system of genomic regions or chromosomes carrying virulence alleles, but they also assumed different mechanisms involved in the acquisition of virulence between field and laboratory-selected virulent nematodes due to the observed differences in stability and spectrum of their virulence (Roberts *et al.*, 1990; Castagnone-Sereno *et al.*, 1994b).

Very recently, virulence studies of *M. chitwoodi* on resistant wild potato *S. fendleri* have shown a different process. Isolates originating from single egg masses occasionally produced on *Rmc2*-resistant plants were able to circumvent completely the resistance based on this resistance gene, but also circumvent resistances in other related and unrelated *Solanum* spp. None the less, there were also some differences between the virulent lines. The virulence against the *Rmc2* gene appears to be simply inherited, but there must be more virulence factors involved to explain the variable response of isolates on other resistance sources (Janssen *et al.*, 1998).

Role in resistance screening

There are several considerations to be made in deciding which nematode isolate(s) to use for resistance screening. First, what is the main agricultural area of the crop or target area for the resistance; and secondly, which *Meloidogyne* species are predominantly present in this area? Both of these parameters should be considered to optimize the initial number of *Meloidogyne* spp. and isolates within the species to be used for screening. In a later phase, more isolates and other *Meloidogyne* spp. can be tested on selected resistant plants as part of the evaluation. Thirdly, is there information available regarding resistant sources, and fourthly, are (partial) resistant crops in culture? A screening programme should be adjusted to detect resistance to virulent isolates against existing resistant breeding lines or cultivars. Fifthly, is complete resistance required or can partial resistance be sufficient? In crops like potato and carrot, the damage threshold level is much lower due to quality loss before actual yield losses and an almost complete resistance level is required.

Screen with mixture of isolates

Screening with a mixture of isolates of a single *Meloidogyn*e species from different geographical areas enables the detection of a broad resistance that can be used over a wide geographical area. It is especially preferred in large screening programmes for a broad purpose and when there is no information available regarding virulence groups towards existing resistant sources. Mixing isolates of different *Meloidogyn*e species should be avoided unless there is no interesting application for resistances to one or two species alone. Marull *et al*. (1994) used a mixture of isolates of *M. incognita*, *M. arenaria* and *M. javanica* to detect broad resistance in *Prunus* rootstocks.

Screen with highly aggressive isolates

When resistance has already been characterized or detected using mixtures of isolates, screening and, more importantly, evaluation of resistance should proceed with well-defined and aggressive root-knot nematode isolates. Highly aggressive isolates will discriminate genotypes with the highest level of resistance. The maintenance of aggressiveness and virulence of nematode isolates is discussed in the section on rearing and preparation of inoculum.

For a genetic analysis of resistance, it is preferable to use nematode isolates with a narrow genetic basis, e.g. originating from single egg masses. The analysis of segregation is complicated by using mixtures of avirulent and virulent nematodes.

Transgenic Plant Resistance

Transgenes and targets

There are several biotechnological strategies to introduce resistance in plants as an alternative to backcross breeding or when resistance is lacking. One approach is to clone natural resistance genes and transfer them into top-performing cultivars by transformation. So far, three nematode resistance genes have been successfully cloned, the *Hs1^{pro}* gene, from the wild beet *Beta procumbens*, which is effective against *Heterodera schachtii* (Cai *et al.*, 1997), the *Gpa2* gene from potato that confers resistance to some isolates of *Globodera pallida* (van der Vossen *et al.*, 2000) and the earlier described *Mi* gene from tomato (Williamson, 1999). One goal of cloning resistance genes is to introduce the genes into crop species damaged by these nematodes and for which no genetic source of resistance has been identified (Williamson, 1998). However, it is not certain that the resistance genes will function effectively in heterologous crop species (Williamson and Hussey, 1996; Williamson, 1998). A further limitation of this strategy is the lack of candidate genes for cloning purposes. Lastly, the use of known resistance genes may lead to an overexploitation of these genes and hence to a higher selection pressure on virulent populations as described above.

An alternative approach to induce resistance is the interference with the initiation and/or development of feeding sites. This can be achieved by activation of phytotoxic genes, like RNases, proteinase-inhibitors or genes which attenuate high metabolic activities, by a highly specific promotor which is upregulated after nematode infection (Atkinson *et al.*, 1995). A related strategy is to generate plants that express a specific pathogen avirulence gene and a resistance gene under the control of a non-specific pathogen inducible promotor. This so-called two component sensor system will be effective against any pathogen – nematodes, fungi, viruses and bacteria – that induces expression of the chosen promoter (De Wit, 1992).

A third approach is to transform plants with transgenes whose products inhibit the parasite without affecting the host plant. Potential targets for interfering with nematode parasitism of plants are proteins synthesized in the oesophageal gland cells that the nematode secretes through its stylet into plant tissues during parasitism (Davis *et al.*, 2000). An example of a transgene that could target nematode stylet secretions is the expression in plants of specific antibody genes that produce monoclonal or single chain antibodies to components of the stylet secretions that are critical for pathogenesis (De Jaeger *et al.*, 2000). Coding sequences for these immunoglobulins could be transferred into a plant resulting in synthesis of antibodies (plantibodies) inside the host tissue that can neutralize a stylet secretion that is

essential in the process of feeding site development or nematode feeding (Baum *et al.*, 1996; Rosso *et al.*, 1996). The most advanced anti-nematode strategy to date has been to target nematode gut proteinases with specific inhibitors to disrupt proper digestion by feeding nematodes (Lilley *et al.*, 1999). The proteinase inhibitor approach targets a basic component of nematode metabolism as opposed to a fundamental adaptation for plant parasitism. As progress is made in the identification of nematode parasitism genes, interfering with multiple fundamental mechanisms of parasitism by nematodes should provide an effective and durable means to develop transgenic plant resistance to nematodes (Davis *et al.*, 2000).

Prospects

The recent developments with recombinant DNA technology have created a wide spectrum of novel resistance strategies supplementary to conventional resistance gene management. Most powerful will be pyramiding distinct nematode resistance genes within a cultivar, thereby minimizing chances of selecting virulent pathotypes and possibly developing a more general resistance to nematodes. However, there are serious barriers to overcome before engineered nematode resistance can be practised in the field. Some defence strategies are dependent on highly specific promotors which are expressed solely in nematode-infected tissue. Comprehensive environmental impact and toxicological and other safety issues need to be addressed before commercial release of transgenic crops, and several crop species have shown a low success of transformation ability.

References

Atkinson, H.J., Urwin, P.E., Hansen, E. and McPherson, M.J. (1995) Designs for engineered resistance to root-parasitic nematodes. *Trends in Biotechnology* 13, 369–374.

Barloy, D., Lemoine, J., Dredryver, F. and Jahier, J. (2000) Molecular markers linked to the *Aegilops variabilis*-derived root-knot nematode resistance gene *Rkn-mn1* in wheat. *Plant Breeding* 119, 169–172.

Baum, T.J., Hiatt, A., Parrot, W.A., Pratt, L.H. and Hussey, R.S. (1996) Expression in tobacco of a functional monoclonal antibody specific to stylet secretions of the root-knot nematode. *Molecular Plant–Microbe Interactions* 9, 382–387.

Boerma, H.R. and Hussey, R.S. (1992) Breeding plants for resistance to nematodes. *Journal of Nematology* 24, 242–252.

Brown, C.R., Mojtahedi, H., Santo, G.S. and Austin-Phillips, S. (1994) Enhancing resistance to root-knot nematodes derived from wild *Solanum* species in potato germplasm. In: Zehnder, G.W., Powelson, M.L., Jansson, R.K. and Raman, K.V. (eds) *Advances in Potato Pest Biology and Management*. APS Press, St Paul, Minnesota, pp. 426–438.

Brown, C.R., Yang, C.-P., Mojtahedi, H. and Santo, G.S. (1996) RFLP analysis of resistance to Columbia root-knot nematode derived from *S. bulbocastanum* in a BC_2 population. *Theoretical and Applied Genetics* 92, 572–576.

Burow, M.D., Simpson, C.E., Paterson, A.H. and Starr, J.L. (1996) Identification of peanut (*Arachis hypogaea* L.) RAPD markers diagnostic of root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood) resistance. *Molecular Breeding* 2, 369–379.

Byrd, D.W., Jr, Ferris, H. and Nusbaum, C.J. (1972) A method for estimating numbers of eggs of *Meloidogyne* spp. in soil. *Journal of Nematology* 3, 378–385.

Cai, D., Kleine, M., Kifle, S., Harloff, H.J., Sandal, N.N., Marcker, K.A., Klein-Lankhorst, R.M., Salentijn, E.M.J., Lange, W., Stiekema, W.J., Wyss, U., Grundler, F.M.W. and Jung, C. (1997) Positional cloning of a gene for nematode resistance in sugar beet. *Science* 275, 832–834.

Castagnone-Sereno, P., Wajnberg, E., Bongiovanni, M., Leroy, F. and Dalmasso, A. (1994a) Genetic variation in *Meloidogyne incognita* virulence against the tomato *Mi* resistance gene: evidence from isofemale line selection studies. *Theoretical and Applied Genetics* 88, 749–753.

Castagnone-Sereno, P., Bongiovanni, M. and Dalmasso, A. (1994b) Reproduction of virulent isolates of *Meloidogyne incognita* on susceptible and *Mi*-resistant tomato. *Journal of Nematology* 26, 324–328.

Castagnone-Sereno, P., Leroy, F., Bongiovanni, M., Zijlstra, C. and Abad, P. (1999) Specific diagnosis of two root-knot nematodes, *Meloidogyne chitwoodi* and *M. fallax*, with satellite DNA probes. *Phytopathology* 89, 380–384.

Church, G.T., Simpson, C.E., Burow, M.D., Paterson, A.H. and Starr, J.L. (2000) Use of RFLP markers for identification of individuals homozygous for resistance to *Meloidogyne arenaria* in peanut. *Nematology* 2, 575–580.

Davis, E.L., Hussey, R.S., Baum, T.J., Bakker, J., Schots, A., Rosso, M.-N. and Abad, P. (2000) Nematode parasitism genes. *Annual Review of Phytopathology* 38, 365–396.

De Jaeger, G., De Wilde, C., Eeckhout, D., Fiers, E. and Depicker, A. (2000) The plantibody approach: expression of antibody genes in plants to modulate plant metabolism or to obtain pathogen resistance. *Plant Molecular Biology* 43, 419–428.

De Wit, P.J.G.M. (1992) Molecular characterization of gene-for-gene systems in plant–fungus interactions and the application of avirulence genes in control of plant pathogens. *Annual Review of Phytopathology* 30, 391–418.

Dickson, D.W. and Strubble, F.B. (1965) A sieving-staining technique for extraction of egg masses of *Meloidogyne incognita* from soil. *Phytopathology* 55, 497.

Ehwaeti, M.E., Phillips, M.S. and Trudgill, D.L. (1998) The viability of *Meloidogyne incognita* eggs released from egg masses of different ages using different concentrations of sodium hypochlorite. *Nematologica* 44, 207–217.

Eisenback, J.D. and Triantaphyllou, H.H. (1991) Root-knot nematodes: *Meloidogyne* species and races. In: Nickle, W.R. (ed.) *Manual of Agricultural Nematology*. Marcel Dekker, New York, pp. 191–274.

Esbenshade, P.R. and Triantaphyllou, A.C. (1985) Use of enzyme phenotypes for identification of *Meloidogyne* species. *Journal of Nematology* 17, 6–20.

Esbenshade, P.R. and Triantaphyllou, A.C. (1986) Partial characterization of esterases in *Meloidogyne* (Nematoda). *Comparative Biochemistry and Physiology* 83B, 31–88.

Esbenshade, P.R. and Triantaphyllou, A.C. (1990) Isozyme phenotypes for the identification of *Meloidogyne* species. *Journal of Nematology* 22, 10–15.

Fassuliotis, G. and Corley, E.L. (1967) Use of seed growth pouches for root-knot nematode resistance tests. *Plant Disease Reporter* 51, 482–486.

Fehr, W.R. (1987) *Principles of Cultivar Development*, Vol. 1, *Theory and Technique*. Macmillan Publishing, New York, 536 pp.

Garcia, G.M., Stalker, H.T., Shroeder, E. and Kochert, G. (1996) Identification of RAPD, SCAR, and RFLP markers tightly linked to nematode resistance genes introgressed from *Arachis cardenasii* into *Arachis hypogaea*. *Genome* 39, 836–845.

Gomez, P.L., Plaisted, R.L. and Brodie, B.B. (1983) Inheritance of resistance to *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* in potatoes. *American Potato Journal* 60, 339–351.

Haroon, S.A., Baki, A.A. and Huettel, R.N. (1993) An *in vitro* test for temperature sensitivity and resistance to *Meloidogyne incognita* in tomato. *Journal of Nematology* 25, 83–88.

Hartman, K.M. and Sasser, J.N. (1985) Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: Barker, K.R., Carter, C.C. and Sasser, J.N. (eds) *An Advanced Treatise on* Meloidogyne, Vol. II, *Methodology*. North Carolina State University, Raleigh, North Carolina, pp. 69–77.

Hussey, R.S. (1985) Host–parasite relationships and associated physiological changes. In: Sasser, J.N. and Carter, C.C. (eds) *An Advanced Treatise on Meloidogyne*, Vol. I, *Biology and Control*, North Carolina State University, Raleigh, North Carolina, pp. 143–153.

Hussey, R.S. and Barker, K.R. (1973) A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter* 57, 1025–1028.

Hussey, R.S. and Boerma, H.R. (1981) A greenhouse screening procedure for root-knot nematode resistance in soybean. *Crop Science* 21, 794–796.

Hussey, R.S. and Boerma, H.R. (1992) Tolerance in soybean. In: Riggs, R.D. and Wrather, J.A. (eds) *Biology and Management of the Soybean Cyst Nematode*, APS Press, St Paul, Minnesota, pp. 169–182.

Hussey, R.S. and Grundler, F.M.W. (1998) Nematode parasitism of plants. In: Perry R.N. and Wright, D.J. (eds) *Physiology and Biochemistry of Free-living and Plant-parasitic Nematodes*. CAB International, Wallingford, UK, pp. 213–243.

Hussey, R.S., Boerma, H.R., Raymer, P.L. and Luzzi, B.M. (1991) Resistance in soybean cultivars from maturity groups V–VIII to soybean cyst and root-knot nematodes. *Journal of Nematology* 23, 76–583.

Hussey, R.S., Davis, E.L. and Ray, C. (1994) *Meloidogyne* stylet secretions. In: Lamberti, F., De Giorgi, C. and Bird, D.M. (eds) *Advances in Molecular Plant Nematology*. Plenum Press, New York, pp. 233–249.

Janssen, G.J.W., Verkerk-Bakker, B., Van Norel, A. and Janssen, R. (1996) Resistance to *Meloidogyne hapla*, *M. fallax* and *M. chitwoodi* in wild tuber-bearing *Solanum* spp. *Euphytica* 92, 287–294.

Janssen, G.J.W., Norel, A.V., Janssen, R. and Hoogendoorn, J. (1997a) Dominant and additive resistance to the root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax* in Central American *Solanum* species. *Theoretical and Applied Genetics* 94, 692–700.

Janssen, G.J.W., Van Norel, A., Verkerk-Bakker, B. and Janssen, R. (1997b) Intra- and interspecific variation of root-knot nematodes, *Meloidogyne* spp., for resistance from wild tuber-bearing *Solanum* spp. *Fundamental and Applied Nematology* 20, 449–457.

Janssen, G.J.W., Scholten, O.E., Van Norel, A. and Hoogendoorn, J. (1998) Selection of virulence in *Meloidogyne chitwoodi* to resistance in the wild potato *Solanum fendleri*. *European Journal of Plant Pathology* 104, 645–651.

Kappelman, A.J. and Bird, L.S. (1981) Indirect selection for resistance to the fusarium wilt–root-knot nematode complex in cotton. *Crop Science* 21, 66–68.

Li, Z., Jakkula, L.R., Hussey, R.S. and Boerma, H.R. (2001) SSR mapping

and confirmation of the QTL from PI96354 conditioning soybean resistance to southern root-knot nematode. *Theoretical and Applied Genetics* (in press).

Lilley, C.J., Urwin, P.E. and Atkinson, H.J. (1999) Characterization of plant nematode genes: identifying targets for a transgenic defence. *Parasitology* 11, S63-S72.

Lu, Z.X., Sosinski, B., Reighard, G.L., Baird, W.V. and Abbott, A.G. (1998) Construction of a genetic linkage map and identification of AFLP markers for resistance to root-knot nematodes in peach rootstocks. *Genome* 41, 199–207.

Luzzi, B.M., Boerma, H.R. and Hussey, R.S. (1987) Resistance to three species of root-knot nematode in soybean. *Crop Science* 27, 258–262.

Luzzi, B.M., Boerma, H.R. and Hussey, R.S. (1994a) A gene for resistance to the southern root-knot nematode in soybean. *Journal of Heredity* 85, 484–486.

Luzzi, B.M., Boerma, H.R. and Hussey, R.S. (1994b) Inheritance of resistance to the southern root-knot nematode in soybean. *Crop Science* 34, 1240–1243.

Luzzi, B.M., Boerma, H.R. and Hussey, R.S. (1995a) Inheritance of resistance to the peanut root-knot nematode in soybean. *Crop Science* 35, 50–53.

Luzzi, B.M., Tamulonis, J.P., Boerma, H.R. and Hussey, R.S. (1995b) Inheritance of resistance to the Javanese root-knot nematode in soybean. *Crop Science* 35, 1372–1375.

Marull, J., Pinochet, J., Felipe, A. and Cenis, J.L. (1994) Resistance verification in *Prunus* selections to a mixture of thirteen *Meloidogyne* isolates and resistance mechanisms of a peach–almond hybrid to *M. javanica*. *Fundamental and Applied Nematology* 17, 85–92.

Mojtahedi, H., Santo, G.S. and Wilson, J.H. (1988) Host tests to differentiate *Meloidogyne chitwoodi* races 1 and 2 and *M. hapla*. *Journal of Nematology* 20, 468–473.

Netscher, C. (1977) Observations and preliminary studies on the occurrence of resistance breaking biotypes of *Meloidogyne* spp. on tomato. *Cahiers ORSTOM Series Biologie* 11, 173–178.

Omwega, C.O., Thomason, I.J. and Roberts, P.A. (1988) A nondestructive technique for screening bean germ plasm for resistance to *Meloidogyne incognita*. *Plant Disease* 72, 970–972.

Powers, T.O. and Harris, T.S. (1993) A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology* 25, 1–6.

Rick, C.M. and Fobes, J. (1974) Association of an allozyme with nematode resistance. *Report of the Tomato Genetics Cooperative* 24, 25.

Riggs, R.D. and Winstead, N.N. (1959) Studies on resistance in tomato to root-knot nematodes and on the occurrence of pathogenic biotypes. *Phytopathology* 49, 716–724.

Roberts, P.A. (1992) Current status of the availability, development, and use of host plant resistance to nematodes. *Journal of Nematology* 24, 213–227.

Roberts, P.A. and Thomason, I.J. (1989) A review of variability in four *Meloidogyne* spp. measured by reproduction on several hosts including *Lycopersicon*. *Agricultural Zoology Reviews* 3, 225–252.

Roberts, P.A., Dalmasso, A., Cap, G.B. and Castagnone-Sereno, P. (1990) Resistance in *Lycopersicon peruvianum* to isolates of *Mi* gene-compatible *Meloidogyne* populations. *Journal of Nematology* 22, 585–589.

Rossi, M., Milligan, S.B., Goggin, F., Kaloshian, I., Ullman, D. and Williamson, V.M. (1998) The nematode resistance gene *Mi* confers resistance to the potato aphid. *Proceedings of the National Academy of Sciences USA* 95, 9750–9754.

Rosso, M.-N., Schouten, A., Roossien, J., Borst-Vrenssen, T., Hussey, R.S., Gommers, F.J., Bakker, J., Schots, A. and Abad, P. (1996) Expression and functional characterization of a single chain FV antibody directed against secretions involved in plant nematode infection process. *Biochemical*

and Biophysical Research Communication 220, 225–263.

Sasser, J.N. (1980) Root-knot nematodes: a global menace to crop production. *Plant Disease* 64, 36–41.

Sasser, J.N. and Freckman, D.W. (1987) A world perspective on nematology: the role of the society. In: Veech, J.A. and Dickson, D.W. (eds) *Vistas on Nematology*. Society of Nematologists, Hyattsville, Maryland, pp. 7–14.

Sidhu, G.S. and Webster, J.M. (1981) The genetics of plant–nematode parasitic systems. *Botanical Review* 47, 387–419.

Smith, P.G. (1944) Embryo culture of a tomato species hybrid. *Proceedings of American Horticultural Science* 44, 431–416.

Staub, J.E., Serquen, F.C. and Gupta, M. (1996) Genetic markers, map construction, and their application to plant breeding. *HortScience* 31, 729–741.

Tamulonis, J.P., Luzzi, B.M., Hussey, R.S., Parrott, W.A. and Boerma, H.R. (1997a) RFLP mapping of resistance to southern root-knot nematode in soybean. *Crop Science* 37, 1903–1909.

Tamulonis, J.P., Luzzi, B.M., Hussey, R.S., Parrott, W.A. and Boerma, H.R. (1997b) DNA markers associated with resistance to Javanese root-knot nematode in soybean. *Crop Science* 37, 783–788.

Tamulonis, J.P., Luzzi, B.M., Hussey, R.S., Parrott, W.A. and Boerma, H.R. (1997c) DNA markers analysis of loci conditioning resistance to peanut root-knot nematode in soybean. *Theoretical and Applied Genetics* 95, 664–670.

Taylor, A.L. and Sasser, J.N. (1978) *Biology, Identification and Control of Root-knot Nematodes (Meloidogyne species).* North Carolina State University, Raleigh, North Carolina, 111 pp.

Taylor, A.L., Dropkin, V.H. and Martin, G.C. (1955) Perineal patterns of root-knot nematodes. *Phytopathology* 45, 26–34.

Triantaphyllou, A.C. (1987) Genetics of parasitism on plants. In: Veech, J.A. and Dickson, D.W. (eds) *Vistas on Nematology*. Society of Nematologists, Hyattsville, Maryland, pp. 354–363.

van der Beek, J.G., Poleij, L.M., Zijlstra, C., Janssen, R. and Janssen, G.J.W. (1998) Variation in virulence within *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* on *Solanum* spp. *Phytopathology* 88, 658–665.

van der Voort, J.N.A.M.R., Janssen, G.J.W., Overmars, H., Van Zandvoort, P.M., Van Norel, A., Scholten, O.E., Janssen, R. and Bakker, J. (1999) Development of a PCR-based selection assay for root-knot nematode resistance (*Rmc1*) by a comparative analysis of the *Solanum bulbocastanum* and *S. tuberosum* genome. *Euphytica* 106, 187–195.

van der Vossen, E.A.G., van der Voort J.N.A.M.R., Kanyuka, K., Bendahmane, A., Sandbrink, H., Baulcombe, D.C., Bakker, J., Stiekeman, W.J., Klein-Lankhorst, R.M. (2000) Homologues of a single resistance-gene cluster in potato confer resistance to distinct pathogens: a virus and a nematode. *Plant Journal* 23, 567–576.

Veremis, J.C. and Roberts, P.A. (1996a) Relationships between *Meloidogyne incognita* resistance genes in *Lycopersicon peruvianum* differentiated by heat sensitivity and nematode virulence. *Theoretical and Applied Genetics* 93, 950–959.

Veremis, J.C. and Roberts, P.A. (1996b) Identification of resistance to *Meloidogyne javanica* in the *Lycopersicon peruvianum* complex. *Theoretical and Applied Genetics* 93, 894–901.

Wang, M. and Goldman, I.L. (1996) Resistance to root knot nematodes (*Meloidogyne hapla* Chitwood) in carrot is controlled by two recessive genes. *Journal of Heredity* 87, 119–123.

Williamson, V.M. (1998) Root-knot nematode resistance genes in tomato and their potential for future use. *Annual Review Phytopathology* 36, 277–293.

Williamson, V.M. (1999) Plant nematode resistance genes. *Current Opinion in Plant Biology* 2, 327–331.

Williamson, V.M. and Hussey, R.S. (1996) Nematode pathogenesis and resistance in plants. *Plant Cell* 8, 1735–1745.

Williamson, V.M., Ho, J.Y., Wu, F.F., Miller, N. and Kaloshian, I. (1994) A PCR-based marker tightly-linked to the nematode resistance gene *Mi* in tomato. *Theoretical and Applied Genetics* 87, 757–763.

Williamson, V.M., Milligan, S.B., Bodeau, J., Yaghoobi, J., Kaloshian, I. and Zabel, P. (1998) The root-knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* 10, 1307–1319.

Yaghoobi, J., Kaloshian, I., Wen, Y. and Williamson, V.M. (1995) Mapping a new nematode resistance locus in *Lycopersicon peruvianum*. *Theoretical and Applied Genetics* 91, 457–464.

Yi, H.Y., Rufty, R.C., Wernsman, E.A. and Conkling, M.C. (1998) Mapping the root-knot nematode resistance gene (Rk) in tobacco with RAPD markers. *Plant Disease* 82, 1319–1322.

Zijlstra, C., Lever, A.E.M., Uenk, B.J. and Van Silfhout, C.H. (1995) Differences between ITS regions of isolates of root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi*. *Phytopathology* 85, 1231–1237.

4

Cyst Nematodes: *Globodera* and *Heterodera* Species

R. Cook[1] and G.R. Noel[2]

[1]Institute of Grassland and Environmental Research, Aberystwyth, SY23 3EB, Wales, UK; [2]USDA ARS, Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA

The cyst-forming nematodes include about 100 known species in six genera. All are specialized parasites of plants, including temperate, subtropical and tropical crops (Sharma, 1998). This chapter deals with the genera *Heterodera* and *Globodera* and species of economic importance controlled by use of resistance. Cyst nematodes are so named because upon death the female body undergoes a tanning process to become a resistant structure that protects eggs from hostile edaphic factors. Species differ in a number of important biological characteristics, associated with adaptation to particular agroecosystems. These characteristics include: mode of reproduction (sexual or asexual); numbers of generations per year or per crop cycle (ranging from a single generation on temperate annual crops to continuous reproduction in suitable conditions on tropical crops); differences in hatching (from responses to temperature change to a requirement for host specific stimuli from root exudates); and tolerance of abiotic stresses. There are also differences among species in numbers of host plants.

Common features of the cyst nematodes include a life cycle in which the second-stage juvenile (J2) emerges from the egg as the infective preparasitic stage that locates and penetrates host roots, migrating through cortical cells towards the stele. No further development takes place unless the J2 stimulates plant cells adjacent to vascular tissues to develop as a syncytium, the feeding site for all juvenile stages and adult females. The J2 grows and undergoes three moults. In many species adult, non-feeding males develop. Mating is necessary before the female produces eggs in some but not all species. First-formed eggs

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may be deposited into a gelatinous matrix, external to the vulva. Whether or not this occurs, some eggs are retained within the female whose dead body forms the cyst. In some species, eggs within the cyst are very persistent when conditions are not favourable for plant growth and infection. Viable eggs can be transported in this way, for example, on potato tubers and in wind-blown soil. Most of these biological differences are relevant to evaluating resistance (Table 4.1).

Identification

Cyst nematodes are usually recognized by the morphology of the adult female (cyst) and by host plant associations (Table 4.2). However, there are risks of misidentification in over-reliance on host plants to assign specific identity. For example, cereals are hosts to the cereal cyst nematode group of *Heterodera*, encompassing a number of morphologically and biologically distinct species. The genera *Heterodera* and *Globodera* are readily distinguished by generally lemon- and round-shaped cysts, respectively. Specific identification usually needs more detailed morphometric studies. Diagnostic characteristics of the cyst include its size, colour and cuticular patterning as well as features associated with the preserved genitalia. These include presence or absence of a vulval cone, the length of the vulval opening, distance between vulva and anus, the nature of the cyst wall around the vulva and anus and the detail of structures inside the vulval cone (bullae, underbridge, vaginal sheath). Specific diagnostics also rely on features of the J2, notably size, number of lateral lines on the cuticle, stylet length and shape, tail length and shape. Good taxonomic keys are available, but it is worth noting that in wild vegetation and perhaps especially in the tropics, there may be as yet undescribed species. Advice is available in many parts of the world from nematode taxonomists, and international collaboration is important to confirm specific identity.

Closely related species, for example, of potato cyst nematodes can be distinguished by isoelectric focusing of proteins and genetic-based approaches are available for most groups. Identification is now assisted by molecular approaches that can distinguish species that are agricultural pests (see, e.g. Subbotin *et al.*, 2000a). In some cases, crop pest species are not yet distinguishable from wild related species solely by molecular technologies, although this will become possible. The attractiveness of the polymerase chain reaction (PCR)-based approaches is that few specimens are needed to confirm identity and that sampling of individuals within populations will reveal differences among species.

Recognition and definition of pathotypes as a subspecific grouping depend on tests with ‘host differentials’ (plants with genetically

Table 4.1. Biological features of cyst nematodes (*Heterodera* and *Globodera* spp.) relevant to screening protocols.

Attribute	State	*H. glycines*	*H. avenae*	*H. schachtii*	*H. trifolii*	*G. rostochiensis*	*G. pallida*
Reproduction	Sexual	+	+	+		+	+
	Asexual				+		
Diapause		+	+	+	(–)	+	+
J2 emergence: response to root diffusates	+ essential (+) some response – not essential	+	(+)		–	+	+
Generations per crop		3–7	1	1–5	1–8	1(2)	1(2)
Optimum temperature (°C)	Hatch	25–30	10–22	25	15–25	20–25	15–20
	Development	26–28	15–20	21–27	15–25		
	Days to mature females	14	65–40	17	45–20		
Eggs in egg mass		+	–	+	–	–	–
Host range	Principal family	*Leguminosae*	*Gramineae*	*Chenopodiaceae* *Cruciferae*	*Leguminosae*	*Solanaceae*	*Solanaceae*
	Other families	Few	None	Very many	Many	Few	Few

Table 4.2. Key morphometric features for distinguishing cyst nematodes, *Heterodera* and *Globodera* spp. (measurements in μm).

		H. glycines	*H. avenae*	*H. schachtii*	*H. trifolii*	*G. rostochiensis*	*G. pallida*
Cyst	Shape	Lemon	Lemon	Lemon	Lemon	Round	Round
	Size	700 × 490	710 × 500	750 × 450	650 × 400	445 × 382	579 × 534
	Length : width	1.43	1.43	1.67	1.63	1.27	1.11
	Subcrystalline layer	++	++	+	+	–	–
	Egg sac	+200	0	+ few	+200	0	0
	Cuticle pattern	Rugose zigzag lines	Rugose zigzag	Minutely rugose			
	Cuticle punctation					Reticulate	
	Colour	Brown	Dark brown	Brown	Brown/dark brown	Golden yellow then brown	Pale yellow then brown
	Vulval cone	Obtuse cone	Obtuse	Prominent	Prominent	None	None
	Length vulva	50	12	> 35	47	10	11.5
	Vulva–anus			77	62	60	45
	Underbridge	++	–	Prominent	Very prominent	–	–
	Bullae	+ elongate	++ high	+	+	–	–
	Fenestration	Ambifenestrate	Ambifenestrate	Ambifenestrate	Ambifenestrate	Circumfenestrate	Circumfenestrate
	Granek's ratio	–	–	–	–	3.6	2.1
J2	Body length	470	575	470	510	468	486
	Lateral lines	4	4	4	4	4	4
	Stylet length	23	27	25	27	22	24
	Tail length	45	56		59	44	51
	Hyaline tail % true tail	50	70		60	60	50
	Stylet knobs shape of anterior face	Flat to anterior projecting	Flat to slight concave	Forward projecting	Concave anterior	Round, slight posterior slope	Forward projection
	Phasmids	Minute	Distinct	Obscure	Small, obscure		Obscure
	Phasmid to anus	10 posterior	Just post.	Just post.	Mid-tail	Mid-tail	
	Dorsal gland outlet to stylet	5.3		3–4	5–9	2.6	3.4

distinct resistance). Different names have been used for these groupings, notably 'race' for soybean cyst nematode, but pathotype for others, including both cereal and potato cyst nematodes. In this chapter, we use the currently accepted words for each species for practical relevance. These subspecific groupings based on virulence phenotypes of populations or individuals are not yet distinguishable by molecular means, but because differences in virulence are based upon the genotype, this will become possible. Potato cyst nematode species are distinguishable in this way and Rouppe van der Voort (1998) was able to distinguish between *Ro1* and *Ro5* using amplified fragment length polymorphism (AFLP). Subbotin *et al.* (2000b) could distinguish closely related species of the cereal cyst nematode group using restriction fragment length polymorphism (RFLP), but not between true pathotypes within a morphological species.

Sources of Resistance

There is a logical approach to the search for sources of resistance. As outlined for root-knot nematodes (see Hussey and Janssen, Chapter 3), resistance in existing cultivars or advanced breeding lines of the crop is likely to be more readily handled within a breeding programme. Moving to less well-adapted sources, including land races, wild relatives of the crop or related species, may increase the work necessary to transfer resistance to agronomically acceptable cultivars. Many pest species of cyst nematodes have had co-evolutionary associations with the host crop or its progenitors. Consequently, there are many host resistance genes (*R*-genes) and complementary avirulence genes (*Avr*-genes) in the nematode populations.

The study of resistant plants has identified different mechanisms by which resistance is expressed and an understanding of the mechanism may enable selection of different sources of resistance. In domestication, selection for traits that have value for consumption or use of the crop usually minimizes mechanisms providing general pest and disease resistance and tolerance. In nature, the extensive polymorphism of plant resistance and nematode avirulence genes is of adaptive value to both plant and parasite survival in heterogeneous populations. Pure line breeding for advanced agriculture may further erode defences by reducing the numbers of *R*-genes in adapted cultivars. This is more likely where variety development is done in the absence of the nematode. This is usually the case in breeders' field nurseries where nematode populations are absent or are controlled by the use of rotations with non-host species to minimize contamination of the soil with crop seeds and to provide soil uniformity for successive selection cycles. Varieties bred in such conditions may subsequently reveal their

susceptibility when exposed to nematode populations resulting from intensification of cropping.

A key consideration in resistance breeding is the extent of heterogeneity in nematode populations. It is clear that some cyst nematode populations identified in agricultural systems have one or a few avirulence genes of relevance to *R*-genes in current crop cultivars. These are identifiable as pathotypes by distinct differences in multiplication on plants with different *R*-genes. In other cases, usually where the *R*- and *Avr*-gene interactions are more diverse, identification of differences in the virulence phenotype of nematode populations is less clear. The extreme examples of these continuously variable genetic interactions may be interpreted as either qualitative or quantitative resistance, respectively.

General Considerations in Screening

General considerations should lead to tests with practically relevant outcomes, i.e. direct assessment of nematode growth and reproduction. Such tests will be practically applicable to traditional screening and be adaptable for other purposes, including screening of transgenic resistant plants. Usually, some measure of reproduction is required, although in testing the effects of transgenes, less direct assessments have been used; for example, Atkinson *et al*. (1996) demonstrated that expression of transgenes reduced the surface area of female potato cyst nematodes.

Rearing and preparation of inoculum

The choice from a variety of approaches is determined by the biology of the nematode; for example, inoculum of potato cyst nematodes can be accumulated as dried cysts. The eggs within these remain viable for long periods without special storage conditions. In other cases, for example *Heterodera avenae*, it is possible to retain viable inoculum in cysts kept moist at temperatures too low for J2 emergence. Species such as *H. glycines*, *H. schachtii* and *H. trifolii*, that deposit eggs in a matrix external to the female, require special attention to collecting eggs for inoculum, and there are differences in hatchability between eggs in matrices and those within the cyst.

Producing desired quantities of inoculum by multiplying nematodes on plants in soil may require more than one crop cycle. However, several generations can be multiplied on a single crop with species without diapause, e.g. *H. trifolii*. Plants for rearing inoculum should be grown in as clean conditions as possible to produce clean inoculum, that is, the desired nematode (species and pathotype(s)) without

contamination either by other nematodes or other plant pathogens, and to ensure that the inoculum remains free of parasites of the nematodes. Fungi infesting cysts and eggs can be problematical when cultures are maintained in field-derived soil. None the less, large quantities of good quality inoculum can be raised in fields or microplots, as well as in more fully controlled conditions in glasshouses or on artificial media.

Selection of isolates

Where there is variation within a species, it is advisable to rear inoculum of each population separately. For example, when the researcher plans to use mixed inocula to select for widely effective resistance, maintaining individually characterized populations allows the same known mixture to be used for successive tests as well as avoiding selecting particular virulence phenotypes.

It is essential to know something about variation. Mixing so-called aggressive populations of a nematode species without knowledge of the range of resistance in the tested plant material may provide the opportunity to select for resistance effective against many populations. But if done speculatively, this approach will discard sources of resistance as insufficiently effective, because they are effective against only a proportion of the inoculum. It is conceivable that such an approach could lead to discarding individual resistance sources that, if combined in a single plant, would provide the required degree of resistance. If all required *R*-genes are known to be present in an interbred plant population, then the mixed inoculum approach will be appropriate to identify plants combining the genes.

Inoculating plants

Nematodes for use as inoculum may be applied as cysts to plant culture media or as eggs and/or hatched juveniles, freed from cysts. The order of greatest control of inoculum quantity and quality is J2 > eggs > cysts; robustness in the face of environmental variation is the converse of this. The appropriate choice of inoculum should optimize these opposing trends, taking account of the extent of control over environmental conditions of the screen, number of entries for evaluation, available space, and the cost of labour.

In controlled screening tests, inoculum is usually hatched juveniles to give precise control over initial population density (P_i) and to synchronize subsequent female development. Application methods, including mixing cysts with soil, adding them in various mesh containers (to allow newly formed cysts to be distinguished from those of the

inoculum), are detailed in the later sections on each nematode species. One important factor is nematode response to plant-produced hatching factors. Potato and soybean cyst nematode populations differ in response to diffusates from roots of different potato and soybean genotypes, respectively (Sikora and Noel, 1996; Dale and de Scurrah, 1998). Such interactions introduce variability in screen results of significance to the choice of inoculum; tests using free eggs or eggs in cysts may give classifications different from tests with hatched juveniles. Applying egg suspensions or eggs within cysts may have the advantage that prolonging the root invasion period may make tests more robust to environmental variation. The key feature is to avoid applying too many nematodes, which may cause such damage from root invasion that subsequent development of plants and nematodes is adversely affected. In stressed plants and when nematodes are competing with each other for feeding sites or other resources, some genetically female J2 develop as intersexes, others may fail to develop.

Generally, the investigator will know how to avoid excessive competition. It is worth allowing plants to develop some roots before adding nematodes, then to limit the period of exposure to infective J2 and to feed plants after inoculation. With some species, particularly cereals and grasses in their vegetative growth phase, and other plants with indeterminate growth, excessive root growth can make subsequent extraction and counting of nematodes difficult.

Evaluating interactions

Resistance

Resistance is a relative concept expressing the effects of plant genotype on nematode reproduction. Even when resistance of major effect is being evaluated, it should be related to reproduction on known susceptible control plants and, where possible, with known resistant plants. Resistance is often described by some form of index of nematode reproduction, usually a Reproduction Index ($RI = 100P_f/P_i$, where P_i and P_f are the initial and final nematode numbers) used in comparison with that on controls of known response. Usually, evaluation is of new generation females: it is easier to count these before they have tanned and formed cysts. When plants have been grown in a rooting medium (soil or other), this may be shaken from the roots before examination for presence/absence or counting of white females. More accurately, root systems may be washed and females adhering to roots as well as those extracted by flotation and sieving from the soil are counted. Various techniques are used to extract females from the soil, from sophisticated apparatus to simple decantation and sieving. Fuller references detailing and comparing techniques are available (Eisenback and Zunke,

1998). It is important that the rooting medium is one from which new generation females and cysts can be readily separated. When soil is used, high sand contents are preferred. The sophistication and accuracy applied to assessment will reflect the known contrast between resistant and susceptible phenotypes and the purpose of the screen. Comparisons of cysts visible on the surfaces of root balls, removed from pots (Colour Plate 6), are sufficient to distinguish between susceptible plants with several hundred visible females and resistant plants with no or few females. In other cases, precise counts are required from known amounts of root.

In some cases, nematode reproduction is measured by counting eggs in cysts extracted from the soil; this is more labour intensive but acceptable when inoculum was applied as eggs or hatched juveniles. When naturally infested soil or soil mixed with cysts has been used, it is much less satisfactory, although with care new cysts can be distinguished from old. Extraction is usually more efficient with drier cysts from air-dried soil than with fresher cysts from moist soil.

Occasionally, plant root responses may be related to resistance. For example, wheat roots inoculated with cereal cyst nematode have distinct 'knots', with swelling and lateral root proliferation at sites where female nematodes are developing. In contrast, oat and barley roots infected by the same species of nematode do not show such distinct morphological responses, so that this form of assessment cannot usually be relied upon.

Tolerance

Plant tolerance of nematode infestation requires a comparison of growth of infected and non-infected plants. In controlled conditions, this may be difficult to achieve at the same time as assessing resistance. In some cases, there are plant responses to infection that seem to be related to tolerance. Thus, in white clover germplasm resistant to *H. trifolii* some plants have marked browning of the roots as a result of necrosis caused presumably by a hypersensitive response. It is possible to select for non-necrosed roots and non-cyst formation.

Plant growth or crop yield as a measure of tolerance is usually characterized initially in field or plot trials. In some cases, small plot trials can be used to screen for tolerance, for example, of cereal cyst nematode in spring-sown oats in Australia and of soybean cyst nematode in the USA. In the case of oats, plants were differentiated that showed all four possible combinations of resistance or susceptibility with tolerance or sensitivity. In potato, a number of traits, including plant growth characteristics as well as root responses to nematode infection, contribute to tolerance to cyst nematodes (Trudgill *et al.*, 1998; van Riel and Mulder, 1998). Tolerance to soybean cyst nematode has been quantified by comparison of plant yields in plots or pots of

infested soil treated or not with nematicides and seems to be effective against more than one pathotype (Hussey and Boerma, 1992). Given the complexity of the trait, it is likely that pot or field plot trials will continue to be necessary to facilitate selection for tolerance, or at least to identify parental combinations from which tolerance may be expected.

The development of host plant genomic maps will allow use of marker-assisted selection for quantitative trait loci (QTLs) for indirect selection of tolerance. It is evident that tolerance and resistance are independent traits (Trudgill, 1991). Many factors, including root size and growth rate, and resistance to other biotic and abiotic stresses, contribute to tolerance. It is a complex character with many genetic and environmental factors influencing yield loss caused by nematodes. Within selection programmes, it is necessary to assess the performance of potential resistant cultivars in nematode-infested soil in the field (Colour Plate 5).

Screening Protocols for Specific Crops

It is essential that the screening protocol should give consistent and repeatable classification between years and, preferably, also between test centres. Accuracy, repeatability and throughput tend to favour selection of controlled techniques rather than field tests, although costs may not. Growing conditions must be adjusted to ensure optimal expression of the susceptible phenotype with which known or potentially resistant responses are to be compared. The following summary of protocols adopted for individual crop/nematode associations gives specific illustration of the general principles but is not meant to be a complete review of breeding methodologies for each nematode.

Heterodera glycines*, soybean cyst nematode (SCN)*

Historically, soybean screening was done in SCN-infested fields, counting white females attached to roots at 1 month after planting. Each test entry was compared with a standard susceptible in a two-row plot (Colour Plate 4) (Ross and Brim, 1957). Pot tests (Epps and Hartwig, 1972; Noel *et al.*, 1990) used 8-cm diameter clay pots to test single seedlings in infested field soil; females on washed roots were counted at 1 month. The technique proposed for race identification (Golden *et al.*, 1970) has been widely adopted for screening. Individual seedlings with 2–3 cm radicals are transplanted into pasteurized sand or soil in 7.5 cm diameter pots, grown for 3–4 days, then inoculated with 1000–5000 eggs collected from fresh white females. After 1 month, new white females, extracted from each pot, are counted and expressed as a female index (the ratio (as a percentage) of female

numbers on test line and susceptible cv. Lee). This index is used to classify cultivars: 0–9%, resistant; 10–30%, moderately resistant; 31–60%, moderately susceptible; and more than this susceptible (Schmitt and Shannon, 1992). This index is reliable when the virulence phenotype of the test population is known but mixtures of the races have caused problems with its interpretation. None the less, more than 200 soybean cultivars, representing maturity groups I–VIII, have been produced in the USA and are effective in reducing yield losses.

For practical breeding in Illinois, USA, individual plants are scored as: 0 = 0, 1 = 1–5, 2 = 6–10, 3 = 11–30 and 4 = > 30 females per root system, and only plants scored as 1 and 2 are kept. This has worked very well and maintained resistance for about 20 years in Illinois, where about 5000 lines are assessed per year.

Molecular marker-assisted selection (MAS), using both RFLPs and random amplified polymorphic DNAs (RAPDs), is being applied in some breeding programmes and will become more widespread. It can screen single plants for their responses to more than one race and allows the development of cultivars with multiple *R*-genes. In cv. Peking, two independently inherited markers pA136 and pA635 on linkage groups A and C, respectively, are associated with resistance to race 3. There is a rapidly increasing number of markers to other soybean cyst nematode *R*-genes (Cregan and Quigley, 1997; Anand *et al.*, 1998). Screening protocols for the markers (see Chapter 12 by Young and Mudge) require an intensive application of a traditional approach to identify single plant reactions; subsequent checks on MAS will also need to run concurrently, to confirm association between marker and response.

Making progress before all genetic interactions are catalogued is clearly possible. Practical breeding in the USA utilizes knowledge of different distributions of predominant races in northern and southern states. In the north, races 1 and 3 predominate (about 25 and 70%, respectively) and the screening protocol first selects for resistance to a population of race 3, and then tests resistant or moderately resistant lines with 1–5 and 14 prior to making a release. In the south, where 87% of SCN infestations are races 2, 4, 5, 6, 9 and 14, the screening is first with races 6 and 2, followed by races 14, 4 and 5. This selects efficiently for effective gene combinations in currently available sources and varieties (Kim *et al.*, 1998).

Heterodera avenae *and the* H. avenae*-group, cereal cyst nematodes (CCN)*

Resistance screening has frequently relied upon naturally infested field soil: very susceptible cultivars may be used to maintain populations on

fields or microplots. In Europe, natural biological control agents may prevent nematode multiplication and endanger inoculum supply. For this reason, some investigators use 'clean' cysts to inoculate susceptible plants to multiply inoculum for one cycle and then store dry soil at 2–4°C to allow regular withdrawal of inoculum. This is then used either as infested soil mixed with sterilized rooting medium, generally sand, or J2 may be hatched from clean cysts to apply to roots in a variety of media. Refinements include the test-tube methods. Ireholm (1994) used 100 hatched J2 to inoculate seedlings growing in 100 ml of sand. The plants are fed and watered carefully. Rivoal *et al*. (1991) grew plants on agar in Petri dishes, adding either four or eight J2 per root tip (depending on the pathotype involved) and was able to distinguish resistant from susceptible plants.

Rivoal *et al*. (1991) also used soil-based methods. The substrate in 70 cm^3 plastic tubes was sand and clay with added mineral fertilizer. Single wheat plants, grown in this substrate, were inoculated with two new cysts preconditioned at 3°C to break diapause and constrained in nylon mesh bags. This inoculum corresponded to 220 J2 per plant. Plants were grown at 16°C and 18 h day length, and watered as required once a week. Females and cysts were washed free of roots on to a 63 µm-pore sieve at 2–5 months. Taylor *et al*. (1998), modifying Fisher (1982), planted seedlings in soil in 27 × 125 mm tubes, and inoculated at planting and at four more times at 3 and 4 day intervals, to give 500 J2 per plant. Plants were grown at 15°C in 16 h day length for 9 weeks after the last inoculation, before counting total cysts.

In Australia, much initial selection could be done in field conditions, by uprooting plants sown in clumps or drills to assess female numbers in comparison with susceptible controls. Higher throughputs are achieved by screening plants sown in racks of 50 pots each filled with 200 g of naturally infested soil mixed with washed sand and fertilizer to deliver between 16 and 32 eggs g^{-1} soil. In these conditions, susceptible plants had from 13–45 cysts on the surface of the root ball (138–902 on the whole root system) compared with 0–6 on partially resistant controls (27–91). Four people were able to assess up to 600 plants day^{-1} on root ball scores. The target throughput is to assess 100,000 plants per season and computer-assisted sowing and recording is invaluable (McKay, 1998). The key to success at these relatively high reproduction levels is good plant nutrition and irrigation in outdoor daylight. It is important to ensure good drainage from the pots and racks of pots, to protect from pest attack, including birds and small mammals, and to remove weeds or volunteer host plants when using naturally infested soils. The assessment of 100–200 plants day^{-1} is readily achieved in a variety of soil-based methods. The restriction of the natural growing season may limit numbers that can be assessed.

Screening procedures must be adapted for the needs of the breeding programme. Tests must be able to characterize responses of single plants, e.g. in heterogeneous sources, and to select parent plants, but also able to test multiples of plants. This allows more than one plant per unit to be assayed and is useful to improve the chances of detecting segregation in later generations. For instance, screening four pots each with four plants gives a probability of 0.95 of detecting the 1 : 3 susceptible : resistant segregation in barley with gene *Rha2*. The probabilities of detecting segregations for other genes are given by Mather (1951).

Frequently, resistance to CCN has been set as < 5% as many cysts as on the susceptible control: flexibility is required when applying this rule. In particular, there should be reproduction (that is $P_f > P_i$) on the susceptible control with about 100 females developing without intraspecific competition. The dilemma of interpreting plants that develop one or two cysts is unresolved (Andersen and Andersen, 1982a). It has not been established whether these are virulent individuals but that outcrossing prevents their progeny from expressing recessive virulence, or whether such cysts result from incomplete expression of resistance. Although the phenomenon cannot be explained, it should not be ignored, and where such females occur there should be planned progeny testing to assess emergence of virulence.

Marker-assisted selection using morphological markers in the plant can be used in some crosses, e.g. in barley, major genes for anthocyanin pigments are closely linked to the *Rha2* gene. DNA markers identify one gene in selected wheat crosses (Eastwood *et al.*, 1994) and in barleys (Williams *et al.*, 1996).

Globodera rostochiensis *and* G. pallida*, potato cyst nematodes (PCN)*

Techniques used to screen for resistance to both potato cyst nematode species are essentially identical. The greater diversity of *G. pallida* has presented the greater challenge to find quick and accurate tests to accurately discriminate between fully susceptible genotypes and ones with partial resistance.

Initially, plants were grown in naturally infested soil in fields. This was refined to screen plants in pots, using root-ball cyst counts to identify sources of resistance. Replication is essential but may be different depending on the target resistance genes. Thus, three replicates are used to identify clones with single major gene resistance to *G. rostochiensis* but up to ten replicates are needed to screen for quantitative resistance to *G. pallida* (Fleming, 1998). Multiplication rates are usually two to three times greater in pots than in the field, but do not usually affect relative rankings of clones. In UK National List trials, a

standardized procedure, described by McKenzie and Turner (1987), relied upon multiplication rates calculated from P_i and P_f expressed as numbers of cysts per pot. A series of critical evaluations of potato cyst nematode screening systems emphasize that relative expressions and rankings of cyst numbers are more accurate and allow better cross-site comparisons than absolute assessments (Fleming, 1998).

More accurate tests of more plants can be handled in a variety of smaller container-based tests. Between 60 and 240 cm^3 of rooting medium is infested with 5–20 eggs cm^{-3}. Potato vegetative sprouts and seedlings grow well in canisters with soil moisture level adjusted to 30%. The transparent-walled canisters are closed and placed in a controlled environment at 20°C for 7 weeks. Developing females are then clearly visible and can be counted through the wall of the clear canister or extracted and counted. Roupe van der Voort *et al.* (1998) also used the Phillips *et al.* (1980) canister method: one tuber per 125 cm^3 container of silver sand was infested with five eggs and J2 cm^{-3} and grown in the dark at 20°C for 3 months, before elutriating and counting cysts.

In testing for resistance in gene bank accessions of cultivated and wild potato species in the Dutch–German Potato Collection, samples of 15–40 plants per accession are tested by one of three approaches: (i) in infested soil; (ii) in pots including an inoculum of 25–30 'full' cysts; or (iii) in pots including an inoculum of three cysts wrapped in nylon mesh. Reproduction was assessed by elutriation and centrifugal extraction of females, plants with more than five females being scored as susceptible, and putative resistant clones being retested. The second simple method (ii) was used for most accessions in later tests. Tests in France used method (iii) which was the most reliable (Rousselle-Bourgeois and Mugniery, 1995). In Europe, at present five virulence groups of *G. rostochiensis* and three of *G. pallida* are recognized. Earlier results had to be adapted to the pathotype identification scheme of Kort *et al.* (1977). More recently, information on the number of plants with 0 cysts (*Pa2*) and 0–2 cysts (*Pa3*, 1981–1985) in relation to the total number of screened plants, has been made available for this collection. In the root-ball test, only the outside of the root ball is screened for the presence of females, but when tests were performed under suboptimal growing conditions (winter season) the inside of the root ball was also examined visually. In tests regarded as more accurate, Mugniery (1983) used Petri dishes with water agar and placed J2 near root tips.

In comparisons of these approaches, although the actual multiplication differs among tests under different conditions, the overall ranking of genotypes is in good agreement. An additional safeguard when using these tests was to include partially resistant clones and also to try to include standard nematode populations for reference purposes. In general, canister methods overestimate, whereas Petri dish methods underestimate the degree of resistance expressed in field conditions.

Rapid progress in mapping the potato genome will allow markers to be used in potato breeding programmes. These will select for genotypes rather than phenotypes to ensure combinations of different genes likely to provide more durable resistance to heterogeneous populations of PCN (Dale and de Scurrah, 1998). RFLP markers linked to major genes have been identified in a number of resistance sources (Fleming, 1998). DNA sequencing of PCN resistance genes will allow their detection in segregating populations by PCR-based screening. There are RFLP markers linked to gene *H1*, and RFLP QTLs to *Ro1* resistance from *Solanum spegazzini*. Progress with at least three different mapping families will greatly enhance the value of these markers for selecting not only major gene resistance but particularly for improving the levels of resistance to *G. pallida*. QTL analysis indicated that a common locus with multiple genes conferred broad spectrum resistance to *G. rostochiensis* and *G. pallida* (Roupe van der Voort *et al.*, 1998). This may provide a better approach than seeking to apply MAS to known *R*-genes. In this work, plants grown *in vitro* were nematode tested after transfer to sterile silver sand and sandy loam mix and inoculated after 2–4 weeks growth. Stem cuttings from individual seedlings were used to replicate tests and for DNA extractions without loss of the parent plant (Rouppe van der Voort *et al.*, 1997). In these tests, 3–4 week-old plants were planted in a loamy sand mixture (900 g per pot) and inoculated with five eggs and J2 g^{-1} soil. At 3 months, cysts were recovered by Fenwick can elutriation, and a root size rating (0–3) was given. Mean numbers of cysts per genotype (log $x + 1$ transformed) were used to distinguish three categories: resistant, unassigned and susceptible (Roupe van der Voort *et al.*, 1998).

Other cyst nematodes

Resistance to other cyst nematodes is evaluated following these principles, adapted as necessary to (i) match the requirements and resources of the programme and (ii) the nature of the plants and nematodes. Sugar beet cyst nematode resistance introduced from wild species was selected in tests using loess soil from 3–5 m deep and supplied with nutrient solutions in PVC tubes ($2 \times 4 \times 12$ cm) stacked in 10 rows each of 12 tubes, sown and inoculated at 14 days with 1000 J2 per tube and grown for 6 weeks at 20°C and 14 h day length. Cysts were collected after washing substrate through 1 and 0.1 mm meshes and treating the debris for 5 min with 20% acetic acid to dissolve loess before counting. Quality assurance required that the susceptible control cv. Desiree had a minimum of 40 cysts per plant and plants with less than 30 cysts were selected as resistant. The key feature is that the frequency distributions of cysts per plant should not overlap between

susceptible controls and putative or control resistant plants (Muller, 1998a).

Kaplan *et al.* (1999) reared beet cyst nematodes on cabbage and then on selective hosts in clay pots filled with river sand inoculated with cysts. Host tests were performed in 80 g of sand (particle size > 250 μm) in vials 3.7 × 6.2 cm with a mesh covered drainage hole. Seeds were sown directly and the resulting seedlings thinned to one per vial. Nutrient solution was applied daily. After expansion of the first true leaf, plants were inoculated with 500 hatched J2 and cysts were counted at 38 days after two generations.

Tobacco cyst nematode resistance has been selected in seedlings germinated in vermiculite, transplanted at 4 weeks to 10 cm diameter clay pots with 250 cm^3 of a sterilized 1 : 1 soil : sand mix over a filter paper in the bottom of the pot. Plants were grown for 2 weeks and inoculated with 6000 eggs per pot. The eggs were collected from crushed field-grown cysts, stored dry in closed containers at ambient temperature, until use. At 8 weeks, pot contents were washed to dislodge females that were collected on a 250 μm aperture mesh. Subsamples (1 g) of roots were washed and stained to detect immature stages. These tests had eight to ten replicates and plants had 0.1–34 females g^{-1} root (Hayes *et al.*, 1997).

Clover cyst nematode resistance is selected in pots of pasteurized sand/soil mix in 6.5 cm diameter pots at 18–25°C, watered by capillary action from trays with liquid fertilizer added as needed. Two-week-old seedlings were inoculated by injecting the soil under the seedling with a syringe with 2000 eggs withdrawn from an agitated suspension of eggs extracted from pot-cultured cysts. At 8 weeks, the roots and soil were washed in an elutriator and cysts collected on a 180 μm aperture sieve and counted. Similar techniques were used for cuttings as for seedlings. Data in these experiments were expressed as the number of cysts per plant and per g dry root weight at assessment (Hussain *et al.*, 1997).

Resistance to *H. sacchari* in rice (*Oryza* spp.) was identified in plants grown in small pots (100 cm^3) and inoculated with J2, and correlated well with the results from field plots in naturally infested soil (Plowright *et al.*, 1999).

Pathotypes and Races

Heterodera glycines*, soybean cyst nematode (SCN)*

Variation in virulence of SCN has been catalogued mainly in the USA where differences between field populations were evident soon after the first resistant cultivars were grown. Subsequently, field

populations were shown to be heterogeneous for virulence. A set of four differential plants with resistance genes describes 16 possible races (Table 4.3), although not all have been isolated from field populations. The scheme classifies a differential plant as resistant to a nematode population if it supports less than 10% as many females as the susceptible control cv. Lee. This underestimates SCN diversity although it is a useful classification for breeder and grower advice in the USA. Closer to the putative centre of origin of SCN, in China, selection of one race for several generations on four cultivars led to the emergence of four different races.

More recently, studies with inbred nematode lines confirmed that major genes determine the outcome of interactions between resistance and avirulence genes in soybeans and cyst nematode (Opperman and Bird, 1998). Incompatible interactions involve dominant *R*-genes and recessive *v*-genes. If this is confirmed, then previous uncertainties over classification of plant responses result from testing plants with nematode populations of complex virulence genotypes.

A new scheme for classification of variability in SCN has been proposed in the USA (Niblack *et al.*, 2001). The new scheme will designate a population 'Hg Type', based on development of females on sources of resistance used either as germplasm or in registered cultivars (see Table 4.6). The differentials (Indicator Lines) will be Peking (now PI548.402), PI88.788, PI90.763, PI437.654, PI209.332, PI89.772 and Cloud (PI548.316). A population that reproduces on none of the lines will be designated Hg Type 0, one that reproduces on PI548.402 and PI88.788 only would be Hg Type 1.2. This scheme will be open-ended, able to include new sources of resistance as these are incorporated into germplasm and varieties, and should be suitable for adoption worldwide.

Table 4.3. Races of soybean cyst nematode recognized on four differentials and expressed in relation to a fifth, susceptible soybean cv. Lee.

Soybean differential	Race[a]															
	1[b]	2[b]	3[b]	4[b]	5[b]	6[b]	7	8	9[b]	10	11	12	13	14[b]	15	16
Pickett	–[c]	+	–	+	+	+	–	–	+	+	–	–	–	+	+	–
Peking	–	+	–	+	–	–	–	–	+	–	+	+	+	+	–	+
PI88.788	+	+	–	+	+	–	+	–	–	–	+	–	–	–	+	+
PI90.763	–	–	–	+	–	–	+	+	–	+	–	+	–	+	+	+

[a]Race designation from Riggs and Schmitt, 1988; [b]Races that occur in the USA; [c]–, susceptible (female index ≥ 10% that of susceptible cv. Lee); +, resistant (female index < 10% cv. Lee).

Heterodera avenae *and* H. avenae*-group, cereal cyst nematodes (CCN)*

Populations of cereal cyst nematodes are very heterogeneous for virulence but also differ in cereal species host range. Thus, in northern Europe most populations reproduce well on oats but in southern Europe, North Africa and parts of Asia, oats are non-hosts to most populations. Even so, there are exceptions and, for example, some Swedish populations do not reproduce on oats. Initial classifications of CCN used differential cereal cultivars of four genera and also nematode populations that included more than one morphological species. The series used was helpful for classifying northern European populations, particularly for the purpose of developing resistant spring barley cultivars (Table 4.4), but underestimated the variation in virulence genes in nematode populations. This was probably a very great underestimate and Ireholm (in Cook and Rivoal, 1998) has shown that on just four differential cultivars each of barley, wheat and oat, 69 populations from around the world could be classed as 30 distinct virulence phenotypes. So, although the Andersen and Andersen (1982b) pathotype scheme has the simplicity of being based on known *R*-genes or at least resistance sources, it suffers from underestimating the polymorphism of resistance and avirulence genes. In practice, many European barley and oat cultivars have resistance genes historically overcome by CCN in the areas in which they have been developed and grown. Whether as a result of residual action of these or of other genes contributing to quantitative resistance, many traditional cultivars have good levels of partial resistance expressed to field populations. For example, the old Swedish oat cv. Sol II (also Sun II) consistently has only 25% as many cysts as the very susceptible UK cv. Milford (Cook and Mizen, 1991). It is possible when screening accessions of exotic genotypes to isolate ones with very much greater susceptibility than those commonly grown and used as susceptible standards. It follows that it will be difficult to define precise pathotypes based on the interactions between incompletely characterized plant genotypes and unknown nematode genotypes. It also follows that virulence of local populations should be evaluated with local susceptible and, where possible, resistant controls as well as with exotic differential varieties. There may be exceptions to this complex picture where an introduced nematode has a limited virulence genotype on a host with limited resistance genotype, e.g. *H. avenae* on cereals in Australia. Elsewhere, it is clear that full virulence characterization will be problematic; the partial interactions that cause problems in pathotype schemes most likely indicate greater heterogeneity in the plant–nematode interaction. This demands continued vigilance on the part of nematologists and breeders.

Continuous cultivation of resistant cereal cultivars has not been much practised. Long term, growing barley with the *Rha2* gene has selected a virulent pathotype of *H. avenae* in Denmark, and of the related species *H. filipjevi* in Sweden. Growing oats with a single dominant resistant gene in France also selected a virulent pathotype of *H. avenae* (Lasserre *et al.*, 1996).

Globodera rostochiensis *and* G. pallida*, potato cyst nematodes (PCN)*

Both species and their pathotypes were originally recognized on European potato cultivars with *R*-genes, on South American cultivated potatoes and on wild *Solanum* species. These distinguished populations differing in virulence phenotypes which were represented by pathotype schemes, based on the gene-for-gene hypothesis. Separate schemes for European (Kort *et al.*, 1977), and South American (Canto Saenz and de Scurrah, 1977) populations were developed independently (Table 4.5). The influences of environment on PCN multiplication, the use of some clones with an apparently quantitative type of resistance and the application of an arbitrary multiplication ratio undermined the basis of the schemes (Trudgill, 1985). It appears that *G. rostiochiensis* populations in Europe can be grouped into three pathotypes but that extensive variation in virulence in *G. pallida* field populations means these are best considered as virulence phenotypes. These populations will likely change virulence phenotype in response to selection pressure imposed by the use of resistant cultivars. Identification of RFLP and RAPD markers linked to avirulence genes will more accurately describe populations in terms of general genetic variation and specifically in terms of virulence genotypes.

Growing potatoes with H1 resistance to *G. rostochiensis* has led to shifts in the PCN populations of Europe to *G. pallida*. Using resistant potatoes selected with European populations, it was shown that European *G. pallida* has as much variation in its virulence phenotype as did populations from South America. The virulence phenotypes of populations differed between the two regions (Phillips and Trudgill, 1998).

Partial resistance to *G. pallida* selects virulent nematodes and, recognizing the finite durability of this resistance (seven to ten potato crops or nematode generations), potato breeders are identifying and introgressing new resistances. The methodology must be flexible to test sources with growth habits differing from clonal tuberous potatoes, and ideally more information should be collected on the interaction between nematode and plant to diversify the genetic basis of resistance.

Table 4.4. Pathotype groups of cereal cyst nematodes defined by an International Test Assortment of cereal species and cultivars. (From Cook and Rivoal, 1998.)

	Ha1 group							*Ha2* group	*Ha3* group		
Differential species	*Ha11*	*Ha21*	*Ha31*	*Ha41*	*Ha51*	*Ha61*	*Ha71*	*Ha12*	*Ha13*	*Ha23*	*Ha33*
Cultivar [*R*-gene][a]											
Barley											
Varde	+[b]	..	..	+	..	+	+	+	+	+	+
Emir [+ ex Emir]	+	+	..	+	..	−	+	+	+	+	+
Ortolan [*Rha1*]	−	−	−	−	−	−	−	+	+	+	+
Morocco [*Rha3*, +]	−	−	−	−	−	−	−	−	−	−	−
Siri [*Rha2* + ex Herta]	−	−	−	+	+	+	−	−	+	+	+
KVL191 [*Rha2*, +]	−	−	−	..	+	+	+	−	..	..	..
Bajo Aragon	−	..	..	−	..	−	−	−	+	+	+
Herta	+	+	−	..	−	..	−	+	+	..	..
Martin 403 [2 dom]	−	..	..	−	..	−	−	−	−	+	+
Dalmatische	(−)	..	..	+	..	−	(+)	+	+	(−)	+
La Estanzuela	..	..	..	..	..	..	+	..	..	(−)	..
Harlan 43	−	..	..	..	..	..	−	−	..	−	+

Oats											
Nidar	+	..	..	(+)	..	+	–	+	+	+	+
Sol II [minor genes]	+	–	–	–	–	+	–	+	+	+	+
Pusa Hybrid BS1 [1 dom]	–	–	..	–	–	–	–	–	+	–	+
A. sterilis l376 [1 to 3 dom]	–	–	..	–	–	–	–	–	–	–	–
Silva [>1 gene]	(–)	..	..	–	..	(–)	–	(–)	(–)	(–)	+
IGV.H 72–646	–	..	..	–	..	–	–	–	+	+	+
Wheat											
Capa	+	+	..	+	..	+	+	+	+	+	+
AUS 10894 [*Ccn1* +]	–	..	..	–	..	–	+	–	(–)	+	+
Loros [*Ccn1* +]	–	–	..	–	..	(–)	–	–	(–)	+	+
Psathias	..	..	..	+	..	..	..	+	+	+	–
Iskamish K-2-light	+	..	..	–	..	(–)	..	+	+	+	+

[a]Resistance genes: those in italic (*Rha1*, *Rha2*, *Rha3*) define the 3 pathotype groups; dom, dominant gene; + additional gene(s) inferred; [b]+, susceptible; –, resistant (< 5% new females on susceptible control); () intermediate; .., no observation.

Table 4.5. Interactions between potatoes with resistance genes and populations of potato cyst nematodes, summarizing pathotype schemes and their modifications. (After Cook and Rivoal, 1998.)

			G. rostochiensis					*G. pallida*							*Globodera* species
			Ro1		*Ro3*		*Ro5*					*Pa2/3*			Virulence grouping
Species and accession	Ploidy	Resistance genes	*Ro1* *R1A*	*Ro4* *R1B*	*Ro2* *R2A*	*Ro3* *R3A*	*Ro5* –	*Pa1* *P1A*	– *P1B*	– *P2A*	– *P3A*	*Pa2* *P4A*	*Pa3* *P5A*	– *P6A*	European pathotype S. American pathotype
S. tuberosum ssp. *tuberosum*	4x	(minor)	+/–[a]	+	+	+	+	+	+	+	+	+	+	+	
S. t. ssp. *andigena* CPC1673	4x	*H1* on chromosome 5	–	–	+	+	+	+	..	..	..	+	+	..	
S. kurtzianum KTT 60.21.19	2x	*K1 K2 A* & *B*	–	(+)	–	(+)	(+)	+	+	+	–	+	+	+	
S. vernei GLKS 58.1642.4	2x	Quantitative	–	+	–	–	+	+	+	–	+	+	+	+	
S. vernei Vt^{n} 62.33.3	2x	Quantitative	–	–	–	–	+	–	+	–	–	–	+	+	
ex *S. multidissectum* hybrid P55/7	2x	> 1 + polygenes *H2*	+	+	+	+	+	–	–/+	–	–	+	+	+	
S. t. ssp. *andigena*	4x	*H3* + polygenes	+	..	..	..	..	(–)	..	..	..	(–)	(–)	..	
CIP 280090.10		Quantitative	..	..	..	..	..	..	..	..	..	–	–	+	
S. vernei hybrid 69.1377/94	2x	Polygenes	–	–	–	–	–	–	..	..	..	–	–	..	
S. vernei hybrid 65.346/19	2x	Polygenes	–	–	–	–	–	+	..	..	..	+	+	..	
S. spegazzini	2x	*Fa* = *H1*	–	–	+	+	+	..	..	..	..	..	..	..	
S. spegazzini	2x	*Fb* + 2 minor	+	–	+	–	–	..	..	..	..	..	..	..	
		Gro1 on chromosome 7	(–)	+	+	+	+	..	..	..	..	..	..	..	

[a]+, Compatible interaction: nematode virulent, potato susceptible; –, incompatible interaction: nematode avirulent, potato resistant; (), indicates partial or uncertain interaction; .., no information.

Other species

Pathotypes of the sugarbeet cyst nematode, have been identified and selected with newly available resistance sources, indicating that there is heterogeneity within native populations of *H. schachtii*. Two new and different virulent pathotypes of *H. schachtii* were selected from two German field populations after six generations of selection on beets with resistance introgressed from related species (Muller, 1998b). In California, USA, crops with different host status selected different genetic markers in different beet cyst nematode populations, demonstrating the heterogeneity of the nematode populations. Such infraspecific variability must be taken into account in management of resistance sources (Kaplan *et al.*, 1999). Plowright *et al.* (1999) observed that field populations of *H. sacchari* formed a few cysts on resistant genotypes of *Oryza glaberrima*, perhaps reflecting heterogeneity, although this nematode reproduces parthenogenetically.

Genetics and Sources of Resistance

Heterodera glycines*, soybean cyst nematode (SCN)*

Resistance of most sources was originally and predominantly regarded as a recessive characteristic. Heterogeneity of sources and nematode populations has complicated genetic interpretations. Molecular mapping has identified a number of complex loci in the soybean genome in which resistance genes are clustered. The practical consequence is that F_1 hybrids between susceptible and resistant plants do not express resistance. To identify resistant plants it is therefore necessary to screen the segregating progeny in F_2 or backcross generations.

Sources of resistance extensively used in the USA include accessions from the US World Collection, which includes 16,000 entries. Dong *et al.* (1997) listed cultivars and sources effective in the USA. Table 4.6 lists those resistance genes from plant introductions (PI) that have been used to develop successful cultivars in the USA. Most of the useful resistance genes available in *Glycine max* have been made available. Molecular markers will likely allow new *R*-gene combinations to be developed and used in breeding programmes, which will greatly increase the range of genes used and should ensure that SCN resistance will continue to contribute to the control of this major pest. Direct genetic screening may be useful, although it may only detect known genes. New sources of resistance are likely to come from perennial soybean *G. tomentella* and other species, representing the ‘treasure harboured in wild perennial relatives of the cultivated soybean’ (Singh *et al.*, 1998) that geneticists

Table 4.6. Chronology of release of sources of resistance to soybean cyst nematode (SCN), *Heterodera glycines*, and examples of their use in public breeding programmes in the USA.

Source of resistance	Cultivar or germplasm	Year released	Resistant to races[a]	Citation
PI548.402 (Peking)	Pickett	1966	1,3	Brim and Ross (1966)
PI88.788 and Peking	Bedford	1977	1,3,4[b]	Hartwig and Epps (1978)
PI88.788	Fayette	1981	3,4[b]	Bernard *et al.* (1988b)
PI90.763, Peking and PI88.788	Cordell	1990	3,4,5	Hartwig and Young (1990)
PI209.332 and Peking	Delsoy 4710	1992	3,4,14	Anand (1992b)
PI209.332	LN89-5699	1993	2,3,4,14	Nickell *et al.* (1994b)
	Fairbault			Orf and MacDonald (1995)
PI437.654 and Peking	Hartwig	1992	1–6,9,14	Anand (1992a)
PI437.654 and PI88.788	Ina	1997	1–3,5,9,14	Nickell *et al.* (1999)
PI89.772	LN89-5717	1993	2,3,4,5,14	Nickell *et al.* (1994a)
PI548.316 (Cloud)	LN89-5612	1993	3,14	Nickell *et al.* (1994c)

[a]R, resistant or partially resistant.
[b]Compare with Table 4.3, where strict application of 10% rule gives PI88.788 susceptible to race 4.

are increasingly able to incorporate into useful germplasm (Riggs *et al.*, 1998).

Heterodera avenae *and the* H. avenae*-group, cereal cyst nematodes (CCN)*

Much use has been made of simply inherited major genes and resistance sources have frequently been chosen with single dominant major genes (Table 4.7). It is likely that many more genes are available within germplasm collections. Those in wheat appear to be at complex *R*-gene loci (Lagudah *et al.*, 1997). Resistance sources in polyploid plants, e.g. oats and wheat, with simple major resistance genes often appear to have other genes of minor effect, for the transfer of the major gene rarely gives derived progeny the complete resistance of the parent. In contrast, single genes are often fully effective in diploid barley.

There is a shortage of good sources of resistance in wheat, but research in Australia, France and Spain has identified related *Triticum* species with genes of potential value to wheat resistance breeding. These have been introgressed by traditional hybridization and selection into new sources with potential widespread effectiveness in both soft and hard wheats. Resistance from *Aegilops ventricosa* is distinct from the Loros resistance and has been called *Cre2* (or *Ccn2*) (Delibes *et al.*, 1993). That from France derives from *Aegilops ventricosa* and is called *Crex*; it is on the homologous chromosome group 2, like *Cre1* and the genes (*Cre3* and *Cre4*) from *T. tauschii* (= *A. squarrosa*).

Table 4.7. Sources of resistance and their use in breeding cereal cultivars resistant to cereal cyst nematodes (CCN), *Heterodera avenae* and other species.

Cereal species	Source		Use and cultivars	Response to pathotypes[a]
	Original	R-gene(s)		
Barley (*Hordeum*)	N. European landraces	*Rha1*	N. Europe cvs 1900–1950s	R to *Ha1*
	Emir	*Rha*?	Susceptible in much of Europe	R to *Ha61*(Norway, NL, India, Siberia)
	N. African accession?	*Rha2*	Cvs in Denmark, Sweden, UK	R to *Ha1* and *Ha2* S to *Ha3*
	Morocco from N. Africa	*Rha3*	Not in cvs	R to *Ha1*, *Ha2* and *Ha3*
	Galleon	Major gene	Australia	R to *Ha13*
Oats (*Avena*)	Sol II, from Sweden	Minor genes	Scandinavia	Partial resistance to many populations
	A. sterilis I376	1–3 major genes	UK	R to all *Ha1*, *Ha2* and *Ha3*: bred cvs susceptible to some populations
	US 1624 (CI3444)	Major gene	Sweden, Denmark, UK	R to *Ha1* and *Ha2* S to *Ha3*
	Avon and several Australian cvs	?	Australia	R in Australia (*Ha13*), S to *Ha1* and *Ha2*
Wheat (*Triticum*)	Loros, AUS10894	*Ccn1*	–	R to *Ha1*, *Ha2* S to *Ha13* in Australia
	T. tauschii	*CcnD1* *CcnD2*		R to *Ha13* Partial R to *Ha13*
	Ae. ventricosa	*Ccn2*		

[a]See also Tables 4.5 and 4.9.

Genes in wheat have been cloned and sequenced and these are being used in breeding in Australia. Marker-assisted selection is being used to incorporate these genes, singly and in concert (Jeffries *et al.*, 1997; Ogbonnaya *et al.*, 1998). There are indications of synteny in that sequences from the wheat *R*-gene, *Cre3*, introgressed from *Triticum tauschii*, detected resistance gene analogues in barley which mapped to loci known to be associated with genes for CCN resistance (Seah *et al.*, 1998).

Globodera rostochiensis *and* G. pallida*, potato cyst nematodes (PCN)*

Potato resistance sources contribute a range of genes (Table 4.8). Initially, progress was made with a single dominant gene that gave fully effective resistance to the then predominant virulence phenotype

Table 4.8. Sources of resistance in *Solanum* spp. to potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, examples of their use in European breeding programmes and some additional resistance sources. (After Anand *et al.*, 1998; Dale and de Scurrah, 1998.)

			Resistance to pathotypes[a]	
Source	R-genes	Cultivars	*G. rostochiensis*	*G. pallida*
S. tuberosum ssp. *andigena* CPC1673	*H1*	Maris Piper UK 1963 Saturna NL 1964 > 70 cvs	Ro1, Ro4	
S. tuberosum ssp. *andigena* CPC2802	*H3* polygenic			(*Pa1*, *Pa2/3*)
S. multidissectum	*H2*			*Pa1*
S. vernei	oligogenic	Morag UK 1985 Glenna NL 1987 Several cvs in D, NL, UK by 1997		
S. brevicaule			Broad spectrum	
S. capsicibaccatum				*P4A*, *P5A*
S. circaefolium				*Pa2*, *Pa3*
S. gourlayi	Major genes and quantitative resistance		Broad spectrum	*P4A*, *P5A*
S. kurtzianum	Major gene		Broad spectrum	
S. leptophytes				*P4A*, *P5A*
S. megastacrolobum				
S. optocense			Broad spectrum	
S. santae-rosae				
S. sparsipilum				*P4A*, *P5A*
S. spegazzinnii	Major gene		Broad spectrum	
S. vernei	Major and quantitative		Broad spectrum	

[a]Also see Tables 4.5 and 4.9.

of *G. rostochiensis*. Other major *R*-genes have been introduced into cultivars. Resistance to *G. pallida* is available from a number of sources of cultivated and wild *Solanum* species, but is rarely fully effective after hybridization.

Other cyst nematodes

Resistance has been introduced from wild beet species into cultivated sugar beet. The translocation appears to have introduced a major single gene complemented by a gene of less effect. Other resistance genes present in the wild species were not transferred into the breeding lines. This gene has been cloned and sequenced and may be a useful synteny for nematode *R*-gene location in other crops. Resistance and tolerance

to beet cyst nematode are generally independently inherited in beet (Muller, 1998a), but there is no information on the genetics of tolerance and no selection methods have been described. Useful resistance to *H. sacchari* in the wild rice, *O. glaberrima*, appeared to be qualitative, suggesting simple genetic control. The resistance was readily transferred to and selected in interspecific hybrids with cultivated rice, *O. sativa* (Plowright *et al.*, 1999).

Germplasm Tests and Collections

Increasingly, searchable databases of plant genetic resources are available on the Internet. Some of those that are particularly relevant to nematode resistance, having some information about variation in response to cyst nematodes, are detailed below (Table 4.9). One good general starting site is the United States Department of Agriculture National Plant Germplasm System (NPGS). This cooperative public and private venture to preserve the genetic diversity of plants is one part of the Germplasm Resources Information Network (GRIN) that allows access to collections and associated databases. Its web site provides links to many related sites, including national and international organizations. Most do not have information about responses to nematodes but allow individually defined ranges of variation to be identified and acquired for screening. Another generally useful starting point is the URL http://www.cgiar.org/ecpgr. From this site, there are links to collections in which nematode resistance has been evaluated, as well as to other attributes of crop plant collections relevant to planning a screening programme. Results of nematode screening included in such databases should be regarded as preliminary, particularly when the germplasm is to be used for a different region.

Heterodera glycines*, soybean cyst nematode (SCN)*

The NPGS soybean database lists responses to SCN in the USDA Soybean Germplasm Collection. All resistant cultivars developed in USA public institutions derive ultimately from one or more of only six plant introductions (Tables 4.6 and 4.9; Bernard *et al.*, 1988a; Dong *et al.*, 1997). Having defined *ror* genes (reproducing on a resistant host) in SCN, Dong and Opperman (1997) concluded that gene for gene interactions do apply in the SCN/soybean system with dominant alleles of *R*-genes in resistant plants and recessive *ror* alleles in virulent nematodes. It follows from this that the intermediate reactions observed in population level interactions result from expression of heterogeneity in plant and animal. This disguises potentially useful gene combinations

Table 4.9. URL addresses of plant genetic resources with particular relevance to cyst nematode resistance.

Germplasm Resources Information Network (GRIN)
Agricultural Research Service-United States Department of Agriculture
National Plant Germplasm Repository, links to USA and international germplasm collections
http://www.ars-grin-gov/npgs
Soybean cyst nematode
USA State sites, list results of SCN screening on varieties, e.g.
http://www.ag.uiuc/~wardt/cover.htm
Cereal cyst nematodes
Nordiska Genbanken (Nordic genebank), maintains and distributes CCN
Differential varieties
http://www.ngb.se/cereal
Potato cyst nematodes
Centro Internacional de la Papa (CIP)
http://www.cgiar.org/cip
Commonwealth Potato Collection
http://scri.sari.ac.uk/cpc
Dutch–German Potato Collection
http://www.cprodlo.nl/cgn

that are increasingly revealed and potentially exploitable by molecular marker-aided selection.

USA cultivars are usually registered with the Crop Science Society of America, and brief descriptions of new cultivars are published in *Crop Science*. There are also lists of resistant cultivars on a number of web pages, some of which give useful methodological links. Some states in the USA frequently have web pages referring to locally resistant soybeans and these usually allow links to other soybean sources (Table 4.9).

Heterodera avenae *and the* H. avenae*-group, cereal cyst nematodes (CCN)*

Some National Lists of Registered Varieties (Europe) give details of cereal cultivars with cyst nematode resistance. Rivoal and Cook (1993) indicate accessions of cereal germplasm with resistance to some of the pathotypes and species with details, where known, of genetic control and relationships (Tables 4.7 and 4.9).

In Australia, barley cultivars with resistance based on Galleon are registered and grown (Wheeler, 1998) and these also have good tolerance compared with that of oats. There are also additional sources of resistance in *Triticum* to a broad range of cereal cyst nematodes (Nicol, 2001). The approach of using gene sequences to screen genomes

directly will identify other genes in due course. A sequence from wheat gene *Cre3* has been used to detect resistance gene analogues on all chromosomes of wheat (Spielmeyer *et al*., 1998). Development of this approach will allow direct gene screening for sequences conditioning nematode resistance. Resistant wheats include Festiguay, whose parentage is not known, and Molineux and recently bred varieties with Loros or AUS10894 alleles.

Oats resistant in Australia are susceptible in the UK (Cook and York, 1988). Most cultivars of rye are generally resistant, but within triticales there are cultivars which have resistance, some like the quantitative type expressed in rye and others with the major gene resistance like that in wheat (Cook and York, 1987).

The USDA World Collection of small grains and the International Maize and Wheat Improvement Center (CIMMYT) collections have not been systematically screened with different isolates of cereal cyst nematodes. The Nordic gene bank (Nordiska Genbanken, Sweden) lists accessions with CCN resistance and also maintains the International Differential series that has been relatively widely characterized.

Ancestral cultivars include old landraces, e.g. cv. Sol II, that may have resistance that is effective against nematodes from outside their zone of origin; e.g. Scandinavian and north-western European sources, although now susceptible to home populations, are effective in southern France, India and Australia.

Globodera rostochiensis *and* G. pallida*, potato cyst nematodes (PCN)*

Several important collections have been screened for resistance to PCN (Tables 4.5 and 4.9). The germplasm includes not only cultivars of the crop species, but also a number of related species in the Commonwealth Potato Collection and Centro Internacional de la Papa (CIP): www.cgiar.org/cip. There are also national lists of potato cultivars, including the Dutch–German Potato Collection, tested in The Netherlands, Germany, and France to pathotypes *Ro1*, *Ro2*, *Ro3*, *Ro5*, *Pa1*, *Pa2*, *Pa3* and (*Ro1* + *Ro2* + *Ro3* + *Ro4* + *Ro5*) in mixture. Other potato collections are accessible via links from the CPC or Dutch–German Potato Collection web sites. There is also considerable variation in wild *Solanum* species (Table 4.8).

Globodera tabacum*, tobacco cyst nematodes*

Tobacco cultivars resistant to *G. tabacum* subspecies are listed by LaMondia (1988) and by Johnson (1990). Twenty-four genotypes of

tobacco included nine with good resistance in glasshouse tests in pots of soil infested with eggs (Herrero *et al*., 1996). Tobacco genotypes resistant to a North Carolina population of the nematode were: cvs Burley 21, PD 4, VA 81, NC 567, Speight G-80, Kutsaga Mammoth 101, Kutsaga 110 and two flue-cured breeding lines. Hayes *et al*. (1997) did similar tests with a Virginia isolate but stained root samples at 8 weeks after inoculation of 6-week-old transplants. The more resistant accessions included TI 1597, TI 1625, and cvs Burley 64, MD 40, Pennbell 69 and Kutsaga Mammoth 10.

Heterodera ciceri*, chickpea cyst nematode*

Chickpea (*Cicer arietinum*) and related species were evaluated for resistance to *Heterodera ciceri* in glasshouse trials. Plants were grown in pots with infestations adjusted to 20 eggs g^{-1} soil. Accessions were evaluated by a 0–5 index, related to numbers of first generation white females and new cysts on roots. Resistance was found in some lines of three species but not in *C. arietinum*, nor in five other species. The resistance of one line of *C. reticulatum*, ILWC 292, is likely to be useful for hybridization with cultivated chick pea (Singh *et al*., 1996) and is maintained at the International Center for Agricultural Research in Dry Areas (ICARDA), Syria.

Heterodera sacchari*, rice cyst nematode*

West African accessions of the African rice (*Oryza glaberrima*) and hybrids with cultivated rice (*O. sativa*) have been identified as valuable resistance sources (Plowright *et al*., 1999). Fifteen of 21 accessions of *O. glaberrima* and seven of nine accessions of the wild species *O. breviligulata* were resistant to *H. sacchari* (Reversat and Destombes, 1998). Like the nematode, all the resistance sources were of African origin.

Heterodera trifolii*, clover cyst nematode*

Resistance to *Heterodera trifolii* has been identified in genotypes from a number of cultivars and populations of white clover in the United Kingdom and in New Zealand. Selection was based on numbers of females per plant, or in New Zealand per g root. Those from New Zealand have been developed as experimental populations and proven to control this nematode in long-term field trials. The proportions of resistant plants increased after repeated selection in successive

progeny generations, and were effective against populations collected from north to south of New Zealand (Mercer *et al.*, 1999). Resistance to this nematode exists in related species, notably in some plants of *Trifolium nigrescens*, and this resistance can be transferred by interspecific hybridization into the cultivated species, *T. repens* (Hussain *et al.*, 1997).

References

Anand, S.C. (1992a) Registration of 'Hartwig' soybean. *Crop Science* 32, 1069–1070.

Anand, S.C. (1992b) Registration of 'Delsoy 4710' soybean. *Crop Science* 32, 1294.

Anand, S.C., Cook, R. and Dale, M.F.B. (1998) Development of varieties resistant and tolerant to cyst nematodes. In: Sharma, S.B. (ed.) *The Cyst Forming Nematodes*. Kluwer Academic, Dordrecht, The Netherlands, pp. 293–321.

Andersen, K. and Andersen, S. (1982a) Classification of plants resistant to *Heterodera avenae*. *EPPO Bulletin* 12, 435–437.

Andersen, S. and Andersen, K. (1982b) Suggestions for determination and terminology of pathotypes and genes for resistance to cyst-forming nematodes, especially *Heterodera avenae*. *EPPO Bulletin* 12, 379–386.

Atkinson, H.J., Urwin, P.E., Clarke, M.C. and McPherson, M.J. (1996) Image analysis of the growth of *Globodera pallida* and *Meloidogyne incognita* on transgenic tomato roots expressing cystatins. *Journal of Nematology* 28, 209–215.

Bernard, R.L., Juvik, G.A., Hartwig, E.H. and Calton, J.E., Jr (1988a) *Origins and Pedigrees of Public Soybean Varieties in the United States and Canada.* United States Department of Agriculture Technical Bulletin No. 1746, 69 pp.

Bernard, R.L., Noel, G.R., Anand, S.C. and Shannon, J.G. (1988b) Registration of 'Fayette' soybean. *Crop Science* 28, 1028.

Brim, C.A. and Ross, J.P. (1966) Registration of 'Pickett' soybeans. *Crop Science* 6, 305.

Canto-Saenz, M. and de Scurrah, M.M. (1977) Races of the potato cyst nematode in the Andean region and a new system of classification. *Nematologica* 23, 340–349.

Cook, R. and Mizen, K.A. (1991) Expression of resistance in oats (*Avena* spp.) and some other cereals to cereal cyst nematode (*Heterodera avenae*). *Helminthologia* 28, 145–150.

Cook, R. and Rivoal, R. (1998) Genetics of resistance and parasitism. In: Sharma, S.B. (ed.) *The Cyst Nematodes*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 322–352.

Cook, R. and York, P.A. (1987) Variation in Triticales in reaction to cereal cyst nematode. *Annals of Applied Biology 110, Tests of Agrochemicals and Cultivars* No. 8, 158–159.

Cook, R. and York, P.A. (1988) Reaction of some European and Australian oat cultivars to cereal cyst nematode. *Annals of Applied Biology 112, Tests of Agrochemicals and Cultivars* No. 9, 84–85.

Cregan, P.B. and Quigley, C.V. (1997) Simple sequence repeat DNA marker analysis. In: Caetano-Anolles, G. and Gresshojj, P.M. (eds) *DNA Markers: Protocols, Applications and Overviews*. John Wiley & Sons, New York, pp. 173–185.

Dale, M.F.B. and de Scurrah, M.M. (1998) Breeding for resistance to the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*: strategies,

mechanisms and genetic resources. In: Marks, R.J. and Brodie, B.B. (eds) *Potato Cyst Nematodes: Biology, Distribution and Control*. CAB International, Wallingford, UK, pp. 167–196.

Delibes, A., Romero, D., Aguaded, S., Duce, A., Mena, M., Lopez-Braña, I., Andres, M.-F., Martin-Sanchez, J.-A. and García-Olmedo, F. (1993) Resistance to the cereal cyst nematode (*Heterodera avenae* Woll.) transferred from the wild grass *Aegilops ventricosa* to hexaploid wheat by a 'stepping-stone' procedure. *Theoretical and Applied Genetics* 87, 402–408.

Dong, K. and Opperman, C.H. (1997) Genetic analysis of parasitism in the soybean cyst nematode *Heterodera glycines*. *Genetics* 146, 1311–1318.

Dong, K., Barker, K.R. and Opperman, C.H. (1997) Genetics of soybean–*Heterodera glycines* interactions. *Journal of Nematology* 29, 509–522.

Eastwood, R.F., Lagudah, E.S. and Appels, R. (1994) A directed search for DNA sequences tightly linked to cereal cyst nematode resistance genes in *Triticum tauschii*. *Genome* 37, 311–319.

Eisenback, J.D. and Zunke, U. (1998) Extraction, culturing and microscopy. In: Sharma, S.B. (ed.) *The Cyst Nematodes*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 141–155.

Epps, J.M. and Hartwig, E.E. (1972) Reaction of soybean varieties and strains to race 4 of the soybean cyst nematode. *Journal of Nematology* 4, 222.

Fisher, J.M. (1982) Towards a consistent laboratory assay for resistance to *Heterodera avenae*. *EPPO Bulletin* 12, 445–449.

Fleming, C.C. (1998) The evaluation and durability of potato cyst nematode resistance in the potato. In: Marks, R.J. and Brodie, B.B. (eds) *Potato Cyst Nematodes: Biology, Distribution and Control*. CAB International, Wallingford, UK, pp. 197–208.

Golden, A.M., Epps, J.M., Riggs, R.D., Duclos, L.A., Fox, J.A. and Bernard, R.L. (1970) Terminology and identity of infraspecific forms of the soybean cyst nematode, *Heterodera glycines*. *Plant Disease Reporter* 54, 544–546.

Hartwig, E.E. and Epps, J.M. (1978) Registration of 'Bedford' soybeans. *Crop Science* 30, 231.

Hartwig, E.E. and Young, L.D. (1990) Registration of 'Cordell' soybean. *Crop Science* 30, 231.

Hayes, A.J., Wilkinson, C.A. and Johnson, C.S. (1997) Evaluation of tobacco accessions for resistance to tobacco cyst nematodes and wildfire. *Crop Science* 37, 586–591.

Herrero, S., Rufty, R. and Barker, K.R. (1996) Evaluation of tobacco germplasm for resistance to the tobacco cyst nematode, *Globodera tabacum solanacearum*. *Plant Disease* 80, 61–65.

Hussain, S.W., Williams, W.M., Mercer, C.F. and White, D.W.R. (1997) Transfer of clover cyst nematode resistance from *Trifolium nigrescens* Viv, to *T. repens* L. by interspecific hybridisation. *Theoretical and Applied Genetics* 95, 1274–1281.

Hussey, R.S. and Boerma, H.R. (1992) Tolerance in soybean. In: Riggs, R.D. and Wrather, A.A. (eds) *Biology and Management of Soybean Cyst Nematode*. APS Press, St Paul, Minnesota, pp. 169–181.

Ireholm, A. (1994) Characterization of pathotypes of cereal cyst nematodes, *Heterodera* spp. in Sweden. *Nematologica* 40, 399–411.

Jeffries, S.P., Barr, A.R., Langridge, P., Chalmers, K.J., Kretschmer, P.G. and Karakousis, A. (1997) Practical application of molecular markers in barley breeding. In: *Proceedings of the 8th Australian Barley Technical Symposium*, Queensland, September 1997.

Johnson, C.S. (1990) Control of *Globodera tabacum solanacearum* by rotating susceptible and resistant flue-cured tobacco cultivars. *Journal of Nematology* 22, 700–706.

Kaplan, M., Caswell Chen, E.P. and Williamson, V.M. (1999) Assessment of host-induced selection on three geographic isolates of *Heterodera schachtii* using RAPD and AFLP markers. *Phytopathology* 89, 68–73.

Kim, D.G., Riggs, R.D. and Mauromoustakos, A. (1998) Variation in resistance of soybean lines to races of *Heterodera glycines*. *Journal of Nematology* 30, 184–191.

Kort, J., Ross, H., Rumpenhorst, H.J. and Stone, A.R. (1977) An international scheme for identifying and classifying pathotypes of potato cyst-nematodes *Globodera rostochiensis* and *G. pallida*. *Nematologica* 23, 333–339.

Lagudah, E.S., Moullet, O. and Appels, R. (1997) Map-based cloning of a gene sequence encoding a nucleotide binding domain and a leucine rich region at the *Cre3* nematode resistance locus of wheat. *Genome* 40, 659–665.

LaMondia, J.A. (1988) Tobacco resistance to *Globodera tabacum*. *Journal of Nematology* 2, 77–80.

Lasserre, F., Gigault, F., Gauthier, J.P., Henry, J.P., Sandmeier, M. and Rivoal, R. (1996) Genetic variation in natural populations of the cereal cyst nematode (*Heterodera avenae* Woll.) submitted to resistant and susceptible cultivars of cereals. *Theoretical and Applied Genetics* 93, 1–8.

Mather, K. (1951) *The Measurement of Linkage in Heredity*, 2nd edn. Methuen, London, 149 pp.

McKay, A. (1998) Rapid test for resistance to cereal cyst nematode in cereal breeders lines. URL: www.sardi.sa.gov.au/crops

McKenzie, M.M. and Turner, S.J. (1987) Assessing reproduction of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) on potato cultivars for National Listing. *EPPO Bulletin* 17, 345–357.

Mercer, C.F., Van den Bosch, J. and Miller, K.J. (1999) Effectiveness of recurrent selection of white clover (*Trifolium repens*) for resistance to New Zealand populations of clover cyst nematode *(Heterodera trifolii*). *Nematology* 1, 449–455.

Mugniery, D. (1983) A method for screening potatoes for resistance to *Globodera* spp. under laboratory conditions. In: Hooker, W.J. (ed.) *Research for the Potato in the Year 2000*. International Potato Center (CIP), Lima, Peru, pp. 137–138.

Muller, J. (1998a) Resistance and tolerance to beet cyst nematodes (*Heterodera schachtii*) in sugar beet cultivars. *Zuckerindustrie* 123, 688–693.

Muller, J. (1998b) New pathotypes of the beet cyst nematode (*Heterodera schachtii*) differentiated on alien genes for resistance in beet (*Beta vulgaris*). *Fundamental and Applied Nematology* 21, 519–526.

Niblack, T.L., Riggs, R.D., Arelli, P.R., Noel, G.R., Opperman, C.H., Orf, J.H., Schmitt, D.P., Shannon, J.G. and Tylka, G.L. (2001) A revised classification scheme for genetically diverse populations of *Heterodera glycines*. *Phytopathology* 91, S139.

Nickell, C.D., Noel, G.R., Bernard, R.L., Thomas, D.J. and Frey, K. (1994a) Registration of soybean germplasm line 'LN89–5717' resistant to soybean cyst nematode. *Crop Science* 34, 1133.

Nickell, C.D., Noel, G.R., Bernard, R.L., Thomas, D.J. and Frey, K (1994b) Registration of soybean germplasm line 'LN89–5699' resistant to soybean cyst nematode. *Crop Science* 34, 1134–1135.

Nickell, C.D., Noel, G.R., Bernard, R.L., Thomas, D.J. and Pracht, J. (1994c) Registration of soybean germplasm line 'LN89–5612' moderately resistant to soybean cyst nematode. *Crop Science* 34, 1134.

Nickell, C.D., Noel, G.R., Cary, T.R., Thomas, D.J. and Leitz, R.A. (1999) Registration of 'Ina' soybean. *Crop Science* 39, 1533.

Nicol, J.M. (2001) Important nematode pests of cereals. In: Curtis, B.C. (ed.) *Wheat Production and Improvement*. FAO Plant Production and Protection, FAO, Rome.

Noel, G.R., Franco, J. and Jatala, P. (1990) Screening for resistance to cyst nematodes, *Globodera* and *Heterodera* species. In: Starr, J. (ed.) *Methods for Evaluation of Plant Species for Resistance to Plant-parasitic Nematodes*. Society of Nematologists, Hyattsville, Maryland, pp. 24–32.

Ogbonnaya, F.C., Moullet, O., Eastwood, R.F., Kollmorgen, J., Eagles, H., Appels, R. and Lagudah, E.S. (1998) The use of molecular markers to pyramid cereal cyst nematode resistance genes in wheat. In: Slinkard, A.E. (ed.) *Proceedings of the 9th International Wheat Genetics Symposium*, pp. 138–139.

Opperman, C.H. and Bird, D.M. (1998) The soybean cyst nematode, *Heterodera glycines*: a genetic model system for the study of plant-parasitic nematodes. *Current Opinion in Plant Biology* 1, 342–346.

Orf, J.H. and MacDonald, D.H. (1995) Registration of 'Fairbault' soybean. *Crop Science* 35, 1227.

Phillips, M.S. and Trudgill, D.L. (1998) Variation of virulence, in terms of quantitative reproduction of *Globodera pallida* populations, from Europe and South America, in relation to resistance from *Solanum vernei* and *S. tuberosum* ssp. *Andigena* CPC 2802. *Nematologica* 44, 409–423.

Phillips, M.S., Forrest, J.M.S. and Wilson, L.A. (1980) Screening for resistance to the potato cyst nematode using closed containers. *Annals of Applied Biology* 96, 317–322.

Plowright, R.A., Coyne, D.L., Nash, P. and Jones, M.P. (1999) Resistance to the rice nematodes *Heterodera sacchari*, *Meloidogyne graminicola* and *H. incognita* in *Oryza glaberrima* and *O. glaberrima* × *O. sativa* hybrids. *Nematology* 1, 745–751.

Reversat, G. and Destombes, D. (1998) Screening for resistance to *Heterodera sacchari* in the two cultivated rice species, *Oryza sativa* and *O. glaberrima*. *Fundamental and Applied Nematology* 21, 307–317.

Riggs, R.D. and Schmitt, D.P. (1988) Complete characterization of the race scheme for *Heterodera glycines*. *Journal of Nematology* 20, 393–395.

Riggs, R.D., Wang, S., Singh, R.J. and Hymowitz, T. (1998) Possible transfer of resistance to *Heterodera glycines* from *Glycine tomentella* to *Glycine max*. *Journal of Nematology* 30, 547–552.

Rivoal, R. and Cook, R. (1993) Nematode pests of cereals. In: Evans, K., Trudgill, D.L. and Webster, J.M. (eds) *Plant Parasitic Nematodes in Temperate Agriculture*. CAB International, Wallingford, UK, pp. 259–303.

Rivoal, R., Lasserre, F., Hulle, M. and Doussinault, G. (1991) Evaluation de la résistance et de la tolèrance á *Heterodera avenae* chex le blé par tests miniatutisés. *Mededelingen van den Faculteit Landbouwwettenschappen* 56, 1281–1292.

Ross, J.P. and Brim, C.A. (1957) Resistance of soybeans to the soybean cyst nematode as determined by a double-row method. *Plant Disease Reporter* 41, 923–924.

Rouppe van der Voort, J.N.A.M. (1998) Mapping genetic factors controlling potato/cyst nematode interactions. PhD thesis, Landbouwuniversiteit te Wageningen, The Netherlands. 135 pp.

Rouppe van der Voort, J., Wolters, P., Folkertsma, R., Hutten, R., van Zandvoort, P., Vinke, H., Kanyuka, K., Bendahmane, A., Jacobsen, E. and Bakker, J. (1997) Mapping of the cyst nematode resistance locus *Gpa2* in potato using a strategy based on comigrating AFLP markers. *Theoretical and Applied Genetics* 95, 874–880.

Rouppe van der Voort, J., Lindeman, W., Folkertsma, R., Hutten, R., Overmars, H., van der Vossen, E., Jacobsen, E. and Bakker, J. (1998) A QTL for broad-spectrum resistance to cyst nematode species (*Globodera* spp.) maps to a resistance gene cluster in potato. *Theoretical and Applied Genetics* 96, 654–661.

Rousselle-Bourgeois, F. and Mugniery, D. (1995) Screening tuber bearing *Solanum* spp. for resistance to *Globodera rostochiensis* Ro1 Woll. and *G. pallida* Pa2/3 Stone. *Potato Research* 38, 241–249.

Schmitt, D.P. and Shannon, G. (1992) Differentiating soybean responses to *Heterodera glycines* races. *Crop Science* 32, 275–277.

Seah, S., Sivasithamparam, K., Karakousis, A. and Lagudah, E.S. (1998) Cloning

and characterisation of a family of disease resistance gene analogs from wheat and barley. *Theoretical and Applied Genetics* 97, 937–945.

Sharma, S.B. (1998) *The Cyst Nematodes*. Kluwer, Dordrecht, The Netherlands, 452 pp.

Sikora, E.J. and Noel, G.R. (1996) Hatch and emergence of *Heterodera glycines* in root leachate from resistant and susceptible soybean cultivars. *Journal of Nematology* 28, 501–509.

Singh, K.B., DiVito, M., Greco, N. and Saxena, M.C. (1996) Registration of ILWC 292, a chickpea cyst nematode-resistant germplasm of *Cicer reticulatum* Ladiz. *Crop Science* 36, 1421–1422.

Singh, R.J., Kolliara, K.P. and Hymowitz, T. (1998) Monosomic alien addition lines derived from *Glycines max* (L) Merr and *G. tomentella* Hayata: production characteristics and breeding behavior. *Crop Science* 38, 1483–1489.

Spielmeyer, W., Robertson, M., Collins, N., Leister, D., SchulzeLefert, P., Seah, S., Moullet, O. and Lagudah, E.S. (1998) A superfamily of disease resistance gene analogs is located on all homoeologous chromosome groups of wheat (*Triticum aestivum*). *Genome* 41, 782–788.

Subbotin, S.A., Waeyenberge, L. and Moens, M. (2000a) Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLP. *Nematology* 2, 153–164.

Subbotin, S.A., Waeyenberge, L., Molokanova, I.A. and Moens, M. (2000b) Identification of *Heterodera avenae* group species by morphometrics and rDNA-RFLPs. *Nematology* 1, 195–207.

Taylor, C., Shepherd, K.W. and Langridge, P. (1998) A molecular genetic map of the long arm of chromosome 6R of rye incorporating the cereal cyst nematode resistance gene *CreR*. *Theoretical and Applied Genetics* 97, 1000–1012.

Trudgill, D.L. (1985) Potato cyst nematodes: a critical review of the current pathotype scheme. *EPPO Bulletin* 15, 273–279.

Trudgill, D.L. (1991) Resistance and tolerance of plant parasitic nematodes in plants. *Annual Review of Phytopathology* 29, 167–192.

Trudgill, D.L., Evans, K. and Phillips, M.S. (1998) Potato cyst nematodes: damage mechanisms and tolerance in the potato. In: Marks, R.J. and Brodie, B.B. (eds) *Potato Cyst Nematodes: Biology, Distribution and Control*. CAB International, Wallingford, UK, pp. 117–133.

van Riel, H.R. and Mulder, A. (1998) Potato cyst nematodes (*Globodera* species) in western Europe. In: Marks, R.J. and Brodie, B.B. (eds) *Potato Cyst Nematodes: Biology, Distribution and Control*. CAB International, Wallingford, UK, pp. 271–298.

Wheeler, R. (1998) Barley varieties sowing guide, 1998. URL: www.sardi.sa.gov.au.

Williams, K.J., Fisher, J.M. and Longridge, P. (1996) Development of a PCR-based allele specific analysis for a RFLP probe linked to resistance to cereal cyst nematode in wheat. *Genome* 39, 798–801.

Ditylenchus Species

5

R.A. Plowright[1], G. Caubel[2] and K.A. Mizen[3]

[1]CABI Bioscience, Bakeham Lane, Egham, Surrey TW20 9TY, UK; [2]INRA, 35653 Le Rheu Cedex, France; [3]Institute of Grassland and Environmental Research, Aberystwyth SY23 3EB, Wales, UK

The genus *Ditylenchus* comprises many species, of which four are currently known to be important pests of crop plants, namely: *Ditylenchus dipsaci* (Kuhn) Filipjev, *D. destructor* Thorne 1945, *D. angustus* (Butler 1913) Filipjev 1936 and *D. africanus* Wendt *et al*. 1995. *D. dipsaci*, the stem and bulb nematode, is prevalent in a wide range of climatic conditions, temperate, subtropical and tropical, where moisture regimes enable nematode infection, multiplication and dispersal. *D. dipsaci* is an important pest of lucerne (alfalfa, *Medicago sativa*), red and white clover (*Trifolium pratense* and *T. repens*), pea (*Pisum sativum*), bean (*Vicia faba*) and bulbous species of *Liliaceae* including garlic (*Allium sativum*), onions (*A. cepa*), tulip (*Tulipa* spp.) and narcissus (*Narcissus* spp.). *D. dipsaci* has been of importance to cereals, particularly oats (*Avena* spp.) and rye (*Secale cereale*). The many host races and populations of *D. dipsaci* are regarded as a species complex (Sturhan, 1971). Resistant cultivars play an important role in nematode management as chemical control is generally uneconomic, seed treatment not always effective and crop rotation complicated by the wide host range of this nematode. Some sources of resistance are available and in lucerne, red and white clover, oat, garlic, strawberry (*Fragaria* × *ananassa*) and sweet potato (*Ipomoea batatas*), resistant cultivars have been developed.

D. destructor, the potato tuber rot nematode, is widespread and locally important. Historically, a pest of potato (*Solanum tuberosum*) in the USA and Europe, the incidence of *D. destructor* is low and the nematode is now of minor importance, except in eastern Europe where

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economic crop losses occur (Sturhan and Brzeski, 1991). *D. destructor* seriously damages sweet potato in China (Lin *et al.*, 1993, 1996) where research has identified sources of resistance, and is one focus of Integrated Pest Management (IPM) research (Anon., 1992).

D. angustus, the ufra disease nematode (Colour Plate 7), was an important pest of deep-water rice (*Oryza sativa*) in south and Southeast Asia, but its importance has declined as the area sown to deep-water rice has declined. *D. angustus* is also widespread in lowland rice systems in the south and particularly the south-west of Bangladesh, where it causes crop failure sporadically in rainfed and irrigated crops. There are good sources of resistance to *D. angustus* and advanced generation breeding material is available for development of resistant cultivars suitable for lowland and deep-water environments.

D. africanus, infecting groundnut (peanut, *Arachis hypogaea*) (Jones and De Waele, 1988) is widespread in South Africa. The nematode reduces the marketable value of groundnut and in some cases can affect crop yield (Venter *et al.*, 1991, 1993). All cultivars tested to date are susceptible to *D. africanus*, although the variety Kwarts is said to be tolerant (Venter *et al.*, 1993).

General Considerations in Screening

Species identification

Precise identification of the target species of plant parasitic nematodes and a thorough understanding of the variability in pathogenicity that can exist in field populations is of crucial importance to resistance breeding and the deployment of cultivars. In this regard, *Ditylenchus* spp. are no exception.

Ditylenchus species are difficult to separate morphologically but, in practice, morphological examination will be the first step in identification. Morphology and clues from field host, site history, the site of infection (root, stem, tuber, seed), symptoms and geographic location, all assist in species identification. A number of organizations offer nematode identification services to aid in this first step.

Intraspecific variation has not been detected in *D. destructor*, *D. africanus* or *D. angustus* but 30 or more biological races exist within *D. dipsaci* (Janssen, 1994). More recently, pathotypes that can break resistance have been found. Some races of *D. dipsaci* have distinct host specialization, particularly the races on herbage legumes and *Liliaceae* but all have considerable variation in host range. The usefulness of host range tests for race designation is thus a subject of discussion but, in practice, the designation of host race or pathotype of field populations

is crucial. Only this will enable correct advice for nematode management to be given. The occurrence of mixtures of races (intraspecific variants) in field populations should be considered to be possible.

Some research has attempted to separate *Ditylenchus* species by using genomic DNA probes (Palmer *et al.*, 1992) or by restriction fragment length polymorphisms (RFLP) of polymerase chain reaction (PCR) amplified ribosomal sequences (Wendt *et al.*, 1994). These techniques currently fail to separate races other than the giant from normal races of *D. dipsaci* and these can readily be separated by adult female size. Some progress has been made towards the development of random amplified polymorphic DNA (RAPD) markers for other host races of *D. dipsaci* (Esquibet *et al.*, 1998), but this approach, too, was more successful in distinguishing giant from normal races. Within these giant and normal groups, races were highly similar. Problems with the reproducibility of RAPDs will probably preclude their use in the field, particularly where populations of mixed races can be expected.

Inoculum

Selection of isolates

Nematode isolates reared for use in resistance screening should be representative of the nematode populations in the target geographic area. *Ditylenchus* spp. can be reared in monoxenic cultures on a variety of 'host' substrates (see below) and, where quarantine regulations permit, the containment provided by these methods enables the development of collections of nematode 'isolates'. These isolates could be different races or pathotypes of *D. dipsaci* or geographically isolated populations of other *Ditylenchus* species. Nematode isolates must be cultured separately, although for screening purposes some advocate the use of mixtures of isolates (Whitehead, 1992; Alcaniz *et al.*, 1996).

Population samples

When nematodes are collected from plants in the field for rearing purposes, there is inevitably some selection from the nematode gene pool that exists in the field. Cultures will, in practice, be established from relatively few nematodes and sometimes just a single female. This selection can be useful, for example, allowing analysis of within-field variation in nematode pathogenicity. In most screening, however, this selection is not desirable. Inoculum for screening may be collected from the field or reared on plants in microplots or in pots. For *D. dipsaci*, this works well in white clover and *V. faba*. Infected plant

tissues can be collected for immediate use or, in the case of *V. faba*, air dried and stored for future use. When inoculum is collected from more than one field, fields with a similar recent cropping history should be selected. Likewise, other species can be obtained from infected tissues: *D. angustus* from fresh rice stems from pots or microplots; *D. destructor* from peel of potato tubers; and *D. africanus* from hulls and seed of groundnut pods. Host plant sources of inoculum, however, may be unreliable and lack the flexibility to supply nematodes when they are needed because the availability of plants infected with many nematodes depends on crop management and season. It is often difficult to collect sufficient *D. dipsaci* from the field when inoculum is required. Even when symptoms are apparent on plants, in new infections, there may be few nematodes present and, in older infections, maturing populations may become contaminated with bacterial feeding nematodes as *D. dipsaci* migrate from the damaged tissues.

Nematode infectivity

Among the species of *Ditylenchus* considered in this manual, *D. dipsaci* and, to a lesser extent, *D. africanus* can survive slow desiccation and persist in plant tissue and seed in an anabiotic state. *D. dipsaci* extracted after rehydration of dried plant tissues and seed will be mainly fourth stage juveniles (J4), the survival stage, whereas fresh tissue will contain all life stages. As tissues senesce, nematode populations have a progressively increasing proportion of J4. *D. africanus* survives in seed predominantly as eggs and some anhydrobiotic nematodes (Venter *et al.*, 1995).

For screening purposes, when inoculum is placed directly on a plant maintained in high relative humidity, it is likely that within each species of *Ditylenchus*, juveniles within eggs, free juveniles and adults have equal infection potential. Thus, in determining inoculum densities all stages can be counted and weighted equally, in terms of infectivity. On the other hand, when inoculum is introduced into soil or water adjacent to a plant, the relative infectivity of different stages influences the numbers that invade. With *D. angustus*, for example, Plowright and Gill (1994) found that second stage juveniles (J2) and hence eggs were not infective using an inoculation system to mimic natural invasion (see below). These issues can be best addressed through consistency of method, particularly in relation to the source of inoculum and the age of cultures which provide the inoculum. Plowright and Gill (1994) found that populations of *D. angustus* achieved a stable demographic equilibrium 60 days after inoculation. Cultures of that age were used to provide inoculum for screening purposes. Some of the difficulties in inoculating plants, encountered by Whitehead *et al.* (1987), may have been due to the range of sources

of inoculum, which included callus cultures, fresh and dried plant material and populations dried on filter papers.

Regardless of the source of nematode inoculum, nematodes should be recovered from extracts daily and used as soon as possible after extraction. They can be stored in shallow water (up to 5 mm deep) in dishes at 2–5°C for 1–2 weeks and still maintain good infectivity. Cook and Evans (1988) avoided the extraction phase by inoculating an aqueous suspension of macerated nematode-infected white clover plant tissue. Similar approaches have been used for *D. angustus* (see below). When extracted from fresh tissue, nematodes should be washed in several changes of sterile water to remove traces of plant phenolics and chlorophyll, which would otherwise render them inactive.

Long-term culture of *Ditylenchus* spp. on callus tissue or an alternative host can influence the ability of nematodes to multiply on the original field host species, as has been reported for *D. angustus* (Ali and Ishibashi, 1997). For this reason, the infectivity or aggressiveness of *Ditylenchus* spp. reared in monoxenic culture should be monitored on the field host. In contrast, a lucerne race of *D. dipsaci* maintained and regularly subcultured on callus, retained its host specificity and ability to induce symptoms on susceptible lucerne for more than 10 years (Eriksson, 1972).

Rearing inoculum

With careful maintenance, monoxenic cultures of *Ditylenchus* spp. can provide a reliable supply of inoculum of consistent quantity and quality. They can also reduce the risk of dissemination of the nematodes in the vicinity of breeding stations.

DITYLENCHUS DIPSACI: MONOXENIC CULTURE. Monoxenic culture methods have been developed for *D. dipsaci* (Hooper, 1986) and are ideal for raising the large numbers of nematodes required for screening. The best methods culture *D. dipsaci* on lucerne callus. Not all callus is suitable for nematode multiplication; indeed resistant and susceptible categorizations derived from whole plants may no longer apply to callus tissues raised from the plant. From callus tissue derived from different lucerne genotypes, those most suitable for nematode multiplication must be selected. Nematodes collected in soil or plant material must be extracted, identified, cleaned and surface sterilized before inoculating on to callus. When collected from field-grown whole plants, *D. dipsaci* must first be identified, after hand-picking with a mounted eyelash or micropipette. When establishing cultures it is important to select only *D. dipsaci*, and to reselect at the first subculture, in order to ensure a monoxenic culture. Nematodes can be separated from debris by migration through either tissue paper on a Whitehead and Hemming

(1965) tray or a 5 mm depth of cellulose sponge filter, cut to fit into an appropriate Baermann funnel modification, and collected in clean water or an antibiotic/antimycotic solution. A suitable antibiotic surface sterilization solution would be: penicillin G sodium (300 units ml^{-1}), streptomycin sulphate (300 μg ml^{-1}), amphotericin B (0.75 μg ml^{-1}). There is a variety of methods for surface sterilizing nematodes. Batches of 1–10 nematodes are quickly transferred, one at a time, using an entomological micro-pin or mounted eyelash, to a few drops of malachite green (0.1% w/v for 15 min) on a sterile glass cavity slide. The nematodes are then quickly transferred to sterile distilled water (SDW) in a sterile excavated glass block. Once sufficient nematodes (30–50) have been axenized in this way they can be transferred to the callus in a drop (5–10 μl) of SDW. Alternatively, larger numbers of nematodes can be rinsed several times in SDW and then, depending on the level of contamination, soaked in 0.5–1.0% Hibitane (chlorhexidine gluconate 20% v/v) for up to 3 h at ambient temperature or overnight at 2–5°C. Following treatment, the nematodes are washed in SDW, collected in a glass block and inoculated on to callus in 5–10 μl of SDW. An excavated glass block is a useful receptacle for surface sterilizing larger batches of nematodes. By gently rotating the block, nematodes in sterilant or rinsing water can be aggregated as they settle. The supernatant liquid can then be removed using a fine Pasteur pipette or a micropipette, leaving the nematodes in a small volume ready for further washing or inoculation. These procedures can also be carried out in sterile centrifuge tubes (e.g. 1.5 ml Eppendorf microtubes) using centrifugation to settle the nematodes.

Protocol for culturing **D. dipsaci** *on callus*

The following protocols describe the scarification and surface sterilization of lucerne or clover seeds, their use in callus production and the techniques for rearing stem nematodes on callus.

General precautions

1. Wear gloves and follow good laboratory practice guidelines.
2. Ensure that as much sulphuric acid as possible is drained from seeds before rinsing with water (exothermic reaction).
3. Collect the concentrated acid separately and dilute in a fume hood by adding acid slowly to an excess of water, neutralize and discard.
4. Mercury vapour is evolved from mercuric chloride in contact with ethanol: prepare in a fume hood.
5. Work in a laminar flow hood and use aseptic techniques throughout.

Method: seed scarification, sterilization and germination

1. Place unscarified seed, two deep to cover the base of a *c.* 100 ml sterilized glass beaker.
2. Add concentrated sulphuric acid to cover seeds.
3. Stir with glass rod and leave to stand for 5–10 min depending on seed size and hardness.
4. Pour off excess H_2SO_4.
5. Rinse seed with excess sterile distilled water (SDW) (to counteract heating effect of dilution of the acid).
6. Repeat rinse with SDW four times.
7. Fill beaker with 1000 p.p.m. $HgCl_2$ in 30% ethanol.
8. Allow to stand in fume hood for 15 min.
9. Pour off $HgCl_2$/ethanol.
10. Rinse with excess SDW.
11. Repeat rinse with SDW four times.
12. Allow seed to stand in SDW in laminar flow hood for 2–3 h to imbibe.
13. Pour off water and rinse with SDW to remove leached tannins.
14. Transfer seed to Petri dishes of nutrient agar and germinate for 2 days at 15–20°C.
15. Discard any contaminated seedlings or plates and transfer healthy seedlings to 30-ml universal tubes of agar and grow on at 20°C and 16 h photoperiod.

Method: callus production, inoculation and nematode collection

1. Sterilized seedlings of lucerne, red or white clover with 3 or 4 trifoliate leaves are cut at the hypocotyl and transferred to callusing medium (B51, that is, Gamborgs B5 medium, pH 5.8, supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) (2 mg l^{-1}), kinetin (0.5 mg l^{-1}), sucrose (2% w/v), agar (8 g l^{-1})). Pinch entire seedling length including laminae lightly between serrated forcep points to create multiple wounds. Insert cut end of hypocotyl into the agar with the rest of the seedling laid on the agar surface, 3 to 4 seedlings per dish and store in the dark for about 7 days. Primary callus will begin to form from each wound point and can be subcultured and inoculated after 2–3 weeks. Inoculate with 100–200 sterilized nematodes delivered in 5–10 µl from a positive displacement pipette. Always inoculate young, actively growing callus by selecting those which have increased in volume. Establishing an initial culture can prove difficult, in which case sterilized seedlings can be inoculated between the cotyledons or in a leaf axil 1–2 days after transfer to the callusing medium. The nematodes then invade and develop at the same time as the primary callus is initiated.

2. Established cultures are maintained in the dark, preferably in an incubator at 20°C. Depending on the size of the initial inoculum and the nematode multiplication rate, callus will require subculture after 40–90 days. Callus with a heavy burden of nematodes tends to discolour and has a watery appearance. Subculture by sterile transfer of pieces of agar and callus containing nematodes on to new, actively growing callus.
3. Nematodes are harvested by breaking up callus and agar with sterile forceps and placing the entire culture with any nematodes washed from the dish on a sterile Baermann funnel or similar device to select active worms. Nematodes should be collected regularly to sterile Petri dishes. Residual 2,4-D can be removed from the nematode suspension in three or four of the rinsing cycles described above, and the clean nematodes stored at 2–4°C until use. Some nematodes can be collected from condensation droplets on the dish lid, where they accumulate after migration from the callus. These should also be selected by passage through the sterile filter system.

DITYLENCHUS DIPSACI: ALTERNATIVE CULTURE METHODS. *D. dipsaci* can be mass cultured in courgettes (Hooper and Cowland, 1988) or onion for some different races, including giant races. Tenente and Evans (1992) found that teasel and red clover races reproduced well in courgettes, but bean, oat and lucerne races failed to multiply. The nematodes should be axenized as above and injected into the tissue in 5–10 µl SDW using a hypodermic needle and sealing the puncture with molten wax. Endogenous bacteria can be a problem leading to rot of some courgettes before nematodes can be recovered.

D. dipsaci can be extracted from fresh tissue or dry plant tissue, the latter being particularly good for *V. faba*. The ability of *D. dipsaci* to survive in a desiccated state, means that dried tissues can be stored in bulk to provide large numbers of nematodes. The plant material, fresh or dry, is cut into 1 cm lengths, placed in a Baermann funnel in shallow water or under automatically controlled, intermittent misting. Nematodes extracted from dried plant tissues will be mainly fourth stage juveniles, whereas those extracted from fresh tissue will contain eggs and all juvenile stages. As fresh tissue senesces, it will contain nematode populations with a progressively increasing proportion of J4.

DITYLENCHUS DESTRUCTOR: MONOXENIC CULTURE. *D. destructor* can feed and reproduce on a wide range of fungi and on callus tissues of carrot, clover, potato and tobacco (Faulkner and Darling, 1961; Hooper, 1986). MacGuidwin and Slack (1991) reared *D. destructor* on *Fusarium roseum* growing on potato dextrose agar. For screening, it is preferable to rear nematodes on plant tissue to reduce the risk of transferring fungal spores with inoculated nematodes. For this purpose, potato

callus maintained at 25°C is suitable. Callus can be initiated from potato stem internodes rinsed with ethanol (70% v/v) or from sterile tissue cultured plants. De Waele *et al*. (1991) propagated callus on Murashige and Skoog's (1962) medium supplemented with 2,4-D (3 mg l^{-1}) and kinetin (0.2 mg l^{-1}). *D. destructor* can be surface sterilized using the methods outlined above for *D. dipsaci* and the same inoculation considerations apply. Cultures are maintained by transferring small pieces of infected callus to new callus cultures.

DITYLENCHUS DESTRUCTOR: ALTERNATIVE METHODS. *D. destructor* can be reared on whole potato tubers. The inoculum is introduced in a solution of carboxymethyl cellulose (2% w/v) into a shallow cavity (*c*. 5 mm deep) cut using a 3 mm diameter cork borer, and sealed with wax (see Hooper, 1986).

DITYLENCHUS ANGUSTUS: MONOXENIC CULTURE. Most screening for resistance to *D. angustus* has been done in the field in microplots, infested with cut pieces of infected rice stems which are reared in irrigated microplots or in pots. Inoculation procedures for rearing *D. angustus* in this way are the same as those for field screening purposes. *D. angustus* can be reliably cultured on rice plantlets grown in monoxenic conditions (Plowright and Akehurst, 1992). It is suggested that a number of fungi also support nematode multiplication (Latif and Mian, 1995). Contrary to earlier work (Plowright and Akehurst, 1992), Ali and Ishibashi (1997) found that *D. angustus* could reproduce on *Botrytis cinerea*. Fecundity was always greater in seedling culture but there was a suggestion that nematodes could adapt and become more fecund on *B. cinerea*.

To establish rice plantlet culture, surface sterilize hulled rice grain in mercuric chloride (0.1% w/v), for 30 min, wash five times in SDW and transfer to Gamborgs B5 medium with sucrose (2% w/v) and solidified with agar (1%) in 9 cm diameter Petri dishes. After 30 days, inoculate a leaf base adjacent to a newly emerging leaf with *c*. 30 adult axenized nematodes in 5 µl of SDW. Seal the Petri dish with elastic PVC tape or Parafilm and maintain at 25°C, 12 h photoperiod. Nematodes migrate from plant tissues and move throughout the Petri dish as numbers increase. Moisture droplets may form on the underside of lids of culture plates, particularly when temperatures fluctuate diurnally by a few degrees. Nematodes can become trapped, starve and die if such droplets persist. When extracting nematodes from such cultures, care should be taken to avoid high proportions of these weak or dead nematodes in the resultant inoculum. Nematodes migrating on the underside of lids can be easily collected in a small volume of SDW (< 1 ml) to provide axenic inoculum to form subcultures. The nematodes collected in this way can be concentrated in a sterile microtube

by centrifugation, if necessary, to achieve an inoculum density of 6 μl^{-1}. A consistent quality of inoculum for screening purposes can be achieved by standardizing the age of cultures used.

DITYLENCHUS AFRICANUS: MONOXENIC CULTURE. *D. africanus* can be reared on groundnut callus derived from groundnut leaves (Van der Walt and De Waele, 1989). Leaves from 4-week-old plants are surface sterilized using ethanol (70% v/v) for 30 s, then sodium hypochlorite (0.5% v/v) containing Tween 20 for 15 min before washing five times in SDW. Leaf sections (1 cm^2) are cut and transferred to Murashige and Skoog's (1962) medium, pH 5.7. Actively growing callus is inoculated after 4 weeks. Callus can show discoloration and requires subculture after 5 weeks. Subculture procedures are the same as those for *D. dipsaci* above.

Inoculating Plants

A wide variety of methods has been developed to achieve stem nematode infection in plants. Stem nematodes can be inoculated on to plants directly or, depending on the species concerned, they can be placed, in soil or water, in the vicinity of plants. The inoculum can be delivered in aqueous suspension with or without a gelling agent such as agar or carboxymethyl cellulose, or by using infected fresh or dry plant material. Similarly, a range of inoculum densities has been used, often without any clear rationale or knowledge of the fate of inoculated nematodes.

Ditylenchus dipsaci

Inoculating plants in the field

In common with most nematodes, infection by *D. dipsaci* in the field can be achieved by establishing a nematode 'sick' plot. Cook and Evans (1988), for example, transplanted pot-grown, white clover accessions and cultivars into a field that had previously been a white clover monoculture, with an established stem nematode infection. Rivoal *et al.* (1978) and Stanton *et al.* (1984) also relied on natural soil-borne infestation of *D. dipsaci* for screening for resistance in cereals. The population dynamics of *D. dipsaci*, however, are strongly influenced by environmental conditions, particularly relative humidity and temperature and thus field results can vary from year to year, with the prevailing conditions. Furthermore, the distribution of nematodes in field soil is known to be heterogeneous which requires that trial designs be replicated and incorporate resistant and susceptible reference cultivars. Working with *V. faba*, Hanounik *et al.* (1986) reduced

the heterogeneity of inoculum in the field, by overlaying seed, sown in rows 1 m long and 50 cm apart, with nematode-infested soil to a depth of 15 cm. The infested soil was prepared by incorporating large quantities of infected stems cut into 2 cm segments. This soil was watered daily and diluted with uninfested soil after 2 weeks, to achieve a population density of about 300 J4 dm^{-3} soil.

Inoculating plants and seedlings in containers

The problem of plants escaping infection for reasons unrelated to host plant resistance can be minimized but rarely eliminated, by inoculating plants or seedlings with infective nematodes. If this is done in conditions optimal for infection, individual plant responses can be characterized and compared. There are many methods of inoculating plants with stem nematodes and different laboratories probably employ variations of standard approaches. Nevertheless, there are some guiding principles.

Inoculum density

The inoculum density should be 30–100 nematodes per plant or seedling. Elgin (1984) preferred 200 but considered 100 to be sufficient. Hooper (1984) used 50,000–100,000 nematodes per pot of field beans, which, although this was added to the seed at sowing, appears to be excessive. For white clover, Cook and Evans (1988) found no difference between 34 or 61 nematodes. There have been few studies on the fate of inoculated nematodes, but losses, i.e. those inoculated individuals that do not invade the host, are probably high. Plowright and Gill (1994) estimated that > 75% of *D. angustus* inoculum were lost, Mercer and Grant (1995) estimated losses of *D. dipsaci* between 67 and 93%.

Inoculum delivery

In all inoculum delivery systems, whether nematodes are introduced to soil or directly on to plants, it is essential that high relative humidity is maintained immediately after inoculation. The first 2–3 days after inoculation are particularly critical. Details of optimal conditions vary with the host plant, but plant growth should be slow for 1–2 weeks after inoculation to allow nematodes to establish an infection site. Actively growing plants may outgrow the infection, so that subsequent secondary thickening can trap nematodes in tissues and prevent or reduce symptom development. It is also advisable to re-inoculate plants after 1–2 weeks to minimize escapes, particularly where nematodes are inoculated in aqueous suspension.

D. dipsaci can be inoculated on to seedlings of lucerne, *V. faba*, clover and pea, in a small drop of carboxymethyl cellulose (CMC) suspension (1–2% w/v) placed in the axil of the first leaves close to the seedling terminal meristem (Figs 5.1 and 5.2). This method can be used

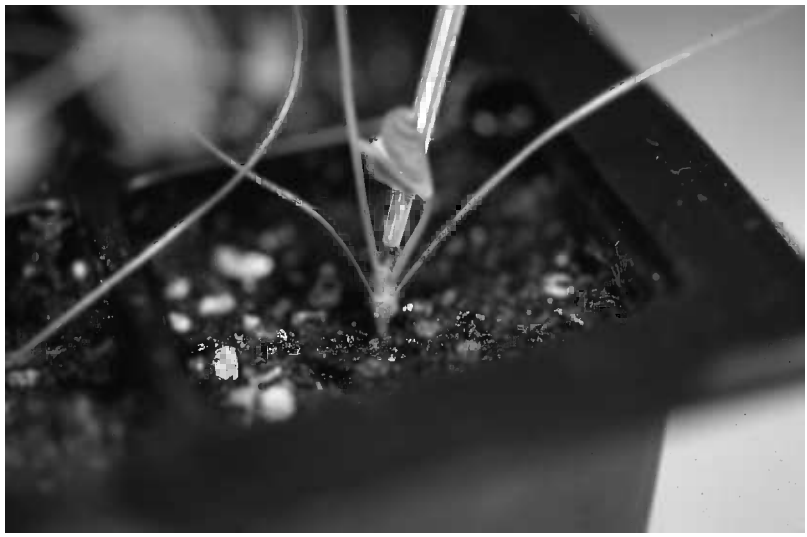

Fig. 5.1. White clover plant being inoculated with *Ditylenchus dipsaci* in controlled environment screening test.

Fig. 5.2. White clover plant immediately after inoculation showing growing point with *Ditylenchus dipsaci* in inoculation droplet.

to inoculate older seedlings in pots or adapted for use with the 'rag doll' protocol (see below). Elgin (1984) inoculated lucerne seedlings in trays or 'flats', 2 weeks after emergence with nematodes delivered either in droplets of water or as an atomized spray, at a rate of 100 nematodes per plant. Mercer and Grant (1995) used a technique for white clover similar to that of Hussey and Krusberg (1968) for pea, in which an

aqueous suspension of nematodes was applied to 3-day-old clover seedlings or germinated pea seeds. Cook and Evans (1988) inoculated white clover stolon-tip cuttings with a suspension of macerated infected white clover containing about 100 nematodes to the soil surface in close proximity of buds. Clover can be injected with a nematode suspension using a hypodermic syringe (Dijkstra, 1957; Cook and Evans, 1988). Cereal seedlings can also be inoculated in this way (Seinhorst, 1952); however, since it is difficult to inject even small volumes of inoculum into plants, the volume to be injected into each seedling must be no more than 5 µl (Cameron, 1963).

Carboxymethyl cellulose provides a number of functions as a carrier for inoculum. It facilitates adhesion to the plant surface, reduces surface tension allowing the inoculum drop to settle into the axil and dries more slowly than water. It can be useful for keeping *D. dipsaci* in suspension in the inoculum bulk and thus ensure more uniform inoculation, although the more active *D. angustus* may form clumps which are hard to disperse. However, automatic micropipettes can now accurately dispense aqueous suspension in microlitre volumes, provided the nematodes can be maintained in suspension. Aqueous inoculum droplets of 5–10 µl maintain their integrity and can be made to adhere at the point of inoculation, although such small drops may dry very quickly and do not settle quickly into the axil. Mercer and Grant (1995) found that infection using droplets was very poor, but this may have been because of the relatively large droplet volume (30 µl).

Inoculation into soil or on young seedlings mimics natural invasion more closely, but, since mechanisms of resistance are thought to operate after infection, direct inoculation methods are equally valid. Better control of the nematode number inoculated to each plant is gained by inoculating individual plants, and the homogeneity of inoculum can be checked regularly by direct observation of sample droplets placed on a glass slide. The spraying/sprinkling approaches used by many are faster, but must sacrifice accuracy unless inoculation is repeated. Cook and Evans (1988) found that the different ways that plants were inoculated led to different levels of overall susceptibility expressed in particular tests, but that there was general agreement in the rank classification of clovers in separate tests. This would suggest that relatively few nematodes are required to establish an infection to classify a genotype.

Ditylenchus angustus

Inoculating plants in the field

Natural infestations of *D. angustus* are very sporadic and can not be relied on for screening purposes. The nematode has no intrinsic ability

to survive desiccation (Ibrahim and Perry, 1993) and soil population densities are virtually undetectable where rice is followed by a dry season, non-irrigated crop. Infested plots can be produced, after draining and allowing the soil to dry, by incorporating infected stubble.

Field microplots of rice are infested by floating cut pieces of infected rice culm on the surface of water around rice seedlings. Sufficient inoculum is introduced to provide at least 100 infective nematodes per seedling (predominantly J4 and adult) (Anon., 1985). The depth of water in relation to seedling height is an important factor in determining the success of inoculation (see protocol and Fig. 5.7).

Inoculating plants in pots

Inoculation of rice coleoptiles was developed in Vietnam (Kinh and Nghiem, 1982). Ten adult nematodes were inoculated in a drop of water on to germinated seed and maintained in a saturated environment for 48 h at 28–30°C. Infected seedlings were potted up and kept in a partly shaded screenhouse at 80–90% relative humidity. Plowright (unpublished) adapted the 'rag doll' method and inoculated *D. angustus* in 2% CMC on rice coleoptiles held in saturated rolls of chromatography paper, but found the method to be very unreliable. *D. angustus* are extremely active and readily aggregate in solution. Aggregates of nematodes can be dispersed by agitation in water, but are difficult to disperse in CMC.

Rahman (1987) inoculated 3–4-week-old plants in pots by introducing nematodes directly into water around plants at a rate of 100 per plant. For routine screening, Plowright and Gill (1994) inoculated 300–500 nematodes per seedling into water and used plastic tubes to confine the inoculum around seedlings (Fig. 5.3). Confining nematodes around the plant ensured more equal infection of plants within a tray and reduced escapes, compared with releasing inoculum into the whole tray of 24 plants at the same rate per plant (Plowright and Gill, 1994). These methods mimic natural invasion from water which takes place at the water surface and it was assumed that with such methods only about 10% of inoculated nematodes would invade the leaf sheath interstices. Rahman and Evans (1987) injected nematodes into the rice leaf sheath, incorporated infected tissues into soil or placed them at the base of the 10-day-old plants in water. The latter method gave the highest proportion of infected plants, but less than 10% of the inoculum invaded.

Ditylenchus destructor *and* D. africanus

There is very little information regarding techniques for inoculating either *D. destructor* or *D. africanus* for the specific purpose of screening for resistance. Both species invade plant tissue below ground and can

Fig. 5.3. Container of rice plants grown and prepared for inoculation with *Ditylenchus angustus* in glasshouse screening test, showing plastic tubes around base of seedlings to concentrate the inocula. (Note host response in foreground.)

be applied in aqueous suspensions to soil around established plants. Venter *et al*. (1993), for example, inoculated 100 or 1000 *D. africanus* of mixed life stages on to 5-week-old plants. They demonstrated very little influence of initial density (P_i) on the relative host status of groundnuts. Many of the considerations regarding the inoculation of plants are the same as those for migratory endoparasitic nematodes (see De Waele and Elsen, Chapter 8). *D. africanus* can invade and multiply in groundnut roots and so inoculation can take place before pod formation. Similarly, *D. destructor* can feed on underground stem tissue before stem tuber development in potato or root tuber development in sweet potato.

Laboratory-based methods for screening root tubers of sweet potato (Anon., 1992) involve the same considerations as those for establishing potato tuber cultures of *D. destructor* (see above and Hooper, 1986). The thickness of the root periderm plays a role in resistance, and mechanical injury of the periderm predisposes the tuber to nematode infection. Inoculating nematodes through the periderm (circumventing this barrier) can enable the differentiation of clones based on a rotting index (Anon., 1992).

Evaluating Genotypes for Resistance

Resistance is interpreted as the ability of some cultivars of an otherwise susceptible plant species to reduce the ability of the nematode to reproduce and multiply to high population densities. Symptoms

are expressed by plants in response to nematode invasion and (or) nematode multiplication. The type of symptom and the severity of expression are generally good indicators of susceptibility or resistance to *Ditylenchus* spp. (Colour Plate 8), although care should always be exercised when categorizing symptomless plants which may be escapes or resistant.

Ditylenchus dipsaci

D. dipsaci feeds on parenchymatous tissue inducing hypertrophy and hyperplasia in susceptible hosts. Infected plant parts containing reproducing populations of nematodes are characteristically stunted, swollen and distorted (Colour Plate 8). Individual plants may exhibit some, although not necessarily all of the typical symptoms. In white clover, for example, symptom development is dependent on the balance between nematode population growth and internode elongation, a balance which is temperature dependent (Griffith *et al.*, 1996). Sub-epidermal infestations are not always correlated with the presence of symptoms. Ectoparasitic infections of stem nematodes can establish and multiply in leaf axils without producing typical susceptible symptoms, e.g. in lucerne and white clover (Griffith *et al.*, 1997). Resistant plants can be symptomless, less swollen than susceptible ones and/or exhibit localized necrosis. For example, the giant race causes limited necrosis on a resistant plant, but never such severe stem swelling as on a susceptible plant (Caubel and Leclercq, 1989). Thus, the reactions of plants after infection may be evaluated by observations of aerial plant parts. Ideally, assessments of symptom expression will be supported by estimates of the rate of nematode multiplication. This can be estimated by soil analysis for field plots and/or by counting nematodes in plants. In field conditions, however, this is time consuming and the distribution of infestation is never regular.

The assessment of symptoms in seedlings facilitates the screening of large amounts of material; in general seedling response, for example, in red clover and lucerne, correlates well with the host suitability of the mature plant (Bingefors, 1970; Lundin and Jonsson, 1975; Caubel *et al.*, 1977). Very early assessments at 2–4 days after inoculation, can be largely a measure of host reaction to invasion and Elgin *et al.* (1975) argued that host responses should be judged weeks after inoculation, whereas Whitehead (1992) considered it necessary to assess plants at flowering and again several months later. Cook and Evans (1988) described an apparent loss of resistance when plants initially categorized as resistant later exhibited susceptibility. This further demonstrates that nematodes can survive in plant tissue without exhibiting symptoms.

Resistance to *D. dipsaci* in *V. faba*, lucerne, and red and white clover is a function of the response of individual plants derived from seed, and the reaction of a cultivar determined by the proportions of resistant plants in a population that expresses both resistant and susceptible symptoms (Caubel and Leclercq, 1989).

Stem nematode multiplication is greatest in plants induced to form massively hypertrophied tissues at infection sites. The probability of this occurring seems to reflect the balance between nematode multiplication rate and plant growth and differentiation. Thus, in spring-sown oats, primary stems quickly become secondarily thickened as stem elongation occurs (Colour Plate 9). In susceptible early heading cultivars, invading stem nematodes rarely induce hypertrophy and do not multiply so much as in susceptible winter oat cultivars whose meristems remain vegetative for long periods. Nematode infection causes massive hypertrophy and symptoms including the infestation of secondary tiller meristems, before lignification and elongation.

Ditylenchus angustus

D. angustus feeds ectoparasitically on the youngest developing leaves of rice, within the leaf sheath. Genotypes of rice can be classified according to the type of symptom on emerging new leaves, following inoculation. Susceptible symptoms are small white speckles on the leaf, which coalesce towards the base of the leaf blade (Colour Plate 7). These can be accompanied by puckering of the leaf surface and distortion of the mid-rib or the leaf periphery. In severe infestations, the entire emerging leaf is white. Plowright and Gill (1994) devised a method of assessing the severity of susceptible symptoms on a new leaf and demonstrated a good correlation between symptom severity and the number of nematodes per plant (Fig. 5.4). Resistant plants are either symptomless or exhibit a rapid browning response to feeding. This browning may be on the leaf mid-rib or within relatively discrete yellow halos on the lamina. Browning also occurs in the susceptible response but it occurs slowly due to the deterioration of affected tissue. The qualitatively different resistant response provides a basis for genotypic selection. Plowright and Gill (1994) also found quantitative variation in susceptibility to *D. angustus.*

Ditylenchus africanus

Resistance to *D. africanus* has not been reported, although some cultivars appear to be more tolerant (Venter *et al.*, 1993). Characterization of groundnut genotypes is based on the severity of pod disease and

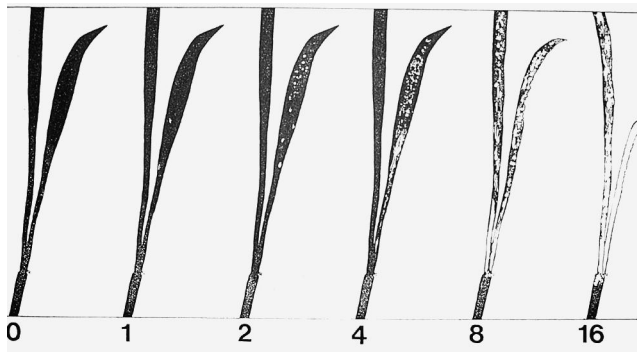

Fig. 5.4. A system for scoring ufra symptom severity on rice susceptible to *Ditylenchus angustus.*

seed disease at harvest. Pod disease severity is rated on a 10-point scale and estimates the proportion of the pod surface which is discoloured, whereas seed disease severity is the proportion of blemished seed (Venter *et al.*, 1991).

Ditylenchus destructor

In China, sweet potato genotypes are assessed according to the extent of browning of the flesh of root tubers, extending from the point of inoculation (Anon., 1992).

Screening Protocols

Screening white clover, red clover and lucerne (alfalfa) for resistance to D. dipsaci *in controlled environment/glasshouse*

Screening is best conducted in controlled environments maintaining 15/12°C (day/night) with a 16 h photoperiod. Alternatively, similarly cool glasshouse or field conditions may be used, if protection from unfavourable conditions (frosts or sun) is available. Field screening may be used but, due to the variability of climatic conditions and difficulty of inoculation, a greater number of escapes should be expected and the replicate number increased accordingly.

1. Set up multiwell (8 column × 5 row) 50 cm^3 plastic pots containing mixed (3:1 v/v) compost and horticultural vermiculite in propagator trays, with drainage holes. Stand these trays in water to soak the compost and after draining, stand each tray in a similar-sized tray without holes and cover with a clear propagator hood.

2. Sow individual germinated seed in each pot, with five seedlings (pots) of each variety per column. Each tray will contain six columns of test entries and one column each of resistant and susceptible controls.
3. Grow seedlings for approximately 3 weeks at 15/12°C day/night with a 16 h photoperiod or until the first trifoliate leaf is expanded, when the seedlings are inoculated.
4. Extract inoculum from infected plants or *in vitro* culture:
Infected plants. Collect well-infected buds and stolons showing symptoms from pots or field, cut off leaves, roots and any dead or rotting tissue (to remove chlorophyll and phenolics). Wash on a 1–2 mm aperture sieve to remove compost, soil and ectoparasites. Chop roughly into 5 mm lengths and extract on a modified Whitehead tray or in a mist extraction chamber.
In vitro *cultures*. Select Petri dishes of infected callus containing active healthy nematodes on the lid and in the agar. Roughly break up the callus and agar with forceps and extract together with the nematodes washed from the lid of the Petri dish on a modified Whitehead tray or in a mist extraction chamber.
5. Collect nematodes after 2, 24 and 48 h. Each time, settle the extract at 2–4°C, siphon off the supernatant and refill with fresh water. This process will remove plant phenolics and chlorophyll which would otherwise inactivate the nematodes.
6. Make up the bulk to a known volume, aerate then remove measured subsamples in which the number of stem nematodes are counted. Calculate the total number of nematodes.
7. Reduce the bulk volume by settling and siphoning the supernatant to give an inoculum density of approximately 10,000 nematodes ml^{-1} (10 μl^{-1}). Aerate, then settle at 2–4°C, before removing half the supernatant and replacing with an equal volume of 2% CMC.
8. Inoculate seedlings by delivering a 10 µl droplet of nematode suspension on to the primary meristem in the axil of the emerging trifoliate leaf (Fig. 5.2). Continue until all seedlings have been inoculated, mixing suspension regularly to ensure even distribution of nematodes. Cover each tray with a clear propagator hood (vents closed for 1 week) to maintain high humidity.
9. Take sample droplets at the start and end of each tray, and count nematodes to monitor the homogeneity of the inoculum.
10. Re-inoculate all seedlings as above, 2–7 days after the first inoculation and in the reverse order, to reduce the chances of escapes.
11. Seedlings may be harvested after about a week and stained whole using the acid fuchsin method (Byrd *et al.*, 1983) to determine invasion and start of egg-laying. Symptom development can be assessed visually at 3 and 6 weeks postinoculation, scoring for hypertrophy, hyperplasia, swelling and stunting in meristems, petioles and leaves.

Fig. 5.5. Field bean (*Vicia faba*) plants after inoculation with the giant race of *Ditylenchus dipsaci*, showing susceptible (left) and resistant phenotypes.

12. Second generation population development can be assessed 4–6 weeks after inoculation by counting life stages of nematodes extracted from individual seedlings.

Screening field beans for resistance to D. dipsaci

Different methods have been developed under field, glasshouse and laboratory conditions, using soil naturally or artificially infested, and direct inoculation of plants.

Field evaluation

1. Collect large quantities of infected stems from fields, cut them into 2 cm segments and mix thoroughly with soil, to give about 300 nematodes dm^{-3} soil.
2. Sow seeds in open drills, 1 m long, 50 cm apart, with a susceptible cultivar repeated every five test entries. Cover all seeds with 15 cm depth of infested soil. Irrigate immediately. Abbad *et al.* (1990) applied an aqueous suspension of 300 nematodes per seedling at 1 month of age.
3. Record disease at about 80% podding, when stem symptoms are well developed on susceptible controls, scoring by extent and nature of symptoms (Hanounik *et al.*, 1986).

Pot tests

1. Place seeds on silicate clay (Vermex M®, Efisol, France) at 23°C: transfer after 4–5 days one seed per pot of steam-treated organic

compost, with 30 pots per tray, and transfer to a controlled environment chamber at 15°C, 16 h photoperiod.
2. Extract inoculum from dry tissues of infected plants to select predominantly preadult stages. Cut dry tissue coarsely on to a 20-µm aperture sieve and soak in water. Collect nematodes every 2 h, settling each extract at 2–4°C. Siphon off supernatant and refill with clean water.
3. Make up bulk of cleaned extracts to known volume, aerate and remove subsamples, count nematodes and calculate total number. Reduce the volume by settling and siphoning to give an inoculum density of 200 nematodes per 15 µl.
4. Add an equal volume of 2% CMC to give 100 nematodes per 15 µl droplet with good adherence to plants allowing increased penetration.
5. Inoculate 10-day-old seedlings by placing a 15 µl droplet in the first stipule. Cover each tray to maintain high humidity for several days.
6. Take a sample droplet at the start and end of each tray to monitor the homogeneity of the inoculum.
7. Assess symptom development at 2 months after inoculation, scoring swelling, stunting or necrosis in stems and leaves (Fig. 5.5).
8. Assess nematode multiplication in each plant. Crush plant tissues in a blender, dilute or concentrate the suspension and count nematodes in subsamples to determine total nematodes per plant. Assess multiplication rate (final population/100) which is correlated with symptom expression.

Screening cereals for resistance to D. dipsaci

Glasshouse screening of cereals is most effective during the natural season for field infection of the cereal type (that is, in winter or early spring). Alternatively, controlled environment conditions can be set to mimic the seasonal regimes of the particular crop to be examined. A long, slow growing period after inoculation allows nematodes to reproduce and symptoms to develop before plant stem differentiation and elongation occur.

1. Sow 8–10 germinated seeds of each entry in a circle 2 cm from the rim of a 15 cm diameter pot containing soil-based compost. Stand each pot in a saucer to allow bottom watering and randomize pot layout. Each block of pots should contain entries to be tested and known resistant and susceptible controls.
2. The seedlings are inoculated once the coleoptile has emerged.
3. Collect infected dried straw, select 3 or 4 tillers and soak in tapwater for about 2 h (this prevents damage to the nematodes when

extracting), rinse, then tease each straw apart longitudinally under a stereomicroscope and extract nematodes overnight on a modified Whitehead tray. Count the nematodes recovered and calculate the tillers required to give an inoculum density of 100–200 nematodes per seedling. Extract (see protocol for legumes), collect nematodes twice daily for 48 h. For each collection, settle at 2–4°C before siphoning off the supernatant and rinsing with clean water to remove plant phenolics which would otherwise inactivate the nematodes.

4. Bulk cleaned collections in a known volume of water and remove measured subsamples for counting and calculate the total number of nematodes in the bulk. Reduce the bulk volume to give an inoculum density of 10–20 nematodes μl^{-1}. Settle, remove half the supernatant, then add an equal volume of 2% CMC and mix.

5. Inoculate by delivering 5–10 µl of the suspension (100–200 nematodes) directly into the coleoptile sheath. This can be achieved using a hypodermic syringe or micropipette. Begin by scraping away some of the compost from the base of the seedlings to be inoculated, then either:

(a) Fill a hypodermic syringe with well-mixed nematode suspension, insert the needle into the exposed base of the coleoptile at 45° angle, and deliver the inoculum directly into the sheath. Some resistance will be felt as the suspension fills the sheath, occasionally a small droplet appears at the top of the coleoptile. Take care not to pass the needle straight through the coleoptile. Practice is required to ensure consistent delivery. Extra inoculum will be needed as the needle sometimes clogs and has to be replaced. Maintaining even distribution of nematodes is more difficult with this method and inoculum density and delivery will be more variable.

(b) Alternatively, use a scalpel to cut a slit longitudinally in the exposed base of the coleoptile, into which the inoculum can be precisely delivered using a micropipette (avoid loss of inoculum by ensuring that it is delivered into the developing leaf tissue within the coleoptile sheath).

6. Replace the compost around the base of the coleoptile and ensure a high humidity is maintained by covering each pot with a plastic bag for 1 week after inoculation.

7. Assess invasion by staining additional whole seedlings in bleach/acid fuchsin (Byrd *et al.*, 1983) 2 weeks after inoculation. Plants should then be characterized by visual observation of symptom development after approximately 4–6 weeks (refer to symptom expression on control pots for exact timing). Population structure is assessed in individual plants by shredding tillers longitudinally (including base) and extracting on a modified Whitehead tray, then counting eggs and life stages.

Screening rice for resistance to D. angustus

Field tests

Field screening for resistance in deep-water rice requires the construction of large tanks which can be flooded in a controlled manner to a depth of 2–3 m (Fig. 5.6). Screening for resistance in lowland rice requires only conventional bunding with soil banks 30–40 cm high. Screening should not be done out of season (e.g. screening for resistance in deep-water rice, using irrigation, during the dry season, because of the sensitivity of nematode population dynamics to the prevailing atmospheric humidity). This protocol has been adapted from Rahman (1982) and Anon. (1985).

1. Divide the deep-water tank into 1 m^2 plots, demarcated by a mud levee 15–20 cm high. There should be paths, 1 m wide, between plots.
2. Sow rice entries in rows 20 cm apart, thinning to 20 seedlings per row. Each plot includes a resistant and a susceptible control and three test entries.
3. Inoculate seedlings with *D. angustus* 2–3 weeks after sowing or when the collar of the leaf sheath is about 10–15 cm above soil level.
4. Raise the water level in the plot to about 10 cm before inoculation. It is important that the plants are not submerged.
5. Collect sufficient infected plants from cultures to provide inoculum for the whole trial. Cut the leaf sheath section, above the uppermost node, of infected culture plants into 3 cm lengths and mix. Remove a subsample, tease apart longitudinally in water and leave to extract overnight. Determine the number of nematodes per stem section

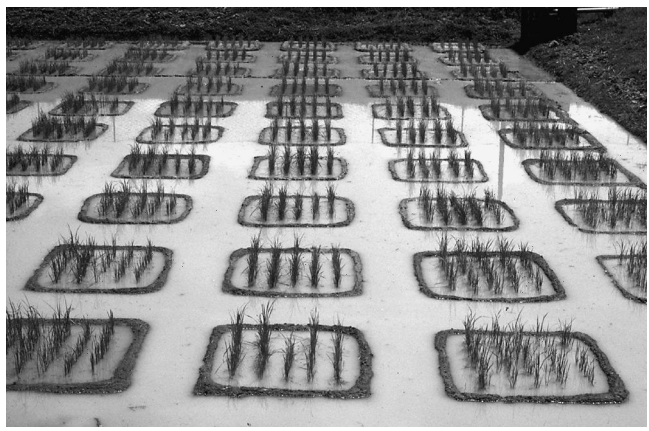

Fig. 5.6. Field plots of deep-water rice screening for resistance to ufra caused by *Ditylenchus angustus*. (Photograph taken at inoculation stage. See also Fig. 5.7.)

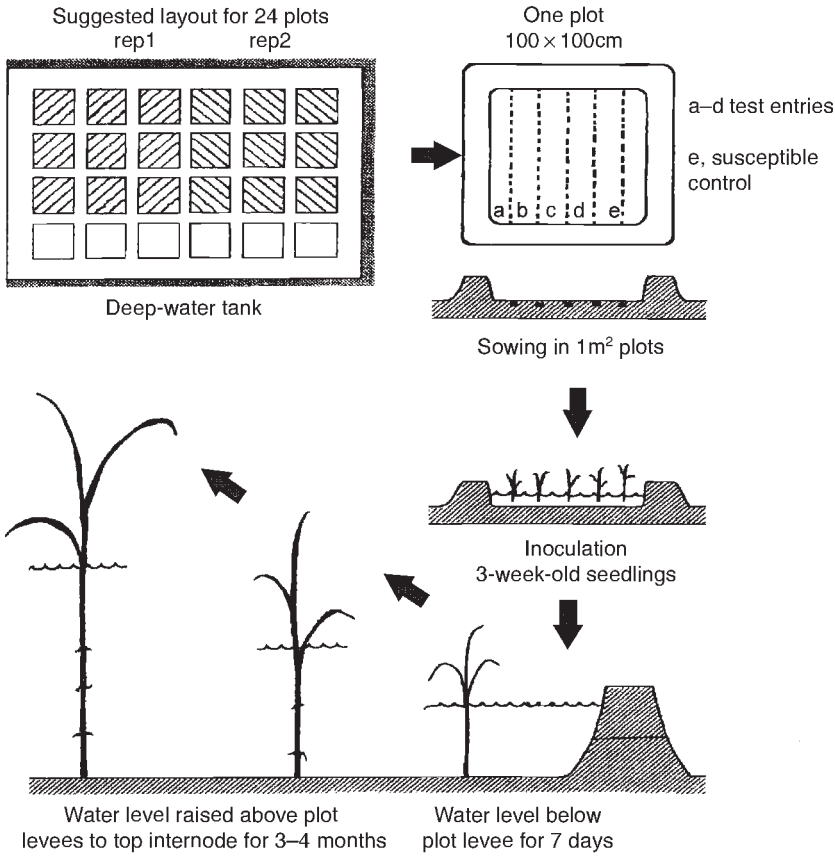

Fig. 5.7. Diagrammatic representation of field screening protocol for resistance to *Ditylenchus angustus* in deep-water rice (see also Fig. 5.6).

and hence the number of stem sections required to provide 100 nematodes per seedling (i.e. 10,000 nematodes per plot).

6. Cut the required number of stems into smaller pieces, split them longitudinally and float them on the water surface evenly across the plot.

7. After inoculation, maintain the water level in the plots 2 cm below the seedling collar for 7 days. If inoculation is delayed, such that plants begin to elongate, raising the meristematic node above the water level, they will *not* become infected.

8. After the 7-day invasion period, the water level must be raised as the plants elongate above the plot levee, so that the water is maintained in the same relative position, above the top meristematic node.

9. After 3–4 months, but before flowering, score symptoms on the most recent leaf of all tillers in each row (Fig. 5.4). Remove ten plants at random and cut back the main tiller to the innermost leaf and obtain a 10 cm length of tiller above the top node. Cut up this section, tease apart in water and leave to extract overnight, if necessary.
10. Determine the proportion of infected tillers per entry and the number of nematodes per tiller.

Glasshouse or screenhouse tests

This protocol is adapted from Plowright and Gill (1994).

1. Sow seed in small (100 cm^3) rectangular pots, in a deep tray, without drainage holes, to allow a water depth of at least 10 cm above the soil surface. Thin seedlings to one per pot after emergence. (In a tray containing 20 pots, include four resistant and four susceptible controls.)
2. Twelve days after sowing, or when seedling collar height is 10 cm, raise the water level to 8 cm. If cold water is used, allow 24 h for temperature equilibration before inoculation.
3. Extract nematodes from monoxenic cultures. *D. angustus* can be rinsed from the Petri dish lid of cultures or from the agar surface and combined with nematodes extracted into water, from rice tissue, on a modified Baermann funnel (Hooper, 1986). After extraction, dilute to achieve an inoculation volume of 1 ml. This suspension will require agitation to prevent the formation of clumps of nematodes.
4. Place a 1–3 cm diameter tube (e.g. plastic piping, cut plastic pipettes, straws, etc.) around each plant in a tray, support the tubes (e.g. with a removable grid of wire placed over the tray) and inoculate each seedling within the tube, with 300–500 J3 to adult stage nematodes (Fig. 5.3).
5. After 7 days, remove the tubes surrounding the inoculated plants.
6. Symptom type and severity (Fig. 5.4) on individual plants can be scored 7 days after inoculation and repeated, for confirmation, 14 days after inoculation.
7. If nematode counts are required, cut plants at soil level 28 days after inoculation, remove the leaves, label and store either by freezing or in formaldehyde (4% v/v). If formaldehyde is used, plants should be immersed in boiling water for 1 min prior to fixing.
8. To count nematodes, cold stain plants overnight in acid fuchsin (0.01% w/v) in a mixture of equal parts lactic acid, glycerol and distilled water. Nematodes can be released by teasing apart the leaf sheath or by blending in a blender. As *D. angustus* is ectoparasitic, it is usually better to use the teasing method as the resulting suspension of nematodes and eggs is cleaner and much easier to count.

Nematode Virulence

The existence of races of *D. dipsaci* with more or less, well defined host preferences has already been mentioned. Evidence for the existence of pathotype-like variation in *D. dipsaci* has emerged over the last 50 years, but is indisputable in only relatively few cases. Evidence for the existence of pathotypes has to be examined critically, for if these occur, screening needs to include a range of nematode populations.

Virulence has been reported in white clover races. The white clovers tested were sixth generation inbred, near isogenic lines, differing from each other only in their resistance or susceptibility, and phenotypically uniform resistant and susceptible F_1 populations bred from pairs of selected characterized parents. The resistance in both sets of clovers was effective against populations of the nematode from white clover in UK, France and New Zealand but was wholly ineffective against a population from Switzerland (K.A. Mizen, 1999, personal communication). The existence of a pathotype is also clear in a second case, where one field population of *D. dipsaci* multiplied and induced a compatible reaction in the highly resistant *V. faba* homozygous line, INRA 29H. This virulence was recorded at a high incidence in a single field population in Morocco, causing severe swelling symptoms and high multiplication.

Resistance breaking pathotypes of lucerne race *D. dipsaci* have been described (e.g. Smith, 1951; Grundbacher and Stanford, 1962; Whitehead, 1984) and of *D. dipsaci* giant race (Sturhan, 1965). Whitehead (1984, 1992) and Whitehead *et al*. (1987) found that supposedly resistant cultivars were susceptible to some European isolates, and concluded that there were pathotype-like variations within races of stem nematode. The problem with these conclusions is that they do not eliminate two important sources of variation which create doubt about the pathotypes. The first is that cultivars in the screen were hetereogeneous and the second the misclassifications brought about by 'escapes' due to failure of inoculation. A high proportion of 'escapes' in the initial characterization of resistant lines could lead to an apparent loss of resistance in subsequent tests with fewer escapes. Lucerne cultivars are heterogeneous and comprise both resistant and susceptible genotypes and hence the number of plants screened must be sufficient to reflect this variation. Furthermore, Whitehead *et al*. (1987) clearly found it very difficult to inoculate plants and the nematode multiplication on susceptible reference cultivars, where they were used, was rather variable. Among other populations, Whitehead (1992) presented evidence, from pot studies, of pathotype variation in *D. dipsaci* sampled from two different fields of the same farm in southern England. Although the management of these fields was not discussed, it seems improbable that they would have been different pathotypes.

Extensive intraspecific variation and genetic polymorphism is known in *D. dipsaci*. Barker and Sasser (1959) and Sturhan (1975) found nematode population × variety interactions in pea, field beans and broad beans, and the latter author described obvious differences in pathogenicity and virulence between nematode populations. North American populations of stem nematode showed some variation in pathogenicity on susceptible plants but did not overcome resistance in cultivars with the Lahontan-derived resistance (Elgin *et al*., 1977). There are different degrees of susceptibility to stem nematode in garlic (Shubina, 1987).

There is no evidence of resistance-breaking pathotypes in *D. angustus* (R.A. Plowright, 1999, personal communication). Differences in multiplication of nematode populations on susceptible rice cultivars can occur but are rarely consistent in repeated experiments. The source of such differences is more often due to practical differences related to the culture, preparation and delivery of nematode inoculum.

Genetics of Resistance and Germplasm

Some UK winter-sown oat cultivars derive their resistance from the land race, Grey Winter. In other oats, resistance may be derived from Uruguayan land races, but ultimately both sources may have originated from wild oat species (Griffiths *et al*., 1957; Goodey and Hooper, 1962). In Grey Winter-derived cultivars, resistance is inherited as a single dominant gene, and has been incorporated into many winter oat cultivars bred in IGER, Wales, UK. The wild oat, *Avena ludoviciana*, has more than one gene for resistance. A number of other cultivars have been reported to be resistant (Table 5.1; Whitehead, 1997) but many of these have only partial resistance or tolerance. Although these reactions may represent different genetic control, it is essential that the sources are retested before use in any new area.

In lucerne, resistance is relatively simply inherited and readily increased by selection within heterogeneous lucerne cultivars. It is easily transferred through backcrossing and by recurrent selection for resistant phenotypes. There appears to be a dominant gene inherited tetrasomically, that is, in a plant there may be one to four resistance alleles. Resistance in the extensive breeding programmes in the USA originates from a lucerne population from Turkestan, grown in Utah. From this a resistant reselection, Nemastan, was made and is the source of mother plants of the variety Lahontan with between 50 and 60% resistant plants. In Sweden, the variety Alfa II with 70% resistant plants originates from 500 resistant plants identified among 25,000 seedlings of the parent population. The resistance of both Lahontan and Vertus is effective against stem nematode populations throughout

Table 5.1. Crop cultivars and accessions resistant to *Ditylenchus dipsaci*.

Crop		Cultivar/accession	Country	Reference
Lucerne	*Medicago sativa*	Vertus	Sweden	Cook and Yeates (1993)
		Nova	Australia	
		Washoe Lahontan	USA	
		Resistador II		
White clover	*Trifolium repens*	Line G49	New Zealand	Mercer and Grant (1995)
	Tolerant	Sabeda	New Zealand	West and Steele (1986)
		Katrina		
		Alice	UK	Cook and Evans (1988)
		Donna		
		Aran		
		Pronitro		
Rye	*Secale cereale*	Ottersum (land race) Heertvelder	The Netherlands	Ritzema-bos (1922)
Vicia bean	*Vicia faba*	INRA 29H	France	Caubel and Le Guen (1983)
		Several		Gastel (1990)
				Hanounik *et al.* (1986)
		Souk el Arba	Morocco	Schreiber (1977)
		Rharb (land race)		
Red clover	*Trifolium pratense*	Sabtoron	UK	
		Norseman		
Oat	*Avena sativa*	Grey Winter		
		Peniarth		
		Anita	Belgium	Clamot (1985)
		Bettong	Australia	MacDaniel and Barr (1994)
	A. ludoviciana	Cc 4346	UK	Griffiths *et al.* (1957)

the world. Resistance in red clover is readily selected from existing and older commercial cultivars through recurrent phenotypic selection. Resistance seems to be effective against all red clover race populations, and although isolates with differing levels of multiplication may be found there is no sound evidence for interactions indicating virulence on resistant genotypes despite intensive selection pressure (Cook and Yeates, 1993). Resistance in some Swedish red clovers appears to be inherited as two dominant genes (Nordenskiold, 1971). A number of breeding programmes have exploited the lucerne and red clover sources to produce named cultivars (Table 5.1; Cook and Yeates, 1993; Whitehead, 1997).

White clover resistance to stem nematode seems to be under relatively simple genetic control as the proportion of resistant

plants can be increased by two generations of phenotypic selection. Intercrosses between resistant parent plants produces an F_1 generation in which most plants are resistant, indicating dominance. Many cultivars have a proportion of resistant plants although few qualify as truly resistant cultivars. Those with some degree of resistance are listed in Table 5.1, in Cook and Yeates (1993) and in Whitehead (1997). Such cultivars, like those of lucerne and red clover, are heterogeneous and require continued reselection during seed production cycles to maintain their stated level of resistance.

In *V. faba*, the high resistance of INRA 29H line (Rinal × Côtes-d'Or) seems to be polygenic and partially cytoplasmically transmitted. The resistant cultivars and accessions (Table 5.1) have been used in France to develop resistant cultivars for use in North Africa. Pea (*Pisum sativum*) cvs Alma and Glenroy are described as tolerant of *D. dipsaci* in Australia (Scurrah *et al.*, 1997). Pea cv. Wando was resistant to one North Carolina population of *D. dipsaci*, but susceptible to a second population (Barker and Sasser, 1959).

Studies on the genetics of resistance to *D. angustus* suggest that resistance is recessive and that two genes may be involved (Anon., 1996). At the International Rice Research Institute, The Philippines and the Bangladesh Rice Research Institute, Gazipur, Bangladesh, several breeding families have been identified to include resistance. These include lowland rice (IR63174) and several families of deep-water rice, as well as a number of other resistant accessions and cultivars (Rahman, 1994). Sweet potatoes with resistance to *D. destructor* have been recognized in China (Anon., 1992; Lin *et al.*, 1996; Whitehead, 1997).

Of the germplasm and cultivars listed above, in Table 5.1 and in reviews by Cook and Yeates (1993) and Whitehead (1997), most would need validating before use as controls, parents or cultivars. This is partly because of the extent of variation in plant nematode interactions, as described in this chapter. Variation, and hence variability in results, also arises through the existence of both different nematode host races and pathotypes. Moreover, many of the resistant crops are out-breeding cultivars which require regular reselection to maintain a high proportion of resistant plants during seed production and multiplication phases. Exceptions appear to be the widely used lucerne and red clover germplasm. But with these, and probably with resistance in other crops to *D. dipsaci* or to other *Ditylenchus* species, locally adapted cultivars may be the best source of resistance. The careful application of the protocols detailed in this chapter should allow researchers to identify individual plants or plant progenies with hereditable, highly effective resistance, in spite of these quantitative variations.

References

Abbad, F.A., Ammati, M. and Alami, R. (1990) Stem nematode in Morocco. Distribution and preliminary screening for resistance. *8th Congress Mediterranean Phytopathological Union*, Agadir, Morocco, pp. 347–349.

Alcaniz, E., Pinochet, J., Fernandez, C., Esmenjaud, D. and Felipe, A. (1996) Evaluation of *Prunus* rootstocks for root-lesion nematode resistance. *HortScience* 31, 1013–1016.

Ali, R. and Ishibashi, N. (1997) Growth and propagation of the rice stem nematode, *Ditylenchus angustus*, on rice seedlings and fungal mat of *Botrytis cinerea*. *Japanese Journal of Nematology* 26, 12–22.

Anon. (1985) The first international rice Ufra screening set (IRUSS). International Rice Research Institute, Philippines.

Anon. (1992) Insect and nematode management. *Annual Report of the International Potato Center*. International Potato Center, Lima, Peru, pp. 81–100.

Anon. (1996) Survey and monitoring of rice diseases. In: *Bangladesh Rice Research Institute, Annual Report for 1993*. Bangladesh Rice Research Institute, Bangladesh, pp. 108–111.

Barker, K.R. and Sasser, J.N. (1959) Biology and control of the stem nematode *Ditylenchus dipsaci*. *Phytopathology* 49, 664 – 670.

Bingefors, S. (1970) Resistance against stem nematodes, *Ditylenchus dipsaci* (Kuhn) Filipjev. *European and Mediterranean Plant Protection Organization Publication Series A*. No. 54, 63–75.

Byrd, D.W., Kirkpatrick, T. and Barker, K.R. (1983) An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* 15, 142–143.

Cameron, D. (1963) *Report of the Scottish Society for Research in Plant Breeding for 1962*. Pentlandfield, Edinburgh, UK.

Caubel, G. and Leclercq, D. (1989) Estimation de la resistance a la race geante de *Ditylenchus dipsaci* par les symptomes chez la feverole (*Vicia faba* L.). *Nematologica* 35, 216–224.

Caubel, G. and Le Guen, J. (1983) Variability of relationships between *Vicia* bean and stem nematode (*Ditylenchus dipsaci*) characterization of varietal resistance. *First European Conference on Grain Legumes*, Angers, France, pp. 337–338.

Caubel, G., Bossis, M., Genier, G. and Guy, P. (1977) Mise au point d'un test de selection de luzernes resistantes a *Ditylenchus dipsaci. Sciences Agronomiques Rennes,* 25–32.

Clamot, G. (1985) Breeding for resistance to the cereal cyst nematode (*Heterodera avenae* Woll.) and to stem nematode (*Ditylenchus dipsaci* (Kühn) Fil.) in Belgium. *Comptes Rendus des Séances de l'Académie d'Agriculture de France* 71, 751–760.

Cook, R. and Evans, D.R. (1988) Observations on resistance in white clover (*Trifolium repens* L.) to the stem nematode (*Ditylenchus dipsaci* (Kühn) Filipjev). *Journal of Agricultural Science* 110, 145–154.

Cook, R. and Yeates, G.W. (1993) Nematode pests of grassland and forage crops. In: Evans, K., Trudgill, D.L. and Webster, J.M. (eds) *Plant Parasitic Nematodes in Temperate Agriculture*. CAB International, Wallingford, UK, pp. 305–350.

De Waele, D., Wilken, R. and Lindeque, J.M. (1991) Response of potato cultivars to *Ditylenchus destructor* isolated from groundnut. *Revue de Nématologie* 14, 123–126.

Dijkstra, J. (1957) Symptoms of susceptibility and resistance in seedlings of red clover attacked by the stem eelworm *Ditylenchus dipsaci* (Kühn) Filipjev. *Nematologica* 2, 228–237.

Elgin, J.H., Jr (1984) Standard tests to characterise pest resistance in alfalfa cultivars. Agricultural Research Service, Beltsville, Maryland, 44 pp.

Elgin, J.H., Jr, Evans, D.W. and Faulkner, L.R. (1975) Swelling response of alfalfa seedlings to initial stem

nematode infection. *Crop Science* 15, 435–437.

Elgin, J.H., Jr, Evans, D.W. and Faulkner, L.R. (1977) Response of resistant and susceptible alfalfa cultivars to regional isolates of stem nematodes. *Crop Science* 17, 957–959.

Eriksson, K.B. (1972) Studies on *Ditylenchus dipsaci* (Kühn) with reference to plant resistance. Dr Agr. thesis, Agricultural College of Sweden, Uppsala, Sweden, 108 pp.

Esquibet, M., Bekal, S., Castagnone-Sereno, P., Gauthier, J.P., Rivoal, R. and Caubel, G. (1998) Differentiation of normal and giant *Vicia faba* populations of the stem nematode *Ditylenchus dipsaci*: agreement between RAPD and phenotypic characteristics. *Heredity* 81, 291–298.

Faulkner, L.R. and Darling, H.M. (1961) Pathological histology, hosts, and culture of the potato rot nematode. *Phytopathology* 51, 778–786.

Gastel, R. (1990) Resistenzprüfung von Ackerbohnene (*Vicia faba*) gegen das Stengelälchen (*Ditylenchus dipsaci*). Dissertation; Universität Hohenheim, Germany, 198 pp.

Goodey, J.B. and Hooper, D.J. (1962) Observations on the attack by *Ditylenchus dipsaci* on varieties of oats. *Nematologica* 8, 33–38.

Griffith, G.S., Cook, R. and Mizen, K.A. (1996) Effects of temperature on the white clover (*Trifolium repens*)/stem nematode (*Ditylenchus dipsaci*) host pest system. *Aspects of Applied Biology* 45, 239–246.

Griffith, G.S., Cook, R. and Mizen, K.A. (1997) *Ditylenchus dipsaci* infestation of *Trifolium repens*. II. Dynamics of infestation development. *Journal of Nematology* 29, 356–369.

Griffiths, D.J., Holden, J.H.W. and Jones, J.M. (1957) Investigations on resistance of oats to stem eelworm, *Ditylenchus dipsaci* (Kühn). *Annals of Applied Biology* 45, 709–720.

Grundbacher, F.J. and Stanford, E.H. (1962) Genetic factors conditioning resistance in alfalfa to the stem nematode. *Crop Science* 2, 211–217.

Hanounik, S.B., Halila, H. and Harrabi, M. (1986) Resistance in *Vicia faba* to stem nematodes (*Ditylenchus dipsaci*). *FABIS Newsletter* 16, 37–39.

Hooper, D.J. (1984) Observations on stem nematodes *Ditylenchus dipsaci* attacking field beans *Vicia faba*. *Rothamsted Experimental Station Report for 1983*, pp. 239–260.

Hooper, D.J. (1986) Culturing nematodes and related experimental techniques. In: Southey, J.F. (ed.) *Laboratory Methods for Work with Plant and Soil Nematodes*. Her Majesty's Stationery Office, London, pp. 133–157.

Hooper, D.J and Cowland, J.A (1988) Courgette marrows for the mass culture of some nematodes. *Nematologica* 33, 488–490.

Hussey, R.S. and Krusberg, L.R. (1968) Histopathology of resistant reactions in Alaska pea seedlings to two populations of *Ditylenchus dipsaci*. *Phytopathology* 58, 1305–1310.

Ibrahim, S.K. and Perry, R.N. (1993) Desiccation survival of the rice stem nematode *Ditylenchus angustus*. *Fundamental and Applied Nematology* 16, 31–38.

Janssen, G.J.W. (1994) The relevance of races in *Ditylenchus dipsaci* (Kühn) Filipjev, the stem nematode. *Fundamental and Applied Nematology* 17, 469–473.

Jones, B.L. and De Waele, D. (1988) First report of *Ditylenchus destructor* in pods and seeds of peanut. *Plant Disease* 72, 453.

Kinh, Dang-ngoc and Nghiem, Nguyen-thi (1982) Reaction of rice varieties to stem nematodes in Vietnam. *International Rice Research Newsletter* 7, 6–7.

Latif, M.A. and Mian, I.H. (1995) Fungal hosts and gnotobiotic culture of *Ditylenchus angustus*. *Japanese Journal of Nematology* 25, 11–15.

Lin, M.S., Fang, Z.D. and Xie, Y.P. (1993) Responses of sweet potato to exudate of potato rot nematodes (*Ditylenchus destructor*). *Acta Phytopathologica Sinica* 23, 157–162.

Lin, M.S., He, L.M., Wen, L., Fang, Z.D. and Song, B. (1996) Mechanism of

morphological structure of sweet potato resistance to potato rot nematode (*Ditylenchus destructor*). *Scientia Agricultura Sinica* 29, 8–12.

Lundin, P. and Jonsson, H.A. (1975) Weibull's Vertus, a lucerne variety with high resistance to stem nematodes and *Verticillium* wilt. *Agri Hortique Genetica* 33, 17–32.

MacDaniel, M.E. and Barr, A.R. (1994) Registration of Australian winter cereal cultivars. *Avena sativa* (oats) cv. Bettong. *Australian Journal of Experimental Agriculture* 34, 701.

MacGuidwin, A.E. and Slack, S.A. (1991) Suitability of alfalfa, corn, oat, red clover, and snapbean as hosts for the potato rot nematode, *Ditylenchus destructor*. *Plant Disease* 75, 37–39.

Mercer, C.F. and Grant, J.L. (1995) Resistance of the white clover variety G49 and its parent lines to stem nematode (*Ditylenchus dipsaci*). *New Zealand Journal of Agricultural Research* 38, 495–499.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15, 473–497.

Nordenskiold, H. (1971) The genetic background of the resistance to nematodes (*Ditylenchus dipsaci*) in red clover (*Trifolium pratense*). *Hereditas* 69, 301–302.

Palmer, H.M., Atkinson, H.J. and Perry, R.N. (1992) Monoclonal antibodies (MAbs) specific to surface expressed antigens of *Ditylenchus dipsaci*. *Fundamental and Applied Nematology* 15, 511–515.

Plowright, R.A. and Akehurst, T.E. (1992) Monoxenic culture of the Ufra nematode *Ditylenchus angustus*. *Fundamental and Applied Nematology* 15, 327–330.

Plowright, R.A. and Gill, J.R. (1994) Aspects of resistance in deepwater rice to the stem nematode *Ditylenchus angustus*. *Fundamental and Applied Nematology* 17, 357–367.

Rahman, M.L. (1982) Screening for ufra resistance in deepwater rice. *International Rice Research Newsletter* 7, 12–13.

Rahman, M.L. (1987) Source of ufra resistant deep water rice. *International Rice Research Newsletter* 12, 8.

Rahman, M.L. (1994) New ufra resistant rice lines. *International Rice Research Notes* 19, 16.

Rahman, M.L. and Evans, A.A.F. (1987) Studies on host–parasite relationships of rice stem nematode *Ditylenchus angustus* (Nematoda: Tylenchida) on rice (*Oryza sativa*, L.). *Nematologica* 33, 451–459.

Ritzema-bos, J. (1922) Het stengelaaltje. *Tijdschrifft Plantenziehten* 28, 159–180.

Rivoal, R., Person, F., Caubel, G. and Scotto la Massese, C. (1978) Methodes d'evaluation de la resistance des cereales au development des nematodes: *Ditylenchus dipsaci*, *Heterodera avenae* et *Pratylenchus* spp. *Annales del l'Amelioration des Plantes* 28, 371–394.

Schreiber, M.L. (1977) Lebenweise, bedeutung und Bekämpfungsmöglischkeiten von *Ditylenchus dipsaci* (Kühn) Filipjev an ackerbohnen *Vicia faba* L. in Morokko. Dissertation; Technical University, Berlin, Germany.

Scurrah, M., Szot, D. and Ali, M. (1997) Selection for tolerant pea lines to stem nematode. *Third International Food Research Conference*, Adelaide, Australia, p. 173.

Seinhorst, J.W. (1952) Eeen nieuwe methode voor de bepaling van vatbaarheid van roggeplanten voor aantasting door stengelaaltjes (*Ditylenchus dipsaci* (Kuhn) Filipjev). *Netherlands Journal of Plant Pathology* 58, 103–108.

Shubina, L.V. (1987) [Susceptibility of various garlic forms to the stem nematode *D. dipsaci*.] *Taksonomiya i biol. fitogel'minotiv* (1984), 152–156. [in Russian]. *From Referativnyi Zhurnal* (1985) 2.79.353. In: *Helminthological Abstracts* Series B (1987) 56, 877.

Smith, O.F. (1951) Biologic races of *Ditylenchus dipsaci* on alfalfa. *Phytopathology* 41, 189–190.

Stanton, J.M., Fisher, J.M. and Britton, R. (1984) Resistance of cultivars of *Avena sativa* to, and host range of, an oat-attacking race of *Ditylenchus dipsaci* in South Australia. *Australian Journal of Experimental Agriculture and Animal Husbandry* 24, 267–271.

Sturhan, D. (1965) Verglichende Wirtspflanzen-untersuchungen and Stengelalchen (*Ditylenchus dipsaci*) aus Ruben verschiedener herkunft. *Mededelingen van de Landbouwhogeschool en de Opzoekinggstations van de Staat te Gent* 30, 1468–1474.

Sturhan, D. (1971) Biological races. In: Zuckerman, B.M., Mai, W.F. and Rohde, R.A. (eds) *Plant Parasitic Nematodes*. Academic Press, New York, pp. 51–71.

Sturhan, D. (1975) Untersuchung von Vicia faba-sorten auf Resistenz gegenuber Stengelalchen (*Ditylenchus dipsaci*). *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* 40, 443–450.

Sturhan, D. and Brzeski, M. (1991) Stem and bulb nematodes, *Ditylenchus* spp. In: Nickle, W.R. (ed.) *Manual of Agricultural Nematology*. Marcel Dekker, New York, pp. 423–464.

Tenente, R.C.V. and Evans, A.A.F. (1992) Reproducao de *Ditylenchus dipsaci* raca 'teasel' sobre calo de alfafa (*Medicago sativa*). *Nematologia Brasileira* 16, 19–26.

Van der Walt, P.C.W. and De Waele, D. (1989) Mass culture of the potato rot nematode *Ditylenchus destructor* on groundnut callus tissue. *Phytophylactica* 21, 79–80.

Venter, C., De Waele, D. and Meyer, A.J. (1991) Reproductive and damage potential of *Ditylenchus destructor* of peanut. *Journal of Nematology* 23, 12–19.

Venter, C., De Waele, D. and Meyer, A.J. (1993) Reproductive and damage potential of *Ditylenchus destructor* on six peanut cultivars. *Journal of Nematology* 25, 59–62.

Venter, C., Van Aswegen, G., Meyer, A.J. and De Waele, D. (1995) Histological studies of *Ditylenchus africanus* within peanut pods. *Journal of Nematology* 27, 284–291.

Wendt, K.R., Vrain, T.C. and Webster, J.M. (1994) Separation of three species of *Ditylenchus* and some host races of *D. dipsaci* by restriction fragment length polymorphism. *Journal of Nematology* 25, 555–563.

Wendt, K.R., Swart, A., Vrain, T.C. and Webster, J.M. (1995) *Ditylenchus africanus* sp. n. from South Africa: a morphological and molecular characterization. *Fundamental and Applied Nematology* 18, 241–250.

West, C.P. and Steele, K.W. (1986) Tolerance of clover cultivars to stem nematode (*Ditylenchus dipsaci*). *New Zealand Journal of Experimental Agriculture* 14, 227–229.

Whitehead, A.G. (1984) Interaction of three lucerne cultivars and eleven English isolates of stem nematode (*Ditylenchus dipsaci*), 'lucerne race'. *Plant Pathology* 33, 33–37.

Whitehead, A.G. (1992) Sources of resistance to stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, in species of *Medicago* and *Trifolium*. *Annals of Applied Biology* 120, 73–81.

Whitehead, A.G. (1997) *Plant Nematode Control*. CAB International, Wallingford, UK, 384 pp.

Whitehead, A.G. and Hemming, J.R. (1965) A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55, 25–38.

Whitehead, A.G., Fraser, J.E. and Nichols, A.J.F. (1987) Variation in the development of stem nematodes, *Ditylenchus dipsaci*, in susceptible and resistant crop plants. *Annals of Applied Biology* 111, 373–383.

6

Foliar Nematodes: *Aphelenchoides* Species

D. De Waele

Laboratory of Tropical Crop Improvement, Catholic University Leuven (K.U.Leuven), Kasteelpark Arenberg 13, 3001 Leuven, Belgium

The nematode genus *Aphelenchoides* includes species parasitic on higher plants, species associated with insects and mycophagous species. The most important plant-parasitic *Aphelenchoides* species are *A. besseyi* Christie, *A. ritzemabosi* (Schwartz) Steiner and Buhrer, *A. fragariae* (Ritzema Bos) Christie and *A. arachidis* Bos.

A. besseyi is mainly known as the cause of white tip of rice, found worldwide in most rice-growing regions (Franklin and Siddiqi, 1972; Fortuner and Orton Williams, 1975). It feeds ectoparasitically on the meristems of stems, leaves, and buds of susceptible plants. On the tillers of affected rice plants, the tips of the leaves whiten for a distance of 3–5 cm, senesce and shred. The upper leaves and the panicle leaf are the most affected; the latter often become twisted and curled, hindering the emergence of the panicle. Infected panicles are shorter, often atrophied at the tips and bear fewer grains. Flowers may be sterile and produce empty grains with white, twisted husks or misshapen grains with a low germination potential. If infection takes place shortly after germination, the height of the plant can be reduced by half. *A. besseyi* is seed borne. The juveniles survive in an anhydrobiotic stage in infected mature seeds. After hydration, they become active, leave the seeds and move to the growing points of stems and leaves of seedlings. Another important host of *A. besseyi* is strawberry on which it causes distortion of the leaves, dwarfing of the whole plant and reduction in flowering and consequently in yield of fruit (Franklin and Siddiqi, 1972).

A. ritzemabosi, the chrysanthemum foliar nematode, is ecto- or endoparasitic in many ornamental plants, but especially

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chrysanthemum on which it has been found in Europe, the former Soviet Union, Latin America (Mexico, Brazil) and Asia (India, China, Japan) (Siddiqi, 1974). Another important host is strawberry on which it usually occurs together with *A. fragariae*. Attacks by *A. ritzemabosi* on several non-ornamental plants such as tobacco (Shepherd and Barker, 1990), lucerne (Gray *et al.*, 1994) and dry bean (Franc *et al.*, 1996) have also been reported. In chrysanthemum, *A. ritzemabosi* is usually found in the leaves but may also be found in axils and innermost parts of the buds. It migrates up the stems and enters the leaves through the stomata. Zones of discoloration, delineated by the veins, appear on the leaves as a result of parasitism by the nematode. Leaves are also crinkled and deformed. Discoloration progresses until necrosis and death of the leaf occurs. Nematodes may become concentrated in the crown of the plant and invade new shoots as they are produced. The nematodes are extremely mobile and can move from plant to plant via splashing water.

A. fragariae is also an ecto- or endoparasite of many plants, but especially of strawberry on which it has been found in Europe, the former Soviet Union, the USA, Latin America (Mexico) and Asia (Japan) (Siddiqi, 1975). On strawberry, it is ectoparasitic, living within the folded crown and runner buds. Only occasionally is the nematode found within the leaf tissue. It causes malformations, such as twisting and puckering of leaves, discoloured areas with a hard and rough surface, undersized leaves with crinkled edges, reddening petioles, short internodes of runners, reduced flower trusses with only one or two flowers and death of the crown bud. Ferns are also important hosts, on which the nematodes enter leaves through the stomata when the surface is covered with a thin film of water. Endoparasitism in leaves results in typical leaf-blotch symptoms. Other ornamental hosts include begonia, lilies, primroses and azaleas (Siddiqi, 1975).

A. arachidis Bos, the groundnut testa nematode, is an endoparasite which invades developing groundnut pods from the soil (Bos, 1977; Bridge and Hunt, 1985). It feeds on internal tissues of the pod shells and parenchymatous tissues of the testa, roots and hypocotyl (Bridge *et al.*, 1977). Significant levels of infestation of *A. arachidis* were originally only found in a limited area around the type locality (Bos, 1977) but a survey of 47 groundnut-growing localities in four ecological zones of Nigeria (Khan and Misari, 1992) revealed this species to be widely distributed throughout the groundnut-growing areas of Nigeria. *A. arachidis* has not been shown to decrease yields but it devalues the confectionary market value of the seeds because infected dried seeds are shrivelled with wrinkled, dark brown testas. Also, infection with this species predisposes seeds to invasion by pathogenic fungi, such as *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseoli* and *Fusarium* spp., which may lead to reduced seed emergence (McDonald

et al., 1979). Large numbers of *A. arachidis* can also occur in roots of maize, sorghum, millet, sugarcane and rice and could be causing economic losses to these crops (Bos, 1977).

Sources of Resistance

Differences in susceptibility of rice to *A. besseyi* had already been reported in 1949 and appear to be widespread because since then resistance (De Oliveira, 1989; Bridge *et al.*, 1990; Da Silveira *et al.*, 1990) or moderate resistance (Sivakumar, 1988) has been found in most rice-growing regions. In Russia, an assessment of the resistance to *A. besseyi* of 1003 rice cultivars from different agro-ecological regions was made in the glasshouse. Three cultivars were immune (Bluebonnet, Bluebonnet 50 and Starbonnet), ten were highly resistant, 164 were moderately resistant and 826 were susceptible or highly susceptible to *A. besseyi* (Popova *et al.*, 1994). Interestingly, several cultivars with multiple resistance to *A. besseyi* and other important pathogens of rice were found. Pecos rice is not only resistant to *A. besseyi* but also to the viral disease hoja blanca and to the fungal diseases rice blast (caused by *Magnaporthe grisea* (*Pyricularia oryzae*)) and rice brown spot (caused by *Cochliobolus miyabeanus*) (Bollich *et al.*, 1985). Namyeongbyeo, bred from crosses involving Milyang 40, Milyang 43, IR10157 and IR5533, is not only moderately resistant to *A. besseyi* but also to several virus diseases, the bacterial disease rice leaf blight (caused by *Xanthomonas campestris* pv. *oryzae*), rice blast and several rice leaf- and planthoppers (Homoptera: Cicadellidae and Delphacidae) (Sohn *et al.*, 1987). Resistance to *A. besseyi* is said to be genetically controlled and carried by the Japanese cultivar Asa-Hi (Nishizawa, 1953). In the USA, the progenitors of almost all the resistant varieties bred in the last 40 years are Fortuna, Nira, Bluebonnet and, in particular, Rexoro. Notable among the offspring of these progenitors is Bonnet 73 with multiple resistance to *A. besseyi* and various other rice pathogens (Zelenskii and Popova, 1991). In addition to the numerous sources of resistance to *A. besseyi* found in rice, resistance to this nematode was also found in 22 of 1919 foxtail millet (*Setaria italica*) accessions screened in China (Cui *et al.*, 1989).

Several authors have listed cultivars of chrysanthemum and strawberry differing in their susceptibility to *A. ritzemabosi* (Siddiqi, 1974, 1975; Szczygiel and Danek, 1975; Nakagome and Kato, 1977; see also the review of the research on *A. fragariae* and *A. ritzemabosi* on strawberry conducted since 1950 in the former Soviet Union by Szczygiel, 1977). Resistance to *A. ritzemabosi* was also found in African violets (Strider, 1979) and in lucerne (Gray *et al.*, 1994).

Wallace (1961) stated that a hypersensitive reaction is the cause of the resistance to *A. ritzemabosi* observed in chrysanthemum.

Seed transmission of *A. arachidis* on groundnut can be prevented by either thorough drying or hot water treatment (Bridge *et al*., 1977). As a consequence, no searches for sources of resistance to this nematode were conducted.

General considerations for screening for *Aphelenchoides* resistance

Identification

The close morphological similarities among species of *Aphelenchoides* makes taxonomic study based on light microscopic examination of morphological features difficult. Moreover, Cayrol and Dalmasso (1975) studied the interspecific relationships among *A. besseyi*, *A. ritzemabosi* and *A. fragariae* and reported that mixtures of one juvenile of one of the species and ten males of one of the other two species gave positive results for five of the six possibilities of crossing. Individuals with intermediate characters were observed. The morphological and morphometrical characters of *A. besseyi*, *A. ritzemabosi*, *A. fragariae* and *A. arachidis* can be found in Franklin and Siddiqi (1972), Siddiqi (1974, 1975) and Bridge and Hunt (1985) and are summarized in Table 6.1.

A study by Ibrahim *et al*. (1994) indicated that gel electrophoresis is useful in establishing a biochemical basis for the separation of *Aphelenchoides* species. They compared non-specific esterase isozymes and protein patterns of three populations of *A. besseyi* (from rice from Sierra Leone, India and the Philippines), *A. bicaudatus* (from *Setaria palmaefolia*, originally from Papua New Guinea), *A. arachidis* (from groundnut from Nigeria), *A. fragariae* (from fern from California), *A. hamatus* (from strawberries from England), *A. nechaleos* (from rice from Vietnam) and *A. paranechaleos* (from rice from Sierra Leone) by native polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE, respectively. The esterase and protein banding patterns of all species and populations examined were highly reproducible and characteristic for each species; distinct banding differences between species were found. The esterase banding patterns appeared to be more useful for separation of species than protein profiles, where there were too many differences in banding for diagnostic purposes. There were no detectable differences in the esterase and protein banding patterns from nematodes cultured on the fungi *Botrytis cinerea* or *Rhizoctonia cerealis*.

Table 6.1. Morphometrical and morphological characters for differentiation of *Aphelenchoides arachidis*, *A. besseyi*, *A. fragariae* and *A. ritzemabosi*.

	A. arachidis	*A. besseyi*	*A. fragariae*	*A. ritzemabosi*
Females				
L[a] (mm)	0.51–1.00	0.57–0.88	0.45–0.80	0.77–1.20
a[b]	39–50	32–58	45–60	40–45
c[c]	25–42	14–21	12–20	18–24
Lateral field: no. of incisures	2	4	2	4
Position of excretory pore	One body width posterior to nerve ring	Level with nerve ring	Level with or close behind nerve ring	0.5–2 body widths posterior to nerve ring
Length of postvulval sac	Extending for about half the vulva–anus distance	Extending for less than one-third vulva–anus distance	Extending for more than half the vulva–anus distance	Extending for more than half the vulva–anus distance
Rows of oocytes on ovary	1	2–4	1	Multiple
Shape of tail	Subcylindroid; with a bluntly rounded tip and a terminal mucron	Conoid, terminus bearing a mucro of diverse shape with 3–4 pointed processes	Conoid, ending in a simple but blunt spike devoid of any processes	Elongate conoid, bearing a terminal peg with 2–4 minute processes
Males				
L (mm)	0.56–1.04	0.44–0.72	0.48–0.86	0.70–0.93
a	37–60	36–47	40–63	31–50
Shape of spicules	Dorsal limb 15–25 μm	Without a dorsal process at proximal end	Dorsal limb 14–17 μm; with moderately developed dorsal and ventral process at proximal end	Dorsal limb 20–22 μm; without a dorsal or ventral process at proximal end

[a]L, total body length.
[b]a, ratio of total body length to maximum body width.
[c]c, ratio of total body length to tail length.

Races, biotypes, pathotypes

Although it has been observed that strawberry plants are not infected by *A. besseyi* populations isolated from chrysanthemum (Noegel and Perry, 1963), there is no proof of the existence of distinct races within *A. besseyi*. In fact, in the reports dealing with resistance of rice cultivars to *A. besseyi*, the existence of races of this nematode species is not mentioned as a problem. Also for *A. ritzemabosi*, *A. fragariae* and *A. arachidis* no races have been reported. In view of their wide host range and variations in their habits on different plants, Burckhardt (1973) examined homogenous (progeny of a single gravid female) populations of *A. ritzemabosi* and *A. fragariae* from different host plants and widely differing geographical origin but observed no differences in their behaviour towards different test plants, dimensions or sex ratios that would indicate the existence of biological races. In a general survey, many *A. arachidis* were found in the roots of maize, sorghum, millet, sugarcane, rice and some wild grasses but only in two samples of groundnut (Bos, 1977). The two infected samples of groundnut were from areas growing seed originating from the type locality of *A. arachidis*. Groundnuts were not infected when intercropped with maize and sorghum heavily infected with *A. arachidis*. Based on these observations, Bos (1977) suggested the existence of two biotypes of *A. arachidis*: one occurring on both groundnut and cereals, the other only on cereals. However, no reports are available to confirm this suggestion.

Inoculum

Culturing

Several methods may be employed to culture and maintain *Aphelenchoides* species. *A. ritzemabosi* can be obtained in gram quantities with relative ease using monoxenic cultures on callus tissue. Lucerne (alfalfa) callus grown on a nutrient agar medium containing 2,4-D, inoculation with 50 nematodes yielded 77,000 nematodes after 2 months (Krusberg, 1961). Lucerne and clover callus are also suitable for culture of *A. ritzemabosi* (Bossis and Caubel, 1982).

Most *Aphelenchoides* species can be readily cultured on various fungi. Feeding and reproduction of *A. besseyi* on *Fusarium solani* (Huang *et al.*, 1972), *Aureobasidium pullulans* (Huang *et al.*, 1979), *Alternaria tenuis* (Todd and Atkins, 1958) and *Alternaria alternata* (Rajan *et al.*, 1989), of *A. ritzemabosi* on *Botrytis cinerea* (Hooper and Cowland, 1986) and of *A. arachidis* on *Macrophomina phaseoli* and *Botrytis cinerea* (Bridge *et al.*, 1977) in agar plates have been reported.

Aphelenchoides-infected plants can be maintained in a glasshouse as sources of inoculum. Hooper and Cowland (1987) describe the mass culturing of *A. ritzemabosi* on courgette marrows. Inoculation with about 1000 individuals in a 0.5 ml suspension yielded 18,000–25,000 nematodes g^{-1} of infected tissue, 6–10 weeks after incubation at 16–18°C. *A. fragariae* also reproduced in courgettes but not as well as *A. ritzemabosi*.

The life cycle of *Aphelenchoides* species is short so that many nematodes can be obtained in a short period. The life cycle of *A. besseyi* takes 10 days at 21°C and only 8 days at 23°C (Franklin and Siddiqi, 1972). At 18°C, the life cycles of *A. ritzemabosi* and *A. fragariae* take 10–15 and 10–11 days, respectively (Siddiqi, 1974, 1975).

Storage

In some studies, juvenile and adult stages of *A. besseyi* in an anhydrobiotic stage are used as the source of inoculum. Dehydration studies showed that moderately humid conditions (allowing slow rate of dehydration) enable all developmental stages of *A. besseyi* to undergo anhydrobiosis (Rajan *et al.*, 1989). *A. ritzemabosi* and *A. fragariae* can also be stored at low temperatures before use as the source of inoculum. Both nematodes can survive in infected plant (strawberry) tissue stored at −1 to −2°C for several months (Hirling, 1972; Tacconi, 1973).

Inoculation and extraction

Inoculum of *Aphelenchoides* species can be most easily obtained by macerating infected plant tissues and extracting the nematodes with a Baermann funnel. Rice plants should be inoculated with *A. besseyi* in a stage conducive to invasion of primordial tissue. According to Qiu *et al.* (1991), *A. besseyi* invades rice plants mainly between sowing and the three-leaf stage. In soil in pots, rice seedlings are usually inoculated with 500–1000 *A. besseyi* each. Popova *et al.* (1994) infected 100 rice plant shoots sown in plastic boxes ($55 \times 25 \times 30$ cm) with *A. besseyi* using either a sprinkle method (500,000 nematodes m^{-2}) or plastic tubes (1.5–2 cm long × 2 mm diameter) attached to the second or third leaf of the rice shoot to which two drops of water suspension containing nematodes were added (500 nematodes per plant). Since *A. ritzemabosi* is extremely mobile and moves in a film of water, nematode inoculum suspended in water can also be sprayed on to test plants with an atomizer or a fine mist sprayer. Although invasion can occur within 15–30 min, plants should be maintained in a high humidity environment (> 95%) for approximately 24 h.

Reproduction of *A. besseyi* is determined by carefully macerating plant tissue in a blender or by teasing plant tissue apart in a dish of water followed by placement on a Baermann funnel. Seeds are first manually hulled. A simple method to detect *A. besseyi* in rice germplasm under exchange was described by Mathur and Lal (1989) who soaked rice seeds in water in Petri dishes for about 6 h, after which the seed coat was removed. The nematodes in the seeds immediately started to float out having recovered from their state of anhydrobiosis. More *A. ritzemabosi* and *A. fragariae* infecting chrysanthemum or strawberry were recovered by funnel extraction using diluted H_2O_2 instead of water (Hirling, 1971a). Increasing the extraction time to 4 weeks increased the yield of nematodes 1.3–7.9 times. Bohmer and Weil (1978) compared four extraction techniques (Baermann funnel with H_2O_2, Seinhorst's spray method, the aeration method of Wyss and a double funnel spray method) for their efficiency in extracting *A. ritzemabosi* and *A. fragariae* from strawberry crowns. Most nematodes were recovered using the double funnel spray method with an extraction time of 96 h.

Assessment of resistance and tolerance

The numbers of *A. besseyi*, *A. ritzemabosi* and *A. fragariae* recovered from infected tissues may vary considerably. For instance, in assessing strawberry hearts (buds, folded leaves and young flower parts) for infection by *A. fragariae* and *A. ritzemabosi*, the number of whole hearts required to make up 20 g varied according to season, weather conditions and degree of nematode infection. The number of nematodes per heart was considered a better standard to evaluate nematode reproduction (Hirling, 1971b). Reproduction is important in determining resistance but it is not the major criterion to use in determining nematode pathogenicity. Plant response in terms of symptom development and yield should also be evaluated. Rate of development of white tip symptoms (four- to five-leaf stage) may be used as an indication of plant response to *A. besseyi*. However, particularly in the field, rice symptom expression can be highly variable due to the strong influence of the environment on nematode development and damage (Bridge *et al*., 1990). Plant susceptibility as determined by nematode reproduction, can be determined with the same extraction methods used to obtain inoculum.

Lee and Evans (1973) studied the attractiveness of seedling extracts of 15 rice varieties to *A. besseyi* and found a correlation between attractiveness and the susceptibility of 8-week-old seedlings of these rice varieties growing in inoculated soil in pots. However, when using attractiveness as a measure of susceptibility one should take into

account the observation by Gokte and Mathur (1988) that the attractiveness of rice seedlings to *A. besseyi* is influenced by the age of the seedlings, the developmental stage of the nematode and temperature.

References

Bohmer, B. and Weil, B. (1978) Methodenvergleich zur Extraktion von Blatt- und Knospennematoden (*Aphelenchoides* spp.) aus Erdbeer Pflanzensprossen. *Nachrichtenblatt des Deutschen Pflanzenschutdienstes* 30, 85–88.

Bollich, C.N., Webb, B.D., Marchetti, M.A. and Scott, J.E. (1985) Registration of Pecos rice. *Crop Science* 25, 885–886.

Bos, W.S. (1977) *Aphelenchoides arachidis* n. sp. (Nematoda: Aphelenchoidea), an endoparasite of the testa of groundnuts in Nigeria. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschütz* 84, 95–99.

Bossis, M. and Caubel, G. (1982) Elevage monoxenique de nématodes phytoparasites sur cals de tissus. *Sciences Agronomiques, Rennes* 2, 115–125.

Bridge, J. and Hunt, D.J. (1985) *Aphelenchoides arachidis*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 8, No. 116.

Bridge, J., Bos, W.S., Page, L.J. and McDonald, D. (1977) The biology and possible importance of *Aphelenchoides arachidis*, a seed-borne endoparasitic nematode of groundnuts from northern Nigeria. *Nematologica* 23, 253–259.

Bridge, J., Luc, M. and Plowright, R.A. (1990) Nematode parasites of rice. In: Luc, M., Sikora, R.A. and Bridge, J. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, UK, pp. 69–108.

Burckhardt, F. (1973) Biologische Rassen bei *Aphelenchoides fragariae* und *Aphelenchoides ritzemabosi*? *Mitteilungen aus der Biologische Bundesanstalt für Land- und Forstwirtschaft* 151, 298.

Cayrol, J.-C. and Dalmasso, A. (1975) Affinités interspécifiques entre trois nématodes de feuilles (*A. fragariae*, *A. ritzemabosi* et *A. besseyi*). *Cahiers O.R.S.T.O.M., série Biologie* 10, 215–225.

Cui, G.X., Zheng, G.C. and Dong, Z.P. (1989) [Screening of foxtail millet materials resistant to *Aphelenchoides besseyi*.] *Acta Agriculturae Boreali-Sinica* Supplement, 145–152.

Da Silveira, S.G.P., Curi, S.M. and Tisseli, O. (1990) [Reaction of rice breeding lines to seed nematode *Aphelenchoides besseyi* Christie, 1942.] *Nematologia Brasileira* 14, 54–60.

De Oliveira, J.V. (1989) [Evaluation of the resistance of four irrigated rice genotypes to the nematode *Aphelenchoides besseyi* Christie, 1942]. *Agronomia Sulriograndense* 25, 11–18.

Fortuner, R. and Orton Williams, K.J. (1975) Review of the literature on *Aphelenchoides besseyi* Christie, 1942, the nematode causing 'white tip' disease in rice. *Helminthological Abstracts Series B, Plant Nematology* 44, 1–40.

Franc, G.D., Beaupré, C.M.-S., Gray, F.A. and Hall, R.D. (1996) Nematode angular leaf spot of dry bean in Wyoming. *Plant Disease* 80, 476–477.

Franklin, M.T. and Siddiqi, M.R. (1972) *Aphelenchoides besseyi*. *C.I.H. Descriptions of Plant-parasitic Nematodes*. Set 1, No. 4.

Gokte, N. and Mathur, V.K. (1988) On the attractiveness of paddy seedlings to *Aphelenchoides besseyi*. *Indian Journal of Nematology* 18, 239–243.

Gray, F.A., Williams, J.L., Griffin, G.D. and Wilson, T.E. (1994) Distribution in the Western United States of alfalfa and cultivar reaction to mixed populations of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi*. *Supplement to Journal of Nematology* 26, 705–719.

Hirling, W. (1971a) Zur Technik der Untersuchung von Erdbeerpflanzen und Chrysanthemumblattern auf Blattalchen (*Aphelenchoides fragariae* und *A. ritzemabosi*). Teil I. *Anzeiger für Schadlingskunde und Pflanzenschütz* 44, 171–174.

Hirling, W. (1971b) Zur Technik der Untersuchung von Erdbeerpflanzen und Chrysanthemumblattern auf Blattalchen (*Aphelenchoides fragariae* und *A. ritzemabosi*). Teil II. *Anzeiger für Schadlingskunde und Pflanzenschütz* 44, 182–185.

Hirling, W. (1972) Zur Technik der Untersuchung von Erdbeerpflanzen und Chrysanthemumblattern auf Blattalchen (*Aphelenchoides fragariae* und *A. ritzemabosi*). Teil III. *Anzeiger für Schadlingskunde und Pflanzenschütz* 45, 6–10.

Hooper, D.J. and Cowland, J.A. (1986) Fungal hosts for the chrysanthemum nematode, *Aphelenchoides ritzemabosi*. *Plant Pathology* 35, 128–129.

Hooper, D.J. and Cowland, J.A. (1987) Courgette marrows for the mass culture of some nematodes. *Nematologica* 33, 488–490.

Huang, C.S., Huang, S.P. and Lin, L.H. (1972) The effect of temperature on development and generation periods of *Aphelenchoides besseyi*. *Nematologica* 18, 432–438.

Huang, C.S., Huang, S.P. and Chiang, Y.C. (1979) Mode of reproduction and sex ratio of rice white-tip nematode, *Aphelenchoides besseyi*. *Nematologica* 25, 255–260.

Ibrahim, S.K., Perry, R.N. and Hooper, D.J. (1994) Use of esterase and protein patterns to differentiate two new species of *Aphelenchoides* on rice from other species of *Aphelenchoides* and from *Ditylenchus angustus* and *D. myceliophagus*. *Nematologica* 40, 267–275.

Khan, F.A. and Misari, S.M. (1992) Plant-parasitic nematodes associated with groundnut crop in four ecological zones of Nigeria. *Journal of African Zoology* 106, 263–272.

Krusberg, L.R. (1961) Studies on the culturing and parasitism of plant-parasitic nematodes, in particular *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* on alfalfa tissues. *Nematologica* 6, 181–200.

Lee, Y.B. and Evans, A.A.F. (1973) Correlation between attractions and susceptibilities of rice varieties to *Aphelenchoides besseyi* Christie, 1942. *Korean Journal of Plant Protection* 12, 147–151.

Mathur, V.K. and Lal, A. (1989) A simple technique for the detection of white tip nematode (*Aphelenchoides besseyi*) in rice germplasm under exchange. *Indian Journal of Nematology* 19, 71.

McDonald, D., Bos, W.S. and Gumel, M.H. (1979) Effects of infestation of peanut (groundnut) seed by the testa nematode, *Aphelenchoides arachidis*, on seed infection by fungi and on seedling emergence. *Plant Disease Reporter* 63, 464–467.

Nakagome, T. and Kato, K. (1977) [Injuries to chrysanthemum cultivars caused by infestation by *Aphelenchoides ritzemabosi*.] *Research Bulletin of the Aichi Ken Agricultural Research Center, B Horticulture* 9, 86–91.

Nishizawa, T. (1953) [Studies on the varietal resistance of rice plants to the rice nematode disease 'Senchu shingare byo' (VI).] *Bulletin of the Kyushu Agricultural Experimental Station* 1, 339–349.

Noegel, K.A. and Perry, V.G. (1963) A foliar disease of Chrysanthemum incited by the strawberry summer crimp nematode. *Proceedings of the Soil Science Society of Florida, 22nd Annual Meeting*, pp. 162–166.

Popova, M.B., Zelenskii, G.L. and Subbotin, S.A. (1994) The assessment of resistance in cultivars of *Oryza sativa* L. to *Aphelenchoides besseyi* Christie, 1942. *Russian Journal of Nematology* 2, 41–44.

Qiu, T.X., Yan, M.F. and Lu, Q. (1991) [Study on the occurrence, regulation and control of *Aphelenchoides besseyi*.] *Zhejiang Nongye Kexue* 6, 290–292.

Rajan, Mathur, V.K. and Lal, A. (1989) Improved culturing technique for

Aphelenchoides besseyi by inducing anhydrobiosis. *Indian Journal of Nematology* 19, 10–13.

Shepherd, J.A. and Barker, K.R. (1990) Nematode parasites of tobacco. In: Luc, M., Sikora, R.A. and Bridge, J. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, UK, pp. 493–517.

Siddiqi, M.R. (1974) *Aphelenchoides ritzemabosi*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 3, No. 32.

Siddiqi, M.R. (1975) *Aphelenchoides fragariae*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 5, No. 74.

Sivakumar, C.V. (1988) White tip nematode (*Aphelenchoides besseyi*) resistance in rice. *Indian Journal of Nematology* 18, 342–344.

Sohn, J.K., Lee, S.K., Koh, J.C., Kim, H.Y., Yang, S.J., Hwang, H.G., Hwang, D.Y. and Chung, G.S. (1987) [A new rice variety with multiple resistance to diseases and insect pests: Namyeongbyeo.] *Research Report of the Rural Development Administration, Crops, Korea Republic* 29, 18–28.

Strider, D.L. (1979) Control of *Aphelenchoides ritzemabosi* in African violet. *Plant Disease Reporter* 63, 378–382.

Szczygiel, A. (1977) Review of Soviet literature on plant parasitic nematodes associated with strawberries. Research Institute of Pomology Experimental Station, Brezna, Poland, 63 pp.

Szczygiel, A. and Danek, J. (1975) Susceptibility of strawberry cultivars to leaf and bud nematodes (*Aphelenchoides* spp.). *Fruit Science Reports* 2, 47–57.

Tacconi, R. (1973) Danni da nematodi alla fragola. *Informatore Fitopatologico* 23, 13–16.

Todd, E.H. and Atkins, J.G. (1958) White tip disease of rice. I. Symptoms, laboratory culture of nematodes and pathogenicity test. *Phytopathology* 48, 632–640.

Wallace, H.R. (1961) Browning of chrysanthemum leaves infested with *Aphelenchoides ritzemabosi*. *Nematologica* 6, 7–16.

Zelenskii, G.L. and Popova, M.B. (1991) [Breeding rice for resistance to the rice leaf nematode in the USA.] *Selektsiya i Semenovodstvo (Moskva)* 5, 59–60.

7

Reniform Nematodes: *Rotylenchulus* Species

A.F. Robinson

Agricultural Research Service, United States Department of Agriculture, 2765 F&B Rd, College Station, TX 77845, USA

Reniform nematodes (genus *Rotylenchulus*) are semi-endoparasites of roots and occur commonly in tropical and subtropical regions throughout the world. Some species also occur in warm temperate climates. The term 'reniform' comes from the name of the type species, *R. reniformis* (Linford and Oliveira, 1940), and refers to the kidney-like shape of the body of sedentary mature females, a shape which characterizes all members of the genus *Rotylenchulus*. The reader is referred to reviews of the species *R. reniformis* by Varaprasad (1986) and Gaur and Perry (1991a) and to a review of the genus *Rotylenchulus* by Robinson *et al*. (1997).

Biology of Reniform Nematodes

Taxonomy and identification

Identification to genus is based primarily on morphological characters of preparasitic vermiform females. Identification to species is possible based on the presence or absence of males and on morphological characters of the immature females, including stylet length, vulva position, shape of head (rounded or truncate conoid) and shape of tail terminus (clavate or bluntly rounded) (Germani, 1978b; Table 7.1). Nine recognized species include *R. anamictis*, *R. borealis*, *R. clavicaudatus*, *R. leptus*, *R. macrodoratus*, *R. macrosoma*, *R. parvus*, *R. reniformis* and

 Plant Resistance to Parasitic Nematodes
(eds J.L. Starr, R. Cook and J. Bridge)

Table 7.1. Key to the *Rotylenchulus* spp. (from Robinson *et al.*, 1997, modified from Germani, 1978b).

1.	Stylet > 27 μm	*R. sacchari*
	Stylet = 10–15 μm	2
	Stylet = 16–26 μm (males present)	5
2.	Males present	3
	Males absent or rare	4
3.	V[a] = 55–66%	*R. borealis*
	V = 67–72%	*R. anamictus*
4.	Head conoid, truncate	*R. leptus*
	Head rounded	*R. parvus*
5.	V = 55–63%	6
	V > 63%	7
6.	Tail with clavate terminus	*R. clavicaudatus*
	Tail with bluntly rounded terminus	*R. macrosoma*
7.	Stylet = 16–21 μm	*R. reniformis*
	Stylet = 22–26 μm	*R. macrodoratus*

[a]V, position of vulva from anterior end along body axis as percentage of body length.

R. sacchari. The type species, *R. reniformis*, is by far the most common. Host ranges of *Rotylenchulus* species differ (Robinson *et al.*, 1997).

Differences have been observed in reproduction and damage caused by 17 populations of *R. reniformis* from the continental United States of America, the Pacific and the Caribbean on cotton (*Gossypium hirsutum*) and soybean (*Glycine max*) (McGawley and Overstreet, 1995). In India, one population of *R. reniformis* differs from others in being unable to reproduce on castor (*Ricinus communis*) or cotton (Dasgupta and Seshadri, 1971). In Japan, three morphologically and reproductively distinct types of populations occur, referred to as 'male-numerous', 'male-rare' and 'male-absent' types (Nakasono, 1983). They differ in host range and ability to reproduce parthenogenetically. However, interpopulation variability of reniform nematodes is poorly understood and standardized tests for distinguishing subspecific variants of *Rotylenchulus reniformis* are lacking. Variability within other species of *Rotylenchulus* has not been studied.

Impact

Host range literature for *Rotylenchulus* spp. is more limited than for root-knot and cyst nematodes. Known hosts for *R. reniformis* include more than 300 species in 77 plant families (Robinson *et al.*, 1997). Only 14% of 364 species tested were non-hosts for *R. reniformis*. Numerous monocots as well as dicots are hosts. Host ranges of other

Rotylenchulus species have only been examined in limited studies, primarily to estimate impact on crops in geographic regions where each species was first found.

The most intensely studied crop damages of *Rotylenchulus* spp. are those of *R. reniformis* in the USA to cotton, soybean and cowpea (*Vigna unguiculata*), and in India to various legumes including blackgram (*Vigna mungo*), chickpea (*Cicer arietinum*), cowpea, greengram (*Phaseolus aureus*), horsegram (*Dolichos biflorus*) and pigeonpea (*Cajanus cajan*). Other crops screened for resistance to *R. reniformis* include castor, papaya (*Carica papaya*), pepper (*Capsicum* spp.), potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*). The distribution of *R. reniformis* in a field typically is uniform, plants are seldom killed and the resulting uniform disease expression often goes unnoticed (Blasingame, 1994; Robinson *et al*., 1999a). Yield loss in an infested field is usually less than 25%; losses as great as 60% occur in cotton in the USA (Jones *et al*., 1959; Overstreet, 1996).

Life cycle

Not all *Rotylenchulus* species have been studied in detail but life cycles appear similar to that reviewed by Sivakumar and Seshadri (1971), Gaur and Perry (1991a) and Robinson *et al*. (1997). Briefly, there are four vermiform juvenile stages (denoted J1, J2, J3, J4), each followed by a moult (Fig. 7.1). The J1 occurs only in the egg, the J2 hatches from the egg, and the J3 and J4 typically remain ensheathed by the remnants of previous cuticles after moulting. None of the juvenile stages feeds. Under favourable conditions, embryonation and eclosion require 5–8 days and subsequent development through the final moult another 8–18 days (Nath *et al*., 1969; Nakasono, 1983) although development can be delayed many weeks by desiccation (Womersley and Ching, 1989; Gaur and Perry, 1991b) or unknown factors. In amphimictic species, the final moult gives rise to equal numbers of sexually differentiated vermiform males and females, both of which are slightly smaller than the J2 (Bird, 1983).

Rotylenchulus spp. typically invade a zone along the root axis where cells have undergone primary differentiation. The vermiform female penetrates the root cortex perpendicularly to the root axis and stops with the anterior end adjacent to the stele and the posterior end protruding from the root surface. The female feeds permanently on a single cell in the endodermis, pericycle or deep cortex, and elicits the formation of a syncytium consisting of a curved sheet of hypertrophied vascular cells (Fig. 7.2), usually in the pericycle, that acquire cytoplasmic confluence through partial dissolution of common cell walls

(Fig. 7.2). Tissues surrounding the syncytium, unlike those in root-knot nematode galls, are not hyperplastic; however, the syncytial cells are similar to the giant-cells induced by root-knot nematodes in having

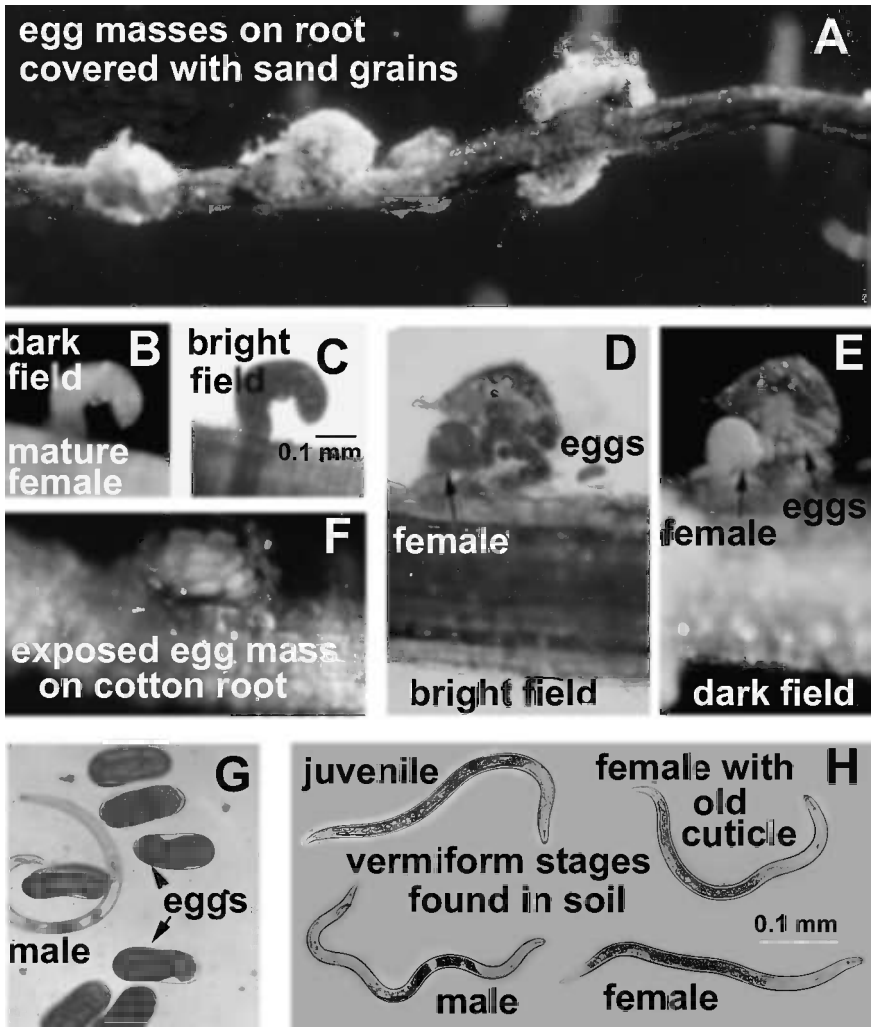

Fig. 7.1. Developmental stages of *Rotylenchulus reniformis*: (A) egg masses on a cotton root; (B) exposed mature female on cotton root after removing egg mass, as seen with dark field illumination; (C) as (B) except with bright field illumination; (D) mature female with egg mass partially disrupted to expose eggs within, as seen with bright field illumination; (E) as (D) but with dark field illumination; (F) eggs seen when top of egg matrix is removed; (G) eggs contrasted with a vermiform adult male; (H) vermiform juveniles, females and males.

safraninophilic cytoplasm with proliferated endoplasmic reticulum, indicative of elevated transcription and protein synthesis. *R. macrodoratus* is an exception inducing in seven plant species studied so far, a single, greatly hypertrophied uninucleate nurse cell (Cohn, 1976; Cohn and Mordechai, 1977; Inserra and Vovlas, 1980).

During the 10–20 days after feeding begins, depending on temperature and species, the posterior of the female swells into a characteristic kidney shape (Fig. 7.2) as the ovaries and uterus mature and 40–200 (usually about 60) eggs are laid in a sticky gelatinous matrix secreted by vaginal glands. The egg mass engulfs much of the exposed female and on disinterred roots soil particles usually adhere to and obscure egg masses. Sedentary females of all species except *R. leptus* and *R. parvus* are thought to be inseminated by males, which occur in large numbers, apparently do not feed, and remain vermiform and free in the soil. *R. parvus*, some Japanese populations of *R. reniformis*, and probably *R. leptus* lack males and are parthenogenetic (Dasgupta and Raski, 1968; Nakasono, 1983).

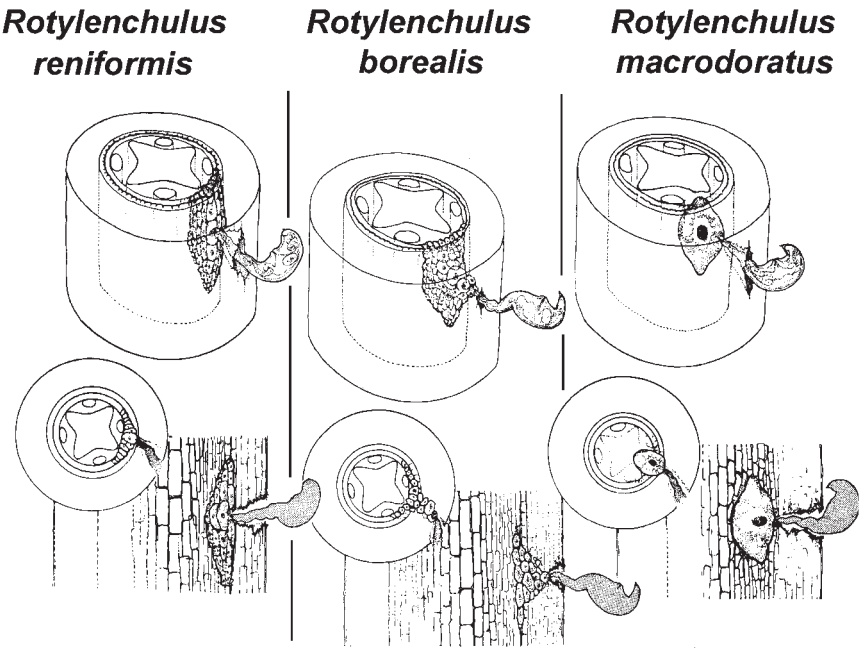

Fig. 7.2. Transverse, longitudinal and three-dimensional views of feeding sites induced by *Rotylenchulus reniformis*, *R. borealis* and *R. macrodoratus* (drawing by A. Triccoli from Robinson *et al*., 1997).

Resistance

Mechanisms

Vermiform females appear to enter roots of susceptible and resistant plants equally and thus resistance appears to depend on plant responses after tissue infection. A transmission electron microscopy study of soybean infection by *R. reniformis* (Rebois *et al.*, 1975) revealed that syncytium development in susceptible plants went through two phases: (i) an initial phase involving partial cell wall lysis and separation, and (ii) an anabolic phase characterized by organelle proliferation and secondary wall deposits. In resistant plants, the initial phase was accelerated, resulting in cell lysis. A similar response was observed in upland cotton (*G. hirsutum*) and in *G. arboreum* (an old world cotton) followed by cell necrosis and walling-off of the nematode (Carter, 1981; Shepherd and Huck, 1989). Resistance in mango (*Mangifera indica*) apparently can be induced by altering auxin levels (Badra and Khattab, 1982).

Sources of resistant germplasm

Germplasm of at least 17 crops has been screened for resistance to *R. reniformis* and many breeding lines, crop species and wild relatives with resistance have been identified (Table 7.2). In addition, about 300 plant species have been evaluated as reservoir hosts, as nematode vectors in the container plant industry, or as useful rotational crops (Robinson *et al.*, 1997). Availability of resistance to *R. reniformis* in crops varies greatly. Mustard (*Brassica nigra*), oat (*Avena sativa*), onion (*Allium cepa*), sugarcane (*Saccharum officinarum*), sunn hemp (*Crotalaria juncea*) and winter wheat (*Triticum aestivum*) are virtually immune to most populations of *R. reniformis*. Some cultivars of maize (*Zea mays*), soybean and tomato are highly resistant. In cowpea, only a few resistant cultivars have been identified. In upland cotton, no cultivars or breeding lines with useful resistance have been found, although tolerant genotypes have been identified that yield well in soil with high population densities of *R. reniformis* (Cook *et al.*, 1997). Four cotton lines developed in Louisiana carry a low degree of resistance (Jones *et al.*, 1988). Incorporating resistance into upland cotton from some wild *Gossypium* species is confounded by ploidy and incompatibility between species. In sweet potato, resistance to *R. reniformis* is genetically linked to nematode-induced cracking of the storage root (Clark and Wright, 1983).

Inheritance of resistance

In most cases, resistance to *R. reniformis* appears inherited, at least in part, independently from resistance to other sedentary nematodes. In

upland cotton only three commercial cultivars and probably less than 20 breeding lines have moderate to high levels of resistance to *Meloidogyne incognita* race 3 (Robinson *et al.*, 1999a). Most derive their resistance from one or more of three sources: the obsolete cultivar Clevewilt 6 (Jones *et al.*, 1991), a Mexican primitive racestock registered as USDA Accession SA 2516 (Shepherd, 1974) and the Acala breeding line N6072 (Hyer *et al.*, 1979). Introgression of resistance from an unidentified *G. barbadense* line also has been achieved (Hyer and Jorgenson, 1978). Apparently, all *M. incognita*-resistant genotypes of *G. hirsutum* support prolific reproduction by *R. reniformis*. Levels of reproduction by *R. reniformis* vary sufficiently so that some breeding lines derived from Clevewilt 6 are considered partly resistant; however, inheritance of resistance is complex and new sources are needed (Muhammad and Jones, 1990).

In soybean, early studies indicated that resistance to *R. reniformis* could be expected in genotypes with resistance to the soybean cyst nematode (Rebois *et al.*, 1968, 1970) This prediction, however, was not supported by subsequent genetic studies (Birchfield *et al.*, 1971; Hartwig and Epps, 1977; Gilman *et al.*, 1979; Harville, 1985; Anand, 1992). Two pairs of genes with unequal effects were proposed to confer resistance to *R. reniformis* in soybean (Williams *et al.*, 1981; Harville, 1985). Recent studies have shown that soybean cyst nematode-resistant soybean cultivars that derive resistance from cv. Peking (e.g. cvs Forrest and Centennial), or PI-437.654 (e.g. cv. Hartwig), or PI-90.763 (e.g. cv. Cordell) are also resistant to *R. reniformis*, whereas those that derive soybean cyst nematode resistance from PI-88.788 are not resistant to *R. reniformis* (Robbins *et al.*, 1994a,b; Davis *et al.*, 1996; Robbins and Rakes, 1996).

In tomato, genes for resistance to *R. reniformis* again appear unlinked to genes for resistance to *Meloidogyne* spp. Resistance of tomato to the sugarbeet cyst nematode, *Heterodera schachtii*, however, was correlated with reniform nematode resistance in 22 cultivars examined by Rebois *et al.* (1973). Immunity to *R. reniformis* was reported in the *L. esculentum* cultivars Kalyampur Sel I, Kalyampur Sel III and LA 121, and in the *L. pimpinellifolium* accession PI-375.937 (Rebois *et al.*, 1977; Balasubramanian and Ramakrishnan, 1983).

Because of the partial coincidence of reniform and cyst nematode resistance observed in soybean (Rebois *et al.*, 1970) and tomato (Rebois *et al.*, 1977), Rebois and Webb (1979) tested for *R. reniformis* resistance in 41 cultivars of potato, most possessing the *H1* gene for resistance to the potato cyst nematode, *Globodera rostochiensis*. Reniform nematode resistance was found in several potato clones including the cultivar La Rouge and possibly Red La Soda. However, reniform nematode resistance segregated independently of the *H1*, *H2* and *H3* genes for resistance to the potato cyst nematode.

Table 7.2. Methods applied to evaluate plant germplasm for resistance to *Rotylenchulus reniformis*.

Crop[a]	Number of entries	Container size[b]	Root growth medium	Replications	Inoculation method[c]
Blackgram	17	10 cm diam. pot (500 g)	Autoclaved soil	5	II
Chickpea	9	15 cm diam. pot	Clay : sand : compost at 7 : 2 : 1	5	I
Chickpea	49	500 g soil (10 cm diam. pot)	Autoclaved soil	5	II
Coffee	3	1 litre pot	Methyl bromide sterilized soil	7	III
Cotton, upland	24	30 cm diam. pot	'Infested soil'	4, 20	III
Cotton, upland	10	Bin	Loamy fine sand	Not stated	III
Cotton, upland	26	Flat	Silt loam soil	Not stated	III
Cotton, upland	840[e]	1 litre pot (500 g)	50 : 50 silt loam : river sand	1[f]	I
Cotton, upland	13	500 cm^3 pot	Sand : peat : vermiculite (3 : 1 : 1 vol)	6	I
Cotton, upland	59	500 cm^3 pot	Sand : vermiculite mixture (6 : 1 v : v), steamed	6	I
Cotton, upland	50	500 cm^3 pot	Sand : vermiculite mixture (6 : 1 v : v), steamed	6	I
Cotton, relatives	200	178 cm^3 cup	Steam sterilized loam soil	6, 2 plants per cup	I
Cotton, Sea Island	16	307 litre wooden box	'Natural field soil'	10	III
Cotton, Sea Island	6	25 cm diam. pot	Steam sterilized loam soil	4	I
Cowpea	20	15 cm diam. pot	3 : 1 : 1 mix of sandy loam : sand : manure	5	II
Cowpea	4	15 cm diam. pot (1300 g)	Steam sterilized soil	3	II
Cowpea	6	15 cm diam. pot	Steam sterilized soil	2	I
Cowpea	7	100 cm^3 paper cup	Steam sterilized sandy loam	10	I
Greengram	1	100 cm^3 paper cup	Steam sterilized sandy loam	10	I
Greengram	17	10 cm diam. pot (500 g)	Autoclaved soil	5	II
Greengram	26	15 cm diam. pot (500 g)	Autoclaved sandy loam soil	5	I
Greengram	53	250 g	Soil	3	I
Horsegram	14	10 cm diam. pot (500 g)	Autoclaved soil	5	II
Olive	6	20 cm diam. pot	Sandy loam soil	4	II
Papaya	4	15 cm diam. pot	Soil	5	I
Pepper	12	10 cm diam. pots (500 g)	Autoclaved soil	5	I or II
Pepper	2	20 or 30 cm diam. pot	Field soil	Not stated	III
Pigeonpea	64	10 cm diam. pot (300 g)	Autoclaved soil	5	I
Pigeonpea	83	10 cm diam. pot (300 g)	Autoclaved soil	5	I
Pigeonpea	296	15 cm diam. pot (500 g)	Infested soil	3	III
Potato	41	20 cm diam. pot	Potting soil mix	4	III
Potato	5	10 cm diam. pot	Potting soil mix	6	I
Potato	4	20 cm diam. pot	Potting soil mix	8	III
Soybean	10	30 cm raised bench	Heat-treated fine sandy loam	4 groups, 5 plants per group	I
Soybean	5	20 cm diam. pot	Fine sandy soil	3 pots, 10 plants per pot	II

Continued

Table 7.2. *Continued.*

Inoculation rate			Duration of	Parameters of	
No. per plant	No. per cm^3 soil	No. per g soil	experiment[d]	resistance[e]	Reference
100	0.13	0.2	7d+12d	ABEH	Routaray *et al.* (1986)
5000	1.3		2m	A	Anver and Alam (1990)
100	0.13		7d+12d	ABEH	Sahoo *et al.* (1986)
Unknown	–		4m	H	Macedo (1974)
Unknown	–		Unstated	HI	Birchfield and Brister (1963)
Unknown	–		To lint harvest	L	Minton *et al.* (1964)
Unknown	9		99d	K	Neal (1954)
2000–3500	2–4	4–7	40–50d or 32d	EF	Muhammad and Jones (1990)
4000	8		2w+6w or 2w+10w	ABC	Cook *et al.* (1997)
4000	8		10d+56d	ABF	Robinson *et al.* (1999b)
4000	8		10d+56d	ABF	Robinson and Percival (1997)
1000	11		35d	C	Yik and Birchfield (1984)
12,000	6		8w	K	Khadr *et al.* (1972)
72,000	7		8w?	K	Khadr *et al.* (1972)
1000	0.25		1w+2m	AI	Khan and Husain (1988)
500	0.2	0.4	15d+45d	ACI	Makadia *et al.* (1987)
200	0.1		5d+20d	HJ	Thakar and Patel (1985)
350	3.5		7d+22d	BH	Gaur (1986)
350	3.5		7d+22d	BH	Gaur (1986)
100	0.13	0.2	7d+12d	ABEH	Routaray *et al.* (1986)
200	0.08	0.4	6d+12d	H	Patel *et al.* (1989a)
100	0.5		20d	BDEH	Patel and Thakar (1986)
100	0.13	0.4	7d+12d	ABEH	Nayak *et al.* (1987)
2000	0.7		2m+4m	A?B?	Al-Sayed and Abdel-Hameed (1991)
200	0.1		12d after inoculation	H	Patel *et al.* (1989b)
500 or 100, resp.	0.7 or 0.13	1 or 0.2	15d+12d or 28d	ABE	Routaray *et al.* (1988)
4200–14,000	0.6–0.8		Not stated	E	Birchfield and Brister (1962)
200	0.7	0.7	6d+12d	H	Patel *et al.* (1987)
200	0.4	0.4	6d+12d	H	Chavda *et al.* (1988)
800?	2		1m	BE	Thakar and Yadav (1985)
9000	1.5		1m+2m	E	Rebois and Webb (1979)
5000	7		8d+3w	E	Rebois and Webb (1979)
9000	1.5		1m+1,2 and 3m	AE	Rebois and Webb (1979)
1500	?		0d+7w	EH	Rebois *et al.* (1970)
1500	2.5		0d+7w	AEH	Rebois *et al.* (1970)

Continued

Table 7.2. *Continued.*

Crop[a]	Number of entries	Container size[b]	Root growth medium	Replications	Inoculation method[c]
Soybean	65	Field plot	Naturally infested clay loam	3	IV
Soybean	8	20 cm diam. pot	Autoclaved soil	3, 6, 6	I
Soybean	20	25 cm diam. pot	Sterilized soil	3	I
Soybean	19	20 cm diam. pot	Naturally infested soil	3 pots, 4 plants per pot	III
Soybean	321[e]	7.6 × 7.6 cm square pot	Sand/soil mixture	5[f]	III
Soybean	572[e]	7.6 × 7.6 cm square pot	Sand/soil mixture	1[f]	III
Soybean	2	7.6 × 7.6 cm square pot	Sand/soil mixture	10	III
Soybean	30	10 cm diam. pot (500 cm^3)	Fine sandy loam (91 : 5 : 4 ssc)	5	I
Soybean	4	2 rows, 30 m long	Silt loam (9 : 84 : 7 ssc)	72[g]	IV
Soybean	288	10 cm diam. pot	Fine loamy sand	5	I
Sweet potato	24	15 cm diam. pot	Soil with known populations	4	III
Sweet potato	44	10 cm diam. pot	Not stated	Not stated	I?
Sweet potato	10	Field plot	Silt loam soil	4, 9, 9	IV
Tobacco	3	15 cm diam. pot	Steam sterilized sandy loam	6	I
Tobacco	13	15 cm diam. pot (800 g)	Soil : manure mix (4 : 1)	4	I
Tomato	37	2.5 × 7.5 cm glass tube	Steam sterilized soil	3	II
Tomato	9	20 cm diam. pot	Loamy sand	2–3 pots, 10–15 plants per pot	I
Tomato	20	15 cm diam. pot	Loamy sand	4	II
Tomato	2	2 litre pot	Soil	10	I

[a]Blackgram = *Phaseolus mungo*, chickpea = *Cicer arietinum*, coffee = *Coffea* spp., upland cotton = *Gossypium hirsutum*, Sea Island cotton = *G. barbadense*, cowpea = *Vigna unguiculata*, greengram = *Phaseolus aureus*, horsegram = *Dolichos biflorus*, olive = *Olea europaea*, papaya = *Carica papaya*, pigeonpea = *Cajanus cajan*, potato = *Solanum tuberosum*, soybean = *Glycine max*, sweet potato = *Ipomoea batatas*, tobacco = *Nicotianum tabacum*, tomato = *Lycopersicon esculentum*.

[b]Pots with diameters of 10, 15, 20, 25 and 30 cm are assumed to hold 0.75, 2.5, 6, 12 and 20 litres and soil is assumed to weigh 1.2 g cm^{-3} unless stated otherwise in the original reference.

[c]Inoculation methods: I, Vermiform stages in aqueous suspension; II, Immature females in aqueous suspension; III, Pre-infested soil in pots; IV, Pre-infested soil in field plots; V, Eggs in aqueous suspension.

Strategy for Evaluating Germplasm

When compared with resistance to other groups of nematodes, particularly root-knot and cyst nematodes, resistance to reniform nematodes has been the subject of a small number of crop germplasm evaluations (Table 7.2). Methods given in the literature are generally suitable but most are adaptations of methods developed originally for testing for resistance to other nematode species. When developing new methods

Table 7.2. *Continued.*

Inoculation rate			Duration of	Parameters of	
No. per plant	No. per cm^3 soil	No. per g soil	experiment[d]	resistance[e]	Reference
–	0.14		11w	DFIH	Lim and Castillo (1979)
300, 1900, 3300	0.2, 0.9, 1.7		Not stated	AH	Rebois *et al.* (1968)
30,000, 20,000	2.5, 1.6		4m or 2m	AG	Birchfield *et al.* (1971)
125 (500 per pot)	0.1		90d	AE	Birchfield and Brister (1969)
1760	4		21d	G	Williams *et al.* (1981)
1760	4		21d	G	Williams *et al.* (1981)
1600–2500	3.6–5.6		14,17,21,24,28, 31d	G	Williams *et al.* (1979)
1000	2		germ+60d	AB	Robbins *et al.* (1994a,b)
–	7.6		104d	A	Robbins *et al.* (1994a,b)
1200	2		1w+11–15w	ABGH	Robbins *et al.* (1999)
500, 640, 2400	0.2, 0.3, 1.0		98,68,145d	A	Martin *et al.* (1966)
600	0.8		58d	A	Clark *et al.* (1980)
–	1.3, 18, 3		115 and 113d	A	Clark and Wright (1983)
25,000	10		1m+63d	AC	Heald and Meredith (1987)
1000	0.4	1.3	8w+45d	ABH	Patel *et al.* (1986)
100	2.7		10d+12d	H	Balasubramanian and Ramakrishnan (1983)
1000	0.17		4–6w	DEH	Rebois *et al.* (1973)
2000	0.8		germ+10d+35d	BDEH	Montasser (1986)
10,000	5		76d	A	Germani (1978a)

[d]d, days; w, weeks; m, months

[e]Resistance parameters:

A, Vermiform nematodes extracted from standard quantity of soil; B, total eggs per plant; C, eggs per gram of root; D, eggs per egg mass; E, egg masses per root system; F, egg masses per gram of root; G, egg mass rating; H, total sedentary females in roots; I, sedentary females per gram of root; J, mature female rating; K, fusarium wilt incidence only; L, yield.

[f]Inheritance study.

[g]Correlation study.

for working with *Rotylenchulus* species, a more or less unique combination of biological characteristics can be considered. *Rotylenchulus* nematodes are characterized by a short life cycle, significant delay between egg hatch and infectivity, possible arrested development prior to host invasion, wide host range, amphimictic reproduction (except *R. parvus*, *R. leptus* and some Japanese populations of *R. reniformis*), adaptation to finely textured soils, large number of vermiform nematodes in soil, high plant damage thresholds and high overwinter survival.

Which are you looking for: immunity, resistance or tolerance?

Immunity

R. reniformis can be an important pest in ornamental industries established in areas where the nematode is present. Plant damage to ornamentals is of less concern than are export restrictions. Arizona, California, New Mexico, Chile and Switzerland consider *R. reniformis* a noxious organism subject to quarantine, and this seriously impacts export of ornamentals from areas of Florida and Texas where the nematode occurs naturally. Due to survival of *R. reniformis* during long periods without a host, it has become critical to determine whether suspect ornamental species are hosts or non-hosts (Starr, 1991; Inserra *et al.*, 1994) and evaluate nematode reproduction on weeds commonly associated with ornamental production.

Confirming immunity requires rigorous scrutiny of roots for nematode development and reproduction. Potential losses are immediate and sampling costs must be weighed carefully against the economic risks of an erroneous host determination (McSorley and Littell, 1993).

Resistance and tolerance

Resistance is any plant property that suppresses or inhibits nematode reproduction to some degree (see Roberts, Chapter 2). A resistant genotype that reduces or eliminates the nematode in the soil must also provide an acceptable crop yield. Tolerant cultivars yield well until very high population densities are reached and in theory may be sufficient in a crop monoculture if biological controls keep nematode population densities below critical levels. These conditions characterize *R. reniformis* in large cotton production regions in the United States (Heald and Robinson, 1990). Several high-yielding breeding lines and one cultivar of upland cotton have been developed that are tolerant to *R. reniformis* (Cook *et al.*, 1997).

Clearly, plants may be selected for tolerance in field plots but resistance should be tested in a container where the number of nematodes introduced can be controlled and the number produced can be measured. Even tolerance can be difficult to measure on individual plants in field plantings where initial densities vary from plant to plant and root systems of adjacent plants are intergrown. Tolerance testing under field conditions, however, is more feasible with reniform than with many nematodes due to the tendency of reniform nematodes to occur uniformly within a field. If pots or microplots are used, large numbers of reniform nematodes (> 5 nematodes cm^{-3} soil) are usually needed to obtain damage and when numerous genotypes are to be screened, it may be necessary to plant or transplant into

previously infested soil rather than inoculate soil with nematodes suspended in water. This will be discussed in the next section, on inoculum preparation.

Inoculum

Maintenance

Stock cultures of reniform nematode populations can be maintained on many hosts in various potting mixes. Tomato, cantaloupe (*Cucumis melo* var. *cantalupensis*) and cotton support high population densities but the best choice probably is a host that is known to grow well with minimal maintenance in the glasshouse facility available and at the high temperatures (26–32°C) that favour reniform nematode reproduction. Large numbers of *R. reniformis* can be maintained easily throughout the year on tomato cv. Rutgers or cotton cv. Stoneville 474 in large, well-drained boxes (0.3 m deep, 1.3 m wide, 2 m long) containing silt loam soil (6% sand, 70% silt, 24% clay).

Preparation

Eggs of *R. reniformis* can be collected, purified and hatched by methods developed for root-knot nematodes. The *R. reniformis* egg mass, however, is five to ten times smaller and so eggs are more likely to be injured by exposure to sodium hypochlorite during the egg dispersal step. If eggs are used as inoculum, it must be remembered that the juveniles that hatch from eggs are not infective, and must moult three times before becoming infective. Balasubramanian and Ramakrishnan (1983) and others (Table 7.2) have solved this problem by holding juveniles in water until moulting was complete before inoculating plants. In this way, developmentally synchronous but differentially starved nematodes are obtained. It is faster to extract vermiform stages directly from the soil. They occur at concentrations comparable to eggs and can be extracted by elutriation followed by sieving and centrifugal flotation or by Baermann funnel. The latter ensures that nematodes are 100% motile, provided they are not allowed to remain in the bottom of the funnel for more than 24 h. Moreover, if the potting mix has become contaminated by insect-borne free-living nematodes or other microfauna, as often happens, most of these can be eliminated by drawing 20–50 ml of water from the bottom of the funnel after 4–6 h, and collecting reniform individuals that descend during the subsequent 18 h. In the funnels described by Robinson and Heald (1991) most of the reniform nematodes descend at 6–24 h after placing soil on the retaining tissue.

The inoculum concentrations used have varied greatly, ranging from 200 nematodes per 15 cm diameter pot (0.1 cm^{-3}) for papaya (Patel *et al*., 1989b) to 2000 nematodes per 178 cm^3 cup (11 cm^{-3}) for cotton (Yik and Birchfield, 1984). I have conducted many successful experiments on cotton with 4000 vermiform nematodes per 500 cm^3 pot (8 cm^{-3}). Most soybean studies have used 1–5 vermiform nematodes cm^{-3} soil. As points of reference, the damage thresholds recommended for reniform nematode in cotton by the Mississippi Agricultural Extension Service are 2 and 10 vermiform nematodes cm^{-3} soil, respectively, for samples collected in the spring and autumn. These values consider extracted vermiform stages only and thus are comparable to inoculum densities that have been used experimentally for both cotton and soybean.

An especially simple approach to host inoculation is to plant or transplant directly into thoroughly mixed, infested soil obtained from glasshouse cultures (Williams *et al*., 1979). Dilution of infested soil with nematode-free soil may be necessary to obtain the desired inoculum concentrations. When using this approach, it is recommended to determine initial population densities of the nematodes by elutriation, centrifugal flotation or Baermann funnel extraction and initially to test seedlings of a standard cultivar of the crop of interest in a dilution series of the infested soil. The dilution series is best prepared by mixing infested soil with uninfested soil of the same texture in a rotary mixer, such as a cement mixer, with care not to damage nematodes by excessive drying or mixing. Thereby, it can be ascertained with some measure of confidence what level of nematode soil population is required with the crop used to achieve a suitable number of infections on seedlings without stunting or killing them during the experiment. The optimum population density will probably lie between 0.1 and 10 nematodes cm^{-3} soil.

Application

A good rule of thumb for inoculum placement is: ‘The more uniform the better as long as it doesn’t injure the nematodes.’ A side-bore syringe needle long enough to reach more than halfway down into the pot can be used to inject about 1 ml of aqueous egg or nematode suspension 100 cm^{-3} soil while twisting and lifting the needle up through the soil. To maximize uniformity, a needle can usually be inserted at three to ten points in a regular pattern around the base of the plant without unduly damaging roots. It is essential, of course, that the suspension of eggs or vermiform stages is stirred thoroughly between plants and that plants are inoculated in random order so that if a change occurs in the inoculum due to stirring or settling it will not affect experimental treatments differently. If inoculations are done in the glasshouse or outdoors, beware that inoculum exposed to the sun in

a covered clear vessel will rapidly reach lethal temperatures! If infested soil is to be used as inoculum, the effect of mixing on viability should be tested initially.

Plant culture

Environment

Reniform nematodes are favoured by moderately high temperatures. The optimum temperature range for movement (Robinson, 1989, 1994; Robinson and Heald, 1993) and development (Rebois, 1973; Nakasono, 1978; Heald and Inserra, 1988) of *R. reniformis* is 25–32°C. Development at 21.5°C is half that at 29.5°C. The lower and upper limits for development are 15°C and 36°C. The threshold for cumulatively lethal effects is 41°C; 50% mortality occurs after 1 h at 45°C and 10 min at 47°C (Heald and Robinson, 1987).

On farmland, reniform nematodes generally occur at highest population densities in finely textured soils (Robinson *et al.*, 1987; Heald *et al.*, 1988; Starr *et al.*, 1993; Blasingame, 1994; Koenning *et al.*, 1996). The best matrix for maintaining stock populations of *R. reniformis* throughout the year is silt loam soil (6% sand, 70% silt, 24% clay). In container-grown plant culture, however, such soils often become unnaturally compacted, provide poor drainage and aeration, and are difficult to clean from roots when roots are removed for inspection and egg extraction. Thus, an intermediately textured soil, such as loam, sandy clay loam or silt loam may be better. In 5000 cotton plant evaluations, good results were obtained with a 6:1 mixture of fine sand and vermiculite supplemented with dolomite and a slow-release formulation of micronutrients (Cook *et al.*, 1997; Robinson and Percival, 1997; Robinson *et al.*, 1999b; A.F. Robinson, unpublished data) (Table 7.2). However, the best choice is probably a trade-off between the nematode, the plant and the objectives of the investigator.

Duration

When conducting initial evaluations of germplasm for resistance to *Rotylenchulus* spp. it is possible to take advantage of the nematode's short life cycle by growing host seedlings for only 35 days at 25–30°C. Thereby, the amount of inoculum, potting medium, space and time required can be greatly minimized. The size of the containers used will be dictated by the growth rate of the crop plant examined. This approach was used successfully to screen soybean (Williams *et al.*, 1979), cotton (Yik and Birchfield, 1984), tomato (Balasubramanian and Ramakrishnan, 1983) and potato (Rebois and Webb, 1979). After potentially resistant genotypes have been identified, resistance can be tested

more rigorously by growing plants to maturity in glasshouse pots and under field conditions.

Repotting plant selections

In plant breeding studies, it may be desirable to replant roots after examining them in order to obtain seed. There are no special requirements other than those of the crop plant. The plant is unlikely to be killed by reniform nematodes. The researcher should be aware that infested root systems cannot be sanitized and transplanting to the field will infest the field.

Measurements

Reproduction

Reproduction may be evaluated indirectly by gently washing and staining roots to count mature females and egg masses. The procedure of Byrd *et al*. (1983) for staining nematodes with acid fuchsin after clearing with sodium hypochlorite solution makes nematodes and egg masses protruding from the roots easier to see and does not require the use of toxic phenol. *R. reniformis* females stain well with acid fuchsin. They can also be stained by the cotton blue methods recommended for root-knot nematodes.

Lim and Castillo (1979) found that in field-grown soybean, the number of nematodes per unit root weight was statistically and economically the most efficient of five parameters used to measure resistance to *R. reniformis*. Alternatively, one may use the soil in which test plants were grown to extract nematodes that have hatched from eggs. Both conventional Baermann funnel and sieving-Baermann pan techniques work well for *Rotylenchulus* spp. A more direct, faster, and probably much easier method was described by Yik (1981). A standard quantity of roots (1–5 g fresh weight) from each plant is cut into 1 cm lengths, soaked in 0.5% sodium hypochlorite for 10 min, and macerated in a standard volume of water (100 ml). A 75 μm aperture (200 mesh) sieve is used to catch the root debris and a 25 μm aperture (500 mesh) sieve is used to catch the eggs. Eggs are counted within 10 ml aliquots from the suspension.

Plant damage

The visually observable effects of reniform nematodes on plants are usually subtle. The most obvious parameters to measure are yield reduction and stunting. In cotton, delayed flowering and fruit set are typical (Jones *et al*., 1959; Lawrence and McLean, 1996). *R. reniformis* also causes chlorosis in many plants and this has been shown to be

related to potassium deficiency in root as well as foliar tissues of cowpea and corn (Heffes *et al.*, 1992).

Markers

Molecular markers for reniform nematode resistance are not yet available but will be particularly powerful tools due to the difficulty of observing reniform nematode symptoms on roots with the unaided eye.

References

Al-Sayed, A.A. and Abdel-Hameed, S.H. (1991) Resistance and susceptibility of olives to *Meloidogyne incognita* and *Rotylenchulus reniformis*. *Annals of Agricultural Science, Moshtohor* 29, 1221–1226.

Anand, S.C. (1992) Registration of 'Hartwig' soybean. *Crop Science* 32, 1069–1070.

Anver, S. and Alam, M.M. (1990) Susceptibility of chickpea accessions to the reniform nematode *Rotylenchulus reniformis*. Tests of agrochemicals and cultivars 11. *Annals of Applied Biology* 116, 94–95.

Badra, T. and Khattab, M.M. (1982) Chemically induced resistance to *Rotylenchulus reniformis* by ethephon growth regulant and relevant pathometabolites in mango seedlings. *Nematologia Mediterranea* 10, 49–56.

Balasubramanian, P. and Ramakrishnan, C. (1983) Resistance to the reniform nematode *Rotylenchulus reniformis* in tomato. *Nematologia Mediterranea* 11, 203–204.

Birchfield, W. and Brister, L.R. (1962) New hosts and nonhosts of reniform nematode. *Plant Disease Reporter* 46, 683–685.

Birchfield, W. and Brister, L.R. (1963) Susceptibility of cotton and relatives to reniform nematode in Louisiana. *Plant Disease Reporter* 47, 990–992.

Birchfield, W. and Brister, L.R. (1969) Reaction of soybean varieties to the reniform nematode, *Rotylenchulus reniformis*. *Plant Disease Reporter* 53, 999–1000.

Birchfield, W., Williams, C., Hartwig, E.E. and Brister, L.R. (1971) Reniform nematode resistance in soybeans. *Plant Disease Reporter* 12, 1043–1045.

Bird, A.F. (1983) Growth and moulting in nematodes: changes in the dimensions and morphology of *Rotylenchulus reniformis* from start to finish of moulting. *International Journal for Parasitology* 13, 201–206.

Blasingame, D. (1994) *Know Your Cotton Nematodes . . . Your Hidden Enemies*. R-P 11255. Cotton Foundation, Memphis, Tennessee.

Byrd, D.W.J., Kirkpatrick, T. and Barker, K.R. (1983) An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* 15, 142–143.

Carter, W.W. (1981) Resistance and resistant reaction of *Gossypium arboreum* to the reniform nematode. *Journal of Nematology* 13, 368–374.

Chavda, J.C., Patel, B.A. and Patel, D.J. (1988) Screening of pigeonpea lines to *Rotylenchulus reniformis*. *International Nematology Network Newsletter* 5, 28–29.

Clark, C.A. and Wright, V.L. (1983) Effect and reproduction of *Rotylenchulus reniformis* on sweet potato selections. *Journal of Nematology* 15, 198–203.

Clark, C.A., Wright, V.L. and Miller, R.L. (1980) Reaction of some sweet potato selections to the reniform nematode, *Rotylenchulus reniformis*. *Journal of Nematology* 12, 218.

Cohn, E. (1976) Cellular changes induced in the roots by two species of the genus *Rotylenchulus*. *Nematologica* 22, 169–172.

Cohn, E. and Mordechai, M. (1977) Uninucleate giant cell induced in

soybean by the nematode *Rotylenchulus macrodoratus*. *Phytoparasitica* 5, 85–93.

Cook, C.G., Robinson, A.F. and Namken, L.N. (1997) Tolerance to *Rotylenchulus reniformis* and resistance to *Meloidogyne incognita* race 3 in high-yielding breeding lines of upland cotton. *Journal of Nematology* 29, 322–328.

Dasgupta, D.R. and Raski, D.J. (1968) The biology of *Rotylenchulus parvus*. *Nematologica* 14, 429–440.

Dasgupta, D.R. and Seshadri, A.R. (1971) Races of the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, 1940. *Indian Journal of Nematology* 1, 21–24.

Davis, E.L., Koenning, S.R., Burton, J.W. and Barker, K.R. (1996) Greenhouse evaluation of selected soybean germplasm for resistance to North Carolina populations of *Heterodera glycines*, *Rotylenchulus reniformis*, and *Meloidogyne* species. *Journal of Nematology* 28, 590–598.

Gaur, H.S. (1986) Invasion and reproduction of the reniform nematode on some cultivars of cowpea and green gram. *International Nematology Network Newsletter* 3, 8–10.

Gaur, H.S. and Perry, R.N. (1991a) The biology and control of the plant parasitic nematode *Rotylenchulus reniformis*. *Agricultural Zoology Reviews* 4, 177–212.

Gaur, H.S. and Perry, R.N. (1991b) The role of the moulted cuticles in the desiccation survival of adults of *Rotylenchulus reniformis*. *Revue de Nématologie* 14, 491–496.

Germani, G. (1978a) Test preliminaires de sensibilité de deux cultivars de tomate et d'un cultivar d'araschide a deus souches de *Rotylenchulus reniformis* (Nematoda: Tylenchida). *Revue de Nématologie* 1, 109–112.

Germani, G. (1978b) Caractéres morphobiométriques de trois espèces ouest-africaines de *Rotylenchulus* Linford and Oliveira, 1940 (Nematoda: Tylenchida). *Revue de Nématologie* 1, 241–250.

Gilman, D.F., Marshall, J.G., Rabb, J.G., Lawrence, J.L., Boquet, D.J. and Bartleson, J.L. (1979) Performance of soybean varieties in Louisiana, 1976–78. *Louisiana Agriculture* 1979, 22.

Hartwig, E.E. and Epps, J.M. (1977) Registration of Centennial soybeans (Reg. No. 114). *Crop Science* 17, 979.

Harville, B.G. (1985) Genetic resistance to reniform nematodes in soybeans. *Plant Disease* 69, 587–589.

Heald, C.M. and Inserra, R.N. (1988) Effect of temperature on infection and survival of *Rotylenchulus reniformis*. *Journal of Nematology* 20, 356–361.

Heald, C.M. and Meredith, J.A. (1987) Response of three tobacco cultivars to three *Rotylenchulus reniformis* populations. *Nematropica* 17, 95–98.

Heald, C.M. and Robinson, A.F. (1987) Effects of soil solarization on *Rotylenchulus reniformis* in the Lower Rio Grande Valley of Texas. *Journal of Nematology* 19, 93–103.

Heald, C.M. and Robinson, A.F. (1990) Survey of current distribution of *Rotylenchulus reniformis* in the United States. *Journal of Nematology* 22, 695–699.

Heald, C.M., Inserra, R.N. and Vovlas, N. (1988) Parasitism and reproduction of *Meloidogyne incognita* and *Rotylenchulus reniformis* on cantaloupe in two soils. *Nematropica* 18, 53–58.

Heffes, T.-A.P., Coates-Beckford, P.L. and Robotham, H. (1992) Effects of *Meloidogyne incognita* and *Rotylenchulus reniformis* on growth and nutrient content of *Vigna unguiculata* and *Zea mays*. *Nematropica* 22, 139–148.

Hyer, A.H. and Jorgenson, E.C. (1978) Notice of release of three root-knot nematode resistant cottons, noncommercial breeding stocks of cotton, N9281, N9308, N9211. USDA-ARS and California Agricultural Experiment Station, University of California.

Hyer, A.H., Jorgenson, E.C., Garber, R.H. and Smith, S. (1979) Resistance to root-knot nematode in control of root-knot nematode–Fusarium wilt disease complex in cotton. *Crop Science* 19, 898–901.

Inserra, R.N. and Vovlas, N. (1980) The biology of *Rotylenchulus macrodoratus*. *Journal of Nematology* 12, 97–102.

Inserra, R.N., Dunn, R.A. and Vovlas, N. (1994) Host response of ornamental palms to *Rotylenchulus reniformis*. *Journal of Nematology* 26, 737–743.

Jones, J.E., Newsom, L.D. and Finley, E.L. (1959) Effect of the reniform nematode on yield, plant characters and fiber properties of upland cotton. *Agronomy Journal* 51, 353–356.

Jones, J.E., Beasley, J.P., Dickson, J.I. and Caldwell, W.D. (1988) Registration of four cotton germplasm lines with resistance to reniform and root-knot nematodes. *Crop Science* 28, 199–200.

Jones, J.E., Dickson, J.I., Aguillard, W., Caldwell, W.D., Moore, S.H., Hutchinson, R.L. and Rogers, R.L. (1991) Registration of 'LA 887' cotton. *Crop Science* 31, 1701.

Khadr, A.S., Salem, A.A. and Oteifa, B.A. (1972) Varietal susceptibility and significance of the reniform nematode, *Rotylenchulus reniformis*, in Fusarium wilt of cotton. *Plant Disease Reporter* 56, 1040–1042.

Khan, T.A. and Husain, S.I. (1988) Response of cowpea cultivars to *Rotylenchulus reniformis*. *Nematropica* 2, 159.

Koenning, S.R., Walters, S.A. and Barker, K.R. (1996) Impact of soil texture on the reproductive and damage potentials of *Rotylenchulus reniformis* and *Meloidogyne incognita* on cotton. *Journal of Nematology* 28, 527–536.

Lawrence, G.W. and McLean, K.S. (1996) Reniform nematode and cotton production in Mississippi. In: Dugger, P. and Richter, D. (eds) *Proceedings of the Beltwide Cotton Conferences*, Nashville, Tennessee, 9–12 January 1996. National Cotton Council of America, Memphis, Tennessee, pp. 251–253.

Lim, B.K. and Castillo, M.B. (1979) Screening soybeans for resistance to reniform nematode disease in the Philippines. *Journal of Nematology* 11, 275–282.

Linford, M.B. and Oliveira, J.M. (1940) *Rotylenchulus reniformis*, a nematode parasite of roots. *Proceedings of the Helminthological Society of Washington* 7, 35–42.

Macedo, M.C.M. (1974) Suscetibilidade de cafeeiros ao nematóide reniforme. *O Solo* 66, 15–16.

Makadia, B.M., Patel, D.J., Jogani, D.K. and Shah, H.M. (1987) Reactions of root-knot nematode resistant and susceptible cowpea varieties to reniform nematode, *Rotylenchulus reniformis*. *GAU Research Journal* 12, 62–64.

Martin, W.J., Birchfield, W. and Hernandez, T.P. (1966) Sweetpotato varietal reaction to the reniform nematode. *Plant Disease Reporter* 50, 500–502.

McGawley, E.C. and Overstreet, C. (1995) Reproduction and pathological variation in populations of *Rotylenchulus reniformis*. *Journal of Nematology* 27, 508.

McSorley, R. and Littell, R.C. (1993) Probability of detecting nematode infestations in quarantine samples. *Nematropica* 23, 177–181.

Minton, E.B., Smith, A.L. and Cairns, E.J. (1964) Population dynamics of 7 nematode species under 10 cotton selections. *Phytopathology* 54, 625.

Montasser, S.A. (1986) Resistance in tomato cultivars to the reniform nematode, *Rotylenchulus reniformis*. *Pakistan Journal of Nematology* 4, 79–82.

Muhammad, N. and Jones, J.E. (1990) Genetics of resistance to reniform nematode in upland cotton. *Crop Science* 30, 13–16.

Nakasono, K. (1978) Effect of soil temperature on development, reproduction and sex ratio of *Rotylenchulus reniformis*: differences between the amphimictic and parthenogenetic populations. *Japanese Journal of Nematology* 8, 32–42.

Nakasono, K. (1983) Studies on morphological and physio-ecological variation of the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, 1940 with an emphasis on differential geographical distribution of amphimictic and parthenogenetic populations in Japan. *Bulletin of the*

National Institute of Agricultural Sciences, Japan 38, 63–67.

Nath, R.P., Swamp, P.G. and Rao, G. (1969) Studies on the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira 1940. *Indian Phytopathology* 22, 99–104.

Nayak, D.K., Routaray, B.N. and Das, S.N. (1987) Relative susceptibility of some horsegram cultivars against reniform nematodes, *Rotylenchulus reniformis*. *International Nematology Network Newsletter* 4, 19–20.

Neal, D.C. (1954) The reniform nematode and its relationship to the incidence of Fusarium wilt of cotton at Baton Rouge, Louisiana. *Phytopathology* 44, 447–450.

Overstreet, C. (1996) The impact of reniform nematode on cotton production in the U.S.A. *Nematropica* 26(3), 216.

Patel, D.S. and Thakar, N.A. (1986) Reaction of some mungbean varieties to the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, 1940. *Indian Journal of Nematology* 15, 239.

Patel, D.J., Desai, M.V. and Patel, D.B. (1986) Susceptibility of tobacco varieties to *Rotylenchulus reniformis*. *Tobacco Research* 12, 202–203.

Patel, B.A., Chavda, J.C., Patel, S.T. and Patel, D.J. (1987) Reaction of some pigeonpea lines to reniform nematode (*Rotylenchulus reniformis*). *International Pigeonpea Newsletter* 6, 57–59.

Patel, B.A., Chavda, J.C. and Patel, D.J. (1989a) Screening of certain greengram lines against reniform nematode, *Rotylenchulus reniformis*. *International Nematology Network Newsletter* 6, 25–26.

Patel, H.V., Patel, D.J. and Patel, B.A. (1989b) Reaction of papaya varieties to *Rotylenchulus reniformis*. *International Nematology Network Newsletter* 6, 24.

Rebois, R.V. (1973) Effect of soil temperature on infectivity and development of *Rotylenchulus reniformis* on resistant and susceptible soybean, *Glycine max*. *Journal of Nematology* 5, 10–13.

Rebois, R.V. and Webb, R.E. (1979) Reniform nematode resistance in potato clones. *American Potato Journal* 56, 313–319.

Rebois, R.V., Johnson, W.C. and Cairns, E.J. (1968) Resistance in soybeans, *Glycine max* L. Merr., to the reniform nematode. *Crop Science* 8, 394–395.

Rebois, R.V., Epps, J.M. and Hartwig, E.E. (1970) Correlation of resistance in soybeans to *Heterodera glycines* and *Rotylenchulus reniformis*. *Phytopathology* 60, 695–700.

Rebois, R.V., Eldridge, B.J., Good, J.M. and Stoner, A.K. (1973) Tomato resistance and susceptibility to the reniform nematode. *Plant Disease Reporter* 57, 169–172.

Rebois, R.V., Madden, P.A. and Eldridge, B.J. (1975) Some ultrastructural changes induced in resistant and susceptible soybean roots following infection by *Rotylenchulus reniformis*. *Journal of Nematology* 7, 122–139.

Rebois, R.V., Steele, A.E., Stoner, A.K. and Eldridge, B.J. (1977) A gene for resistance to *Rotylenchulus reniformis* in tomato, and a possible correlation with resistance to *Heterodera schachtii*. *Journal of Nematology* 9, 280–281.

Robbins, R.T. and Rakes, L. (1996) Resistance to the reniform nematode in selected soybean cultivars and germplasm lines. *Journal of Nematology* 28, 612–615.

Robbins, R.T., Rakes, L. and Elkins, C.R. (1994a) Reniform nematode reproduction and soybean yield of four soybean cultivars in Arkansas. *Journal of Nematology* 26, 656–658.

Robbins, R.T., Rakes, L. and Elkins, C.R. (1994b) Reproduction of the reniform nematode on thirty soybean cultivars. *Journal of Nematology* 26, 659–664.

Robbins, R.T., Rakes, L. and Jackson, L. (1999) Reniform nematode resistance ratings for 288 soybean cultivars to help select rotational crops for reniform infested fields. *Proceedings, Beltwide Cotton Conferences*, January 4–7, 1999, Orlando, Florida.

Robinson, A.F. (1989) Thermotactic adaptation of two foliar and two root-parasitic nematodes. *Revue de Nématologie* 12, 125–131.

Robinson, A.F. (1994) Movement of five nematode species through sand subjected to natural temperature gradient fluctuations. *Journal of Nematology* 26, 46–58.

Robinson, A.F. and Heald, C.M. (1991) Carbon dioxide and temperature gradients in Baermann funnel extraction of *Rotylenchulus reniformis*. *Journal of Nematology* 23, 28–38.

Robinson, A.F. and Heald, C.M. (1993) Movement of *Rotylenchulus reniformis* through sand and agar in response to temperature, and some observations on vertical descent. *Nematologica* 39, 92–103.

Robinson, A.F. and Percival, A.E. (1997) Resistance to *Meloidogyne incognita* Race 3 and *Rotylenchulus reniformis* in wild accessions of *Gossypium hirsutum* and *G. barbadense* from Mexico. *Journal of Nematology* 29, 746–755.

Robinson, A.F., Heald, C.M., Flanagan, S.L., Thames, W.H. and Amador, J. (1987) Geographical distributions of *Rotylenchulus reniformis*, *Meloidogyne incognita*, and *Tylenchulus semipenetrans* in the Lower Rio Grande Valley as related to soil texture and land use. *Journal of Nematology* 1, 20–25.

Robinson, A.F., Inserra, R.N., Caswell-Chen, E.P., Vovlas, N. and Triccoli, A. (1997) *Rotylenchulus* species: identification, distribution, host ranges, and crop plant resistance. *Nematropica* 27, 127–180.

Robinson, A., Bowman, D., Cook, C., Jenkins, J., Jones, J., May, O., Oakley, S., Oliver, M., Robinson, M., Smith, C., Starr, J. and Stewart, J.McD. (1999a) Nematode resistance. In: Kirkpatrick, T. and Rothrock, C. (eds) *Compendium of Cotton Diseases*. American Phytopathological Society, St Paul, Minnesota.

Robinson, A.F., Cook, C.G. and Percival, A.E. (1999b) Resistance to *Rotylenchulus reniformis* and *Meloidogyne incognita* race 3 in the major cotton cultivars planted since 1950. *Crop Science* 39(3), 850–858.

Routaray, B.N., Sahoo, H. and Das, S.N. (1986) Evaluation of greengram and blackgram varieties against reniform nematode, *Rotylenchulus reniformis*. *Indian Journal of Nematology* 16, 27–29.

Routaray, B.N., Sahoo, H. and Das, S.N. (1988) Evaluation of some chilli cultivars against *Meloidogyne incognita* and *Rotylenchulus reniformis*. *Journal of the Indian Botanical Society* 67, 220–221.

Sahoo, H., Routaray, B.N. and Das, S.N. (1986) Evaluation of bengalgram varieties against reniform nematode, *Rotylenchulus reniformis*. *Journal of the Indian Botanical Society* 65, 86–89.

Shepherd, R.L. (1974) Registration of Auburn 623 RNR cotton germplasm (Reg. no. GP20). *Crop Science* 14, 911.

Shepherd, R.L. and Huck, M.G. (1989) Progression of root-knot nematode symptoms and infection on resistant and susceptible cottons. *Journal of Nematology* 21, 235–241.

Sivakumar, C.V. and Seshadri, A.R. (1971) Life history of the reniform nematode *Rotylenchulus reniformis* Linford and Oliveira, 1940. *Indian Journal of Nematology* 14, 148–151.

Starr, J.L. (1991) *Rotylenchulus reniformis* on greenhouse-grown foliage plants: host range and sources of inoculum. *Journal of Nematology* 23, 634–638.

Starr, J.L., Heald, C.M., Robinson, A.F., Smith, R.G. and Krausz, J.P. (1993) *Meloidogyne incognita* and *Rotylenchulus reniformis* and associated soil textures from some cotton production areas of Texas. *Journal of Nematology* 25, 895–899.

Thakar, N.A. and Patel, C.C. (1985) Screening of few cowpea varieties against reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, 1940. *Indian Journal of Nematology* 14, 204.

Thakar, N.A. and Yadav, B.S. (1985) Screening pigeonpeas for their resistance to reniform nematode. *International Pigeonpea Newsletter* 4, 42–43.

Varaprasad, S. (1986) *Rotylenchulus reniformis* Linford and Oliveira, 1940 – a

comprehensive account of systematics, biology and management. In: Swarup, G. and Dasgupta, D.R. (eds) *Plant Parasitic Nematodes of India, Problems and Progress*. Indian Agricultural Research Institute, New Delhi, pp. 194–210.

Williams, C., Gilman, D.F., Fontenot, D.S. and Birchfield, W. (1979) A rapid technique for screening soybean for reniform nematode resistance. *Plant Disease Reporter* 63, 827–829.

Williams, C., Gilman, D.F., Fontenot, D.S. and Birchfield, W. (1981) Inheritance of reaction to the reniform nematode in soybean. *Crop Science* 21, 93–94.

Womersley, C. and Ching, C. (1989) Natural dehydration regimes as a prerequisite for the successful induction of anhydrobiosis in the nematode *Rotylenchulus reniformis*. *Journal of Experimental Biology* 143, 359–372.

Yik, C.P. (1981) Resistant germplasm in *Gossypium* species and related plants to the reniform nematode. Louisiana State University Dissertation, Department of Plant Pathology and Crop Physiology.

Yik, C.P. and Birchfield, W. (1984) Resistant germplasm in *Gossypium* species and related plants to *Rotylenchulus reniformis*. *Journal of Nematology* 16, 146–153.

8

Migratory Endoparasites: *Pratylenchus* and *Radopholus* Species

D. De Waele and A. Elsen

Laboratory of Tropical Crop Improvement, Catholic University Leuven (K.U. Leuven), Kasteelpark Arenberg 13, 3001 Leuven, Belgium

Species of *Pratylenchus* and *Radopholus* are small (adults < 1 mm long) and polyphagous. They migrate inter- and intracellularly in roots, corms or tubers and feed mainly on the cytoplasm of cortical cells. Cell walls collapse and cause cavities and tunnels, which evolve as necrotic lesions that may extend to the whole cortex. The lesions are dark brown, reddish-purple to black, elliptical in shape with the axis parallel to the root axis. The reduction of root tissues reduces the uptake of water and nutrients by the plant. Above-ground symptoms of attack include chlorosis and stunting. In banana and plantain, a weakened root system also affects plant anchorage resulting in plant toppling, especially at bunch filling and when strong storms are prevailing. In nematode-infested fields, losses caused by root-lesion nematodes can be very high.

The most important *Pratylenchus* species are *P. crenatus* Loof, *P. hexincisus* Taylor and Jenkins, *P. neglectus* (Rensch) Filipjev and Schuurmans Stekhoven, *P. penetrans* (Cobb) Filipjev and Schuurmans Stekhoven, *P. scribneri* Steiner, *P. thornei* Sher and Allen and *P. vulnus* Allen and Jensen in temperate zones, subtropics and cooler regions of the tropics, and *P. brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven, *P. coffeae* (Zimmermann) Filipjev and Schuurmans Stekhoven, *P. goodeyi* Sher and Allen and *P. zeae* Graham in the tropics. *Pratylenchus* species seem to occur everywhere where conditions, especially temperature, permit them to thrive. In general, it can be said that their geographical distribution is zonal: when a species is found in a given climatic zone it will occur throughout this zone. The same

 Plant Resistance to Parasitic Nematodes (eds J.L. Starr, R. Cook and J. Bridge)

holds for the tropics. The most important *Radopholus* species is *Radopholus similis* Cobb, the burrowing nematode. Since Valette *et al.* (1998) proposed to consider *R. citrophilus* Huettel, Dickson and Kaplan as a junior synonym of *R. similis*, *R. similis* now also includes the *Radopholus* populations which, mainly in Florida, USA, are able to infect citrus roots. *R. similis* is widespread in tropical and subtropical zones and is also present in glasshouses in Europe. All the other *Radopholus* species only occur in natural habitats in Australasia.

The life cycle of these genera is simple. Eggs are laid in roots, corms or tubers. The first-stage juveniles moult inside the eggs, the second-stage juveniles hatch, moult through the third and fourth stages and become adults. Amphimictic reproduction occurs in species in which males are common (such as *R. similis*, *P. coffeae*, *P. goodeyi*, *P. penetrans* and *P. vulnus*). Other species in which males are rare (such as *P. brachyurus*, *P. neglectus*, *P. thornei* and *P. zeae*) reproduce parthenogenetically. The duration of the life cycle differs between species and is temperature dependent. Tropical species, such as *P. brachyurus* and *P. zeae*, can complete their life cycle in about 3–4 weeks at 30°C (Olowe and Corbett, 1976). Species that prefer cooler climates, such as *P. penetrans*, complete their life cycle in about 6–7 weeks at 20°C (Mamiya, 1971).

Efforts to screen agricultural crop germplasm for resistance to plant-parasitic nematodes have mainly been aimed at identifying resistance to sedentary endoparasitic nematodes, such as root-knot (*Meloidogyne* spp.) and cyst (*Globodera* and *Heterodera* spp.) nematodes. As a consequence, resistance to nematodes has primarily been identified in this group of nematodes which has, moreover, the most specialized host–parasite relationships (Cook and Evans, 1987; Roberts, 1992; De Waele, 1996). Because host–parasite relationships are genetically controlled, the natural selection of resistance genes is more likely to occur in the most complex interactions (Sidhu and Webster, 1981; Roberts, 1992). In contrast, fewer efforts have been made to incorporate resistance into agricultural crops that suffer economic losses caused by *Pratylenchus* and *Radopholus* spp. Because the host–parasite relationships of this group of migratory endoparasitic nematodes are less specialized than the sedentary endoparasitic nematodes, these efforts have also been less successful. Nevertheless, sources of resistance and tolerance to *Radopholus* and *Pratylenchus* species have been and can be found.

Evaluation of agricultural crop germplasm for nematode reproduction and damage is based on the terms ‘resistance and susceptibility’ and ‘tolerance and sensitivity’. Resistance/susceptibility on the one hand and tolerance/sensitivity on the other hand are defined by Bos and Parlevliet (1995) as independent, relative qualities of a host plant based on comparison between genotypes. A host plant may either

suppress (resistance) or allow (susceptibility) nematode development and reproduction; it may suffer either little injury (tolerance), even when quite heavily infected with nematodes, or much injury (sensitivity), even when relatively lightly infected with nematodes. The comparison between genotypes results in such indications as completely, highly and partially resistant genotypes describing, respectively, genotypes supporting no, little or an intermediate level of nematode reproduction. A non-resistant or susceptible genotype allows nematodes to reproduce freely (see Roberts, Chapter 2).

Sources and Genetics

Cereals

Resistance to several *Pratylenchus* species was found in maize (*Zea mays*) and in the teosintes *Z. diploperennis* and *Z. perennis*. The perennial teosintes are closely related to and sometimes considered ancestral to maize. The diploid *Z. diploperennis* crosses readily with maize (Pohl and Albertsen, 1981) and fertile hybrids have been obtained by many breeders. In the glasshouse, *Z. diploperennis* and *Z. perennis* supported significantly fewer *P. hexincisus* than did some common maize lines, although these differences were not confirmed in the field (Norton, 1989). In the USA, improved maize hybrids with resistance to *P. hexincisus* have been registered, including SD101 (PI533.658), SD102 (PI533.659) and SD103 (PI533.660) (Wicks *et al.*, 1990a,b). SD101 is not only resistant to *P. hexincisus* but also to *P. scribneri* and the fungi *Exserohilum turcicum* (*Setosphaera turcica*) and *Diplodia* (*Stenocarpella*) *maydis*. Resistance to *P. brachyurus* and *P. zeae* was also found in maize and differences in susceptibility of maize to *P. penetrans* were observed. Qing-Yu *et al.* (1998) evaluated 13 randomly selected maize cultivars for their ability to support a population of *P. penetrans* in growth chamber and glasshouse studies. The cultivars Earlivee, Seneca Horizon, Lyric, Grant and King Arthur supported the fewest nematodes in both tests. The inheritance of the resistance in maize to *P. zeae* and *P. brachyurus* was studied by Sawazaki *et al.* (1987) using segregating populations obtained from crosses between the lines Col 2(22) (resistant) and Ip 48-5-3 (susceptible) grown in a field naturally infested with *P. zeae* and *P. brachyurus*. Based on the number of nematodes g^{-1} roots at 80 days after planting, Sawazaki *et al.* (1987) concluded that the resistance was due to two dominant genes with an additive effect.

In Australia, sources of resistance or tolerance to *P. thornei* and *P. neglectus* were found and several wheat (*Triticum aestivum*) cultivars with tolerance to one or both nematodes were released.

Sunvale, Baxter, Sturt, Kennedy, Pelsart, Tasman and Houtman are not only tolerant to *P. thornei* but also possess the linked resistant genes against *Puccinia striiformis*, *P. graminis* and *P. recondita*. Sunvale is also moderately tolerant to crown rot (*Gibberella zeae*), Pelsart is resistant to flag smut (*Urocystis agropyri*) and moderately tolerant to *Heterodera avenae*, and Houtman resistant to flag smut, partially resistant to crown rot and common root rot (*Cochliobolus sativus*) (Anon., 1994, 1997a,b,c; Brennan *et al*., 1994a,b,c; Ellison *et al*., 1995). Resistance to *P. thornei* has been found in wild cereal species such as *Aegilops tauschii*. Thompson and Haak (1997) tested 244 accessions of *A. tauschii* from Central Asia for resistance to *P. thornei* in a series of glasshouse experiments. *A. tauschii* is one of the grass-like wild progenitors of wheat and a rich source of resistance to pests and diseases (Cox *et al*., 1992). Of the accessions tested by Thompson and Haak (1997) 39 had fewer *P. thornei* than GS50a, a partially resistant line of wheat used as a reference standard. Resistance was most common in *A. tauschii* subsp. *strangulata*, with 20 out of 40 *strangulata* accessions classed as resistant and none as susceptible. Three out of four accessions of *A. tauschii* var. *meyeri* with the *Cre3* gene for resistance to *H. avenae* were also resistant to *P. thornei*. If *Cre3* confers resistance to *P. thornei*, it will be valuable in breeding wheat for areas where both species occur. All the *A. tauschii* subsp. *strangulata* accessions shown to be resistant to *P. thornei* were susceptible to *H. avenae*. Nombela and Romero (1999) examined the host response to *P. thornei* of the introgression wheat line H93-8, bearer of the *Cre2* gene conferring resistance to *H. avenae*. In the growth chamber, line H93-8 was resistant to *P. thornei* but in a 5-month field experiment this line was as susceptible to *P. thornei* as were susceptible reference standards included in the experiment. Eighteen wheat cultivars varying in reaction to *P. thornei*, substitution lines and lines with the whole genome of rye (including the triticales) were screened in the glasshouse for their resistance to *P. neglectus* by Farsi *et al*. (1995). Significant differences in the number of nematodes per plant and per g dry root between the three main groups were observed. The triticale lines Abacus and Muir had the fewest nematodes and triticales (hybrids between wheat and rye) are therefore considered a useful rotation crop for fields infested with *P. neglectus*. None of the wheat cultivars varying in reaction to *P. thornei* was resistant indicating that the genetic mechanisms conferring resistance or tolerance to *P. thornei* are not effective against *P. neglectus*. Vanstone *et al*. (1998) ranked nine wheat cultivars for their susceptibility to *P. thornei* and *P. neglectus*. The tolerant variety Excalibur yielded 33% (*P. thornei*) and 19–23% (*P. neglectus*) more and had 63–69% fewer nematodes than the intolerant cultivars included in the field experiments. In contrast to Farsi *et al*. (1995), the varietal reactions were unexpectedly similar for the two *Pratylenchus*

species. Nevertheless, Vanstone *et al*. (1998) considered it doubtful that resistance or tolerance to one *Pratylenchus* species necessarily confers resistance or tolerance to the other species and unlikely that this similarity would occur with all wheat cultivars. Growth of tolerant cultivars that are also resistant enhances not only yield but also reduces the need for growers to implement other nematode management strategies.

In rice (*Oryza sativa*), tolerance to *P. zeae* linked to drought-avoidance strategies, such as deep roots, might be present (Plowright *et al.*, 1990).

Townshend (1989) examined the host response of two oat (*Avena sativa*) cultivars, Saia and OAC Woodstock, to *P. neglectus*, *P. crenatus*, *P. penetrans* and *P. sensillatus* in the glasshouse. Both cultivars were non-hosts for *P. crenatus* and susceptible to *P. sensillatus*. Saia was less susceptible to *P. neglectus* and *P. penetrans* than OAC Woodstock.

In 1990, Sato *et al*. registered Natsukaze, a guineagrass cultivar with resistance not only to *P. brachyurus* but also to several *Meloidogyne* spp. This cultivar was derived from clones of *Panicum maximum*.

Root and tuber crops

A detailed search for resistance to *P. penetrans* among diverse potato (*Solanum tuberosum*) germplasm was undertaken when Brodie and Plaisted (1993) evaluated potato clones from five different breeding populations for their resistance to *P. penetrans*. Brodie and Plaisted (1993) found that clones that supported the least *P. penetrans* were from a breeding population derived from *S. tuberosum* ssp. *andigena* crossed to a *S. tuberosum* ssp. *tuberosum* hybrid that contained some *S. vernei* germplasm originally selected for its resistance to the potato cyst nematode *Globodera pallida*. Most, but not all, of the clones that were less susceptible to *P. penetrans* were also resistant to *G. pallida* (P_4A and P_5A) and to *G. rostochiensis* (Ro1). Although the genetics of resistance to *P. penetrans* was not investigated, the variation in the different levels of resistance exhibited between experiments suggest that this response is quantitatively inherited and not controlled by a single major gene. As such, this resistance is subject to genetic and environmental interactions. Such interactions could account for the observed relatively large variation in the number of nematodes per root unit of resistant plants in the different tests. Previously, less detailed studies had evaluated commercial potato cultivars for their ability to support reproduction of *P. penetrans* (Bernard and Laughlin, 1976; Olthof, 1986). The potato cultivars Peconic and Hudson were initially described as less susceptible to *P. penetrans* but this could not be confirmed (Brodie and Plaisted, 1993). According to Brodie and

Plaisted (1993), the ability of Hudson to support different amounts of reproduction of geographically isolated populations of *P. penetrans* suggested the existence of biological races of this nematode species. The potato cultivar Butte was reported to be highly resistant to *P. neglectus* and to possess some resistance to *P. penetrans* (Davis *et al.*, 1992).

In Japan, screening sweet potato (*Ipomoea batatas*) germplasm for resistance to *P. coffeae* has resulted in the release of the resistant cultivars Fusabeni, Joy White, J-Red and Sunny Red (Tarumoto *et al.*, 1990; Yamakawa *et al.*, 1995, 1998, 1999). Interestingly, these four cultivars are also resistant to *M. incognita*. Moreover, Fusabeni is moderately resistant to soil and stem rot, caused, respectively, by *Streptomyces ipomoea* and *Fusarium oxysporum*, whereas Joy White is moderately resistant to *Ceratocystis fimbriata*. In glasshouse trials, *P. flakkensis* was unable to reproduce on all 20 sweet potato cultivars tested (Anguiz and Canto-Saenz, 1991).

*Banana and plantain (*Musa *spp.)*

In *Musa*, two widely confirmed sources of resistance to *R. similis* are known: Pisang Jari Buaya and Yangambi km5 (Wehunt *et al.*, 1978; Pinochet and Rowe, 1979; Sarah *et al.*, 1992; Price, 1994b; Viaene *et al.*, 1997; Fogain and Gowen, 1998; Stoffelen *et al.*, 2000a,b). The Pisang Jari Buaya group consists of diploid AA genotypes of which several cultivars showed no lesions when planted in *R. similis*-infested soil (Wehunt *et al.*, 1978). The use of Pisang Jari Buaya in the *Musa* breeding programme of the Fundación Hondureña de Investigación Agrícola (FHIA) in La Lima, Honduras, resulted in the *R. similis*-resistant diploid AA hybrid SH-3142 (Pinochet and Rowe, 1979). Crossing SH-3142 with the triploid AAB cultivar Prata Aña produced the tetraploid AAAB hybrid FHIA-01 (Goldfinger; Rowe and Rosales, 1993). FHIA-01 was partially resistant to *R. similis* when 3–4-month-old plants grown from corms were evaluated, but was as susceptible as the reference standards when plants of the same age grown from *in vitro* maintained tissue culture plants were used as the source of planting material (Viaene *et al.*, 1998). Yangambi km5 is a triploid AAA genotype collected in the Democratic Republic of Congo and is possibly related to some Malaysian genotypes. Although male and female fertile, this genotype is not being used in *Musa* breeding because all progenies produce abnormal leaves and/or erect and semi-erect bunches. In addition to these two widely confirmed sources of resistance to *R. similis*, several other possible sources of resistance have been reported but these need to be confirmed. In Cameroon, three diploids from the wild

Musa balbisiana (BB-) group were found to be as resistant to *R. similis* as Yangambi km5 in glasshouse trials whereas three triploids from the AAB-group, Pisang Kelat, Foconah (Pome group) and Pisang Celan (Mysore group), were less susceptible to *R. similis* (Fogain, 1996). The lesser susceptibility of the latter three genotypes was also observed in field trials (Price and McLaren, 1996). In Nigeria, PITA-8, a tetraploid (AAAB) plantain hybrid resistant to the black Sigatoka disease caused by the fungus *Mycosphaerella fijiensis*, appeared not to be infected with *R. similis* during field trials (Afreh-Nuamah *et al.*, 1996). In glasshouse experiments in Belgium, evaluation of the host plant reaction to *R. similis* of 25 banana cultivars of the section *Eumusa* (AA-group) and seven of the section *Australimusa* (Fe'i-group) collected in Papua New Guinea revealed the Fe'i cultivar Rimina to be resistant to *R. similis* and the Fe'i cultivar Menei to be a possible source of resistance to *R. similis* (Stoffelen *et al.*, 1999b, 2000b). Although Fe'i bananas are highly seed- and pollen-sterile and have a rather low harvest index and erect bunch orientation, their resistance to *R. similis* warrants investigation of their combining ability with *Eumusa* bananas. Gros Michel, a triploid AAA cultivar, has been reported as less susceptible to *R. similis* in some studies (Mateille, 1992; Price, 1994b) but this host reaction was not confirmed (Stoffelen *et al.*, 2000a).

Several histopathological differences were observed between *R. similis*-resistant and -susceptible *Musa* genotypes. In Gros Michel, movement of the nematodes and the development of necrosis in the outer cortex along the root axis seemed to be slowed down compared with migration and necrosis formation in the susceptible cultivar Poyo (Mateille, 1994). In Yangambi km5, *R. similis* was only observed in the cortex and not in the stele as in Poyo (Valette *et al.*, 1997). The genetic basis of the resistance to *R. similis* in *Musa* has not been established. Preliminary tests demonstrated that the genetic resistance to *R. similis* in Pisang Jari Buaya is controlled by one or more dominant genes (Pinochet, 1988a).

Yangambi km5 is not only resistant to *R. similis*, it has also been reported as partially resistant to *P. goodeyi* (Fogain and Gowen, 1998; Pinochet *et al.*, 1998). Based on field trials, both *M. acuminata* and *M. balbisiana* were less susceptible to *P. goodeyi* and so was the cooking banana Banane Cochon (AAA-group) of the Lujugira subgroup (Price, 1994a). In the Kagera Region, Tanzania, replacement of local cooking and beer bananas with exotic cultivars like Gros Michel, Pisang Awak (ABB-group) and Kanana (AB-group) which are less susceptible to *P. goodeyi*, the major nematode associated with banana in that region, suggests, according to Speijer and Bosch (1996), that farmers, unconsciously, selected these genotypes because of the presence of the nematode.

*Lucerne (*Medicago sativa*)*

In lucerne, differences between genotypes in host reaction to *P. penetrans* have been reported (Townshend and Baenziger, 1977; Nelson *et al*., 1985; Christie and Townshend, 1992). Within genotypes, individual plants may differ for many inherited characteristics, including nematode resistance (Thies *et al*., 1994). The cross-pollinated, tetraploid-inheritance characteristics of lucerne contribute to the existence of this type of variability. In 1989, two lucerne genotypes, MNGRN-2 and MNGRN-4, with tolerance to *P. penetrans* were released in the USA (Barnes *et al*., 1990). Both genotypes showed superior performance in fields infested with large populations of *P. penetrans*. Laboratory and field studies showed that they supported about 20–30% fewer *P. penetrans* per g fresh root weight than did the susceptible cultivar Baker. The tolerant genotypes had many fibrous roots even in the presence of nematodes. MNGRN-2 and MNGRN-4 also showed some resistance to *Clavibacter michiganensis* subsp. *insidiosus*, *Fusarium oxysporum* f. sp. *medicaginis* and *Phytophthora megasperma* f. sp. *medicaginis*. The inheritance of the resistance to *P. penetrans* in MNGRN-4 was studied using a diallel mating design (Thies *et al*., 1994). The high correlation between parental clone means and S_1 progeny means for numbers of nematodes in the roots indicated that resistance to *P. penetrans* is conditioned by additive gene action.

*Rootstocks (*Prunus *spp.,* Rosa *spp.,* Citrus *spp.)*

In *Prunus*, resistance and tolerance to *P. penetrans* has been found in seedlings of the peach (*Prunus persica*) rootstock cultivars Bailey, BY520–8, Chui Lum Tao, Guardian, Higama, Rubira, Pisa, Rutgers Red Leaf, Tzim Pee Tao and in hybrids of Rutgers Red Leaf × Tzim Pee Tao (Potter *et al*., 1984; Layne, 1987; McFadden-Smith *et al*., 1998). In contrast, the search for resistance to *P. vulnus* has been less successful. Efforts to find resistance to this nematode in the USA (Culver *et al*., 1989; Ledbetter and Shonnard, 1991; Ledbetter, 1994), France (Scotto La Massese, 1975; Crossa-Raynaud and Audergon, 1987; Stalin *et al*., 1994) and Spain (Marull and Pinochet, 1991; Pinochet *et al*., 1996) have resulted in the detection of potential sources of resistance to *P. vulnus* in Bokhara and Shalil peach seedlings (Okie, 1987) and in a few wild and hybrid plums (*Prunus domestica*), apricots (*Prunus armeniaca*) and interspecific hybrids (Scotto La Massese, 1975; Ledbetter, 1994; Pinochet *et al*., 1996). In France and Spain, resistance to root-knot nematodes, the most common group of nematodes associated with stone fruit production in the Mediterranean, has been incorporated into new *Prunus* rootstocks. In most instances, *Prunus*

rootstocks which were resistant to one or several *Meloidogyne* species were not resistant to *P. vulnus* (Marull and Pinochet, 1991; Stalin *et al.*, 1998). Exceptions are the plum hybrid Bruce, one of the few rootstocks that exhibit resistance to both *Meloidogyne incognita* and *P. vulnus* (Pinochet *et al.*, 1996). Based on a glasshouse evaluation of five cultivars, almond (*Prunus amygdalus*) was considered a poor host of *P. neglectus* and a non-host of *P. thornei* (Marull *et al.*, 1990).

In *Rosa*, resistance to *P. vulnus* has been incorporated in Ludiek, a *Rosa multiflora* rootstock cultivar (Schneider *et al.*, 1995).

In *Citrus*, the first rootstocks resistant to *R. similis* populations able to infect citrus roots were released in 1964 (Cook and Evans, 1987). Resistance was identified in only 15 out of 1400 clones screened. Milam (a selection of rough lemon *C. limon*), Ridge pineapple and Algerian navel (sweet oranges *C. sinensis*) are resistant; Estes rough lemon (*C. jambhiri*) is tolerant but susceptible whereas Carrizo (*C. sinensis* × *Poncirus trifoliata*) has some resistance and appears to be tolerant. *Balsamocitrus dawaii* is another source of resistance to these *R. similis* populations (Kaplan, 1990).

*Strawberry (*Fragaria *spp.) and raspberry (*Rubus *spp.)*

In strawberry (*Fragaria* × *ananassa*), variation in cultivar responses to *P. penetrans* has been reported. Cultivars which are less susceptible to *P. penetrans* include Guardian, Redchief, Senga Sengana and Micmac (Dale and Potter, 1998). Cultivars related to the Lassen family group from California appeared to be the most resistant suggesting that, within the North American breeding programmes, the Californian breeders had inadvertently selected for resistance to *P. penetrans* whereas the breeders in other parts of North America had not (Dale and Potter, 1998). The continuous nature of the variation in response, the relatively distinct family grouping for resistance and the demonstration by Potter and Dale (1994) that intraspecific crossing of a susceptible (Midway) and moderately resistant (Guardian) parent produced offspring some of which were as resistant as Guardian, suggest that the resistance of strawberry to *P. penetrans* can be improved by breeding. Variation in resistance and tolerance to *P. penetrans* was found in wild *Fragaria* spp.: beach strawberry (*F. chiloensis*) and woodland strawberry (*F. virginiana*) (Potter and Dale, 1994).

Resistance to *P. penetrans* was also identified in red raspberry (*Rubus* spp.; Bristow *et al.*, 1980; Vrain and Daubeny, 1986). The inheritance of the resistance was studied in a four-member half diallel crossing involving two resistant genotypes, Nootka and Dalhouse Lake, a North American red raspberry (*Rubus strigosus*) selection and two susceptible genotypes (Vrain *et al.*, 1994). Bimodality of the

distribution of each variable was not found, so the inheritance of resistance was assumed to be quantitative. Estimates of the general combining abilities for top weight and root weight suggested that additive gene action is involved in the tolerance observed and that either or both measures could be used for selection for nematode tolerance in raspberry breeding programmes. Thus, parents could be chosen based on phenotypic performance.

Other plants

Resistance to *R. similis* populations able to infect citrus was found in *Anthurium* (Wang *et al.*, 1997). In India, resistance and tolerance to *R. similis* was observed in arecanut (*Areca catechu*) and coconut (*Cocos nucifera*) (Sosamma *et al.*, 1988; Sundararaju and Koshy, 1988). Resistance or tolerance to *P. brachyurus* was reported in Barbados cherry (*Malpighia glabra*) (Ferraz *et al.*, 1989), soybean (*Glycine max*) (Ferraz, 1996), groundnut (Smith *et al.*, 1978), sugarcane (*Saccharum officinarum*) (Dinardo-Miranda and Ferraz, 1991) and coffee (*Coffea* spp.) germplasm (Oliveira *et al.*, 1999). Interestingly, in several coffee genotypes an intolerant reaction to *P. brachyurus* was observed in which very few nematodes caused a lot of damage to coffee seedlings (Inomoto *et al.*, 1998). Several *Crotalaria* spp. were poor hosts for *P. brachyurus* (Da Silva *et al.*, 1989). Resistance to *P. coffeae* was reported in Robusta coffee (*Coffea canephora*) (Wiryadiputra, 1996). Toruan-Mathius *et al.* (1995) examined the anatomy and total polyphenol content of the roots and the polymorphism of root proteins and genomic DNA of six Robusta coffee clones that were either susceptible, moderately resistant or resistant to *P. coffeae*. Resistant clones had hairy roots, thicker cell walls in the root epidermis and endodermis and higher polyphenol contents. A specific protein marker of molecular weight 29 kDa was found in the resistant clones, indicating that resistant clones had specific enzymes as products of DNA associated with resistance. Resistance to *P. scribneri* was found in lima bean (*Phaseolus lunatus*) (Rich *et al.*, 1977). Tolerance to *P. penetrans* was observed in grapevine (*Vitis* spp.) cultivars (Ramsdell *et al.*, 1996). Resistance to *P. sefaensis* was reported in cowpea (*Vigna unguiculata*) (Sarr and Baujard, 1988). Resistance or tolerance to *P. thornei* was found in *Cicer arietinum*, *Cicer bijugum*, *Cicer cuneatum*, *Cicer judaicum* and *Cicer yamashitae* (Tiwari *et al.*, 1992; Simeone *et al.*, 1995; Castillo *et al.*, 1998). Resistance to *P. vulnus* was observed in kiwi fruit (*Actinidia chinensis*) (Simeone *et al.*, 1995). Resistance or tolerance to *P. zeae* was reported in sugarcane (Novaretti *et al.*, 1988; Dinardo-Miranda and Ferraz, 1991; Mehta *et al.*, 1994; Dinardo-Miranda *et al.*, 1996). Sunflower (*Helianthus annuus*) hybrids and

several *Crotalaria* spp. were poor hosts for *P. zeae* (Bolton and De Waele, 1989; Da Silva *et al.*, 1989). In winter rapeseed (*Brassica napus* ssp. *oleifera*), resistance and (or) tolerance to *P. scribneri*, *P. neglectus*, *P. fallax*, *P. crenatus*, *P. penetrans* and *P. pinguicaudatus* has been observed (Bernard and Montgomery-Dee, 1993; Webb, 1996).

Identification

It is essential that the nematode populations used in screening are identified accurately at the species level. *Radopholus similis* can be recognized relatively easily by light microscopy but identification of *Pratylenchus* species is considerably more difficult. The general morphology of all *Pratylenchus* species is uniform. There are only a restricted number of characters that have taxonomical value and, without exception, these show large intraspecific variation. Even species that have been well decribed present difficulties. Diagnostics based on biochemical (isozyme phenotypes) or genetic (DNA sequences) differences are not available so that morphological characters and morphometrics are still being used for species identification.

R. similis is recognized by the combination of the following morphological characters: sexual dimorphism in the anterior region (in females the head region is low, hemispherical, continuous or slightly offset with strong cephalic sclerotization and stylet; in males the head region is high, often knob-like, more offset with weak cephalic sclerotization and degenerated stylet); the rather rare occurrence of males; median position of the vulva (at about 50–60% of body length); presence of two equally developed genital branches; female tail shape somewhat elongate-conoid but with a rounded or indented terminus; male tail elongate, conoid, ventrally arcuate with bursa extending over two-thirds of tail length. A full description of *R. similis* can be found in Orton Williams and Siddiqi (1973).

Taxonomic keys to the species of the genus *Pratylenchus* have been published by Loof (1978), Café Filho and Huang (1989) and Handoo and Golden (1989). *P. brachyurus* can be found in Corbett (1976), *P. coffeae* in Siddiqi (1972), *P. goodeyi* in Machon and Hunt (1985), *P. neglectus* in Townshend and Anderson (1976), *P. penetrans* in Corbett (1973), *P. thornei* in Fortuner (1977), *P. vulnus* in Corbett (1974) and *P. zeae* in Fortuner (1976).

To mount whole specimens suitable for light microscopy, good results may be obtained when the nematodes are quickly killed and fixed immediately in hot 4% formaldehyde (after Seinhorst, 1966), transferred to glycerol by the ethanol–glycerol method (after Seinhorst, 1959) and mounted on glass slides with the wax-ring method (after De Maeseneer and D'Herde, 1963). Unmounted specimens can be

preserved in 2–4% formaldehyde for shipping to laboratories with taxonomic expertise.

Screening: General Considerations

During screening for resistance or tolerance to *Pratylenchus* and *Radopholus* spp., nematologists are confronted with several problems: (i) the existence of differences in reproductive fitness and pathogenicity among populations of *R. similis* and among populations of the same *Pratylenchus* species; (ii) differences in host response and nematode reproduction between experiments; (iii) the lack of information concerning the effect of root development on host response and nematode reproduction.

Intraspecific differences in reproductive fitness and pathogenicity between Pratylenchus *and* Radopholus *populations*

In *R. similis*, biological diversity among populations was first demonstrated by studies based on morphology, cytogenetics, host range, reproductive and damage potential (reviewed by Pinochet, 1988b). Examination of *R. similis* populations from the major banana-growing areas of the world has revealed a large variability in pathogenicity to banana and plantain, maize and other plants (Pinochet, 1979; Tarte *et al.*, 1981; Sarah *et al.*, 1993; Fallas and Sarah, 1995; Fallas *et al.*, 1995; Fogain and Gowen, 1995; Hahn *et al.*, 1996). A direct relationship was found between the reproductive fitness (multiplication rate) on carrot discs of populations and their pathogenicity (induced damage) on banana roots: the higher the reproductive fitness on carrot discs, the greater the pathogenicity on banana roots (Sarah *et al.*, 1993; Fallas *et al.*, 1995). However, high reproductive fitness is not necessarily related to high pathogenicity (Tarte *et al.*, 1981; Hahn *et al.*, 1996). Usually, the reproductive fitness of *R. similis* and *Pratylenchus* spp. is compared at a fixed time after inoculation. However, at that moment only one measurement related to nematode reproduction is available. A more comprehensive characterization of the reproduction (maximum growth rate, presence of a lag phase and the commencement of the stationary growth phase) was obtained by Stoffelen *et al.* (1999a) who studied the dynamics of the reproduction using the Gompertz model. A high maximum growth rate and an early stationary phase were characteristic of populations with a high reproductive fitness. Molecular techniques such as isozyme patterns, RFLP and RAPD have also been used to further study biological diversity among *R. similis* populations (Hahn *et al.*, 1994, 1996; Fallas *et al.*, 1996). These molecular studies

have shown a high degree of genetic similarity among *R. similis* populations from different areas of the world. In cluster analysis of random amplified polymerase DNA (RAPD) profiles, two separate clusters were found (Fallas *et al.*, 1996). The two genomic groups are being spread independently and no clear relationship is present between molecular and biological diversity. Apparently, reproductive fitness and pathogenicity evolved independently but similarly in both genomic groups under the influence of local environmental conditions.

Initially, *R. similis* populations that were able to infect citrus in Florida were considered morphologically indistinguishable from those that attacked banana worldwide and thus were considered the citrus race of *R. similis* (Ducharme and Birchfield, 1956). Based on differences in karyotype, isozymes, proteins and sexual behaviour (Huettel and Dickson, 1981; Huettel *et al.*, 1982, 1983a,b, 1984a), the sibling species *R. similis* and *R. citrophilus* were established from the banana and citrus races of *R. similis*, respectively (Huettel *et al.*, 1984b). In 1988, minor morphological differences in the tail regions of *R. similis* and *R. citrophilus* males were described by Huettel and Yaegashi (1988), who claimed that both species could be differentiated based on these minute external characters. However, recent studies have shown that populations of *R. similis* from banana and citrus are pheno- and genotypically very similar (Hahn *et al.*, 1996; Kaplan, 1999). Citrus parasitism in Florida appeared to be associated with limited changes in the genome (Kaplan, 1994; Kaplan *et al.*, 1996, 1997; Kaplan and Opperman, 1997). In addition, the inheritance of a specific marker and ability to parasitize citrus by reproductively viable progeny derived from matings of selected nematode populations suggested that gene-flow is not restricted between the populations infecting banana and citrus (Kaplan *et al.*, 1997). When Valette *et al.* (1998) also observed that the minor morphological differences in the tail region of *R. similis* and *R. citrophilus* males described by Huettel and Yaegashi (1988) all showed variation overlapping the differences between the two species described by these authors, *R. citrophilus* was proposed as a junior synonym of *R. similis*, the citrus and banana races representing different pathotypes.

In *Pratylenchus*, biological diversity among populations of the same species has been reported in *P. brachyurus* (Payan and Dickson, 1990), *P. coffeae* (Wehunt and Edwards in Stover, 1972; Mizukubo, 1995; Bridge *et al.*, 1997; Waeyenberghe *et al.*, 2000), *P. goodeyi* (Pinochet, 1998c), *P. loosi* (Waeyenberghe *et al.*, 2000), *P. neglectus* (Griffin, 1991; Hafez *et al.*, 1999), *P. penetrans* (Olthof, 1968; Griffin and Gray, 1990; Griffin, 1991; Brodie and Plaisted, 1993; France and Brodie, 1995, 1996; Hafez *et al.*, 1999) and *P. vulnus* (Pinochet *et al.*, 1992, 1993, 1994). In *P. coffeae*, the taxonomic status of populations designated as *P. coffeae* is currently under scrutiny since studies have

shown that *P. coffeae* most likely represents a species complex (Mizukubo, 1992; Duncan *et al.*, 1999; Waeyenberghe *et al.*, 2000). Therefore, it is possible that the biological diversity observed among *P. coffeae* populations represents in fact differences among species.

The variability in reproductive fitness (and thus pathogenicity) among both *R. similis* and *Pratylenchus* populations belonging to the same species can influence the interpretation of screening experiments for resistance and tolerance. Therefore, the reproductive fitness of the population used in the screening experiments should be determined, eventually compared with other populations, and the same population should be used in all screening experiments. By preference a population with a high reproductive fitness should be used. The existence of differences in reproductive fitness between root-lesion nematode populations from the field also means that, ultimately, promising plant genotypes should be evaluated on their host response using a mixture of populations from different geographical regions (Alcañiz *et al.*, 1996).

Differences in host response and nematode reproduction between experiments

When screening for resistance to *R. similis* and *Pratylenchus* species, it is not uncommon to observe differences in host response and nematode reproduction between glasshouse and field experiments, and even between a series of successive experiments conducted under similar, controlled conditions. In some instances, resistance to *Pratylenchus* spp. has been observed in the glasshouse but not in the field or *vice versa* (see e.g. Norton, 1989; Nombela and Romero, 1999). Differences in nematode reproduction can be high as shown by numbers of *R. similis* and *P. thornei* extracted from the roots of different *Musa* and wheat genotypes, respectively, in glasshouse experiments carried out almost simultaneously (*Musa*) or successively (wheat), under similar, controlled conditions. On the same cultivar (Cavendish 901), the final numbers of *R. similis* averaged 22,347, 732 and 27,201 in three tests (Stoffelen *et al.*, 2000b). On three wheat cultivars (GS50a, Gatcher and Potam), the final numbers of *P. thornei* per plant were 10,085 and 33,390, 36,650 and 95,680, and 37,750 and 128,665, respectively, in two experiments (Thompson and Haak, 1997). Such differences may arise from differences in the abiotic and biotic environmental conditions, in the developmental stage of the plants, and in infectivity of the nematode inoculum. Even in a glasshouse, environmental conditions may fluctuate enough to influence the outcome of successive experiments (Stoffelen, 2000). It is known that temperature, soil moisture,

soil texture and other edaphic factors, greatly influence hatching, penetration and development of *Pratylenchus* spp. (see e.g. Florini *et al.*, 1987; Castillo *et al.*, 1996; Mizukubo and Adachi, 1997). Favourable conditions in small pots can alter the apparent reproductive rate of *Pratylenchus* species as discussed by Farsi *et al.* (1995). Abiotic and biotic environmental conditions not only influence the nematodes but also plant development. The occurrence of these differences in host response and nematode reproduction underlines the importance of including the same susceptible reference cultivar in each experiment, plus, if available, a resistant (or less susceptible) reference cultivar. The reproduction of nematodes on the genotypes to be screened can then not only be compared with the nematode reproduction on the reference cultivars, but also comparison of the results obtained from different batches or experiments is then possible. Furthermore, resistance to *Pratylenchus* spp. should not only be assessed in glasshouse experiments but also under field conditions examining plants in the same developmental stage.

Effect of root development and root system structure on host response and nematode reproduction

Pratylenchus and *Radopholus* spp. penetrate, feed, develop and multiply in roots as well as migrating within and between roots. Root development will influence this dynamic process and thus nematode reproduction, on which resistance assessment is based. Root development may also explain tolerance. In *Musa*, differences in both reproduction of *R. similis* and root damage (number of functional roots, percentage of dead roots and necrosis) on 2-month-old sword sucker-derived plants and on sword suckers of established mats were observed during early field screening suggesting a different host response to nematode infection of young and old root systems (Speijer *et al.*, 1999). Also in *Musa*, differences were observed during early glasshouse screening for reaction to both *R. similis* and *P. coffeae* between *in vitro* propagated plants and plants derived from corms of the same genotype (Viaene *et al.*, 1998). In spite of its importance, the complex interaction between nematodes and root development has seldom been studied. Stoffelen (2000) examined root development and root systems of several *Musa* genotypes in order to optimize early nematode resistance and tolerance screening. To reduce the effect of root growth on nematode reproduction, she recommended that nematode inoculation should be postponed until the second flush of primary root emergence which in Grande Naine is at about 8 weeks after planting of *in vitro* propagated plants.

Screening: Protocols

Nematode inoculum

Carrot discs (*Daucus carota* L.) are widely used for rearing root-lesion nematodes (O'Bannon and Taylor, 1968; Moody *et al.*, 1973; Verdejo-Lucas and Pinochet, 1992; Fallas and Sarah, 1994; Pinochet *et al.*, 1995). Cultures of *R. similis* and *Pratylenchus* species can be established from a single gravid female. The initiation of *in vitro* carrot disc cultures of nematodes requires four steps: (i) extraction of the nematodes from infected roots; (ii) sterilization of the nematodes; (iii) surface-sterilization of the carrot discs; (iv) inoculation of the carrot discs with the nematodes.

Protocol 1: culturing root-lesion nematodes on carrot discs

1. EXTRACTION OF NEMATODES FROM INFECTED ROOTS. Juveniles and adults of migratory endoparasitic nematodes can be extracted from roots by several methods. Two of these methods, the maceration–Baermann funnel and the maceration–sieving method are described below. In contrast to the centrifugal-flotation method, these two methods require little equipment. A description of the maceration–sieving technique can be found in Coolen and D'Herde (1972). Other methods are the use of mist chambers and incubation with aeration or agitation (Seinhorst, 1950; Young, 1954).

Maceration–Baermann funnel method. Wash the roots with tap water and cut them in 1 cm pieces. Put the root pieces in a kitchen-type blender with distilled water and blend three times for a total of 10 s with a short pause between cycles (duration of blending depends on the root type). Pour the blended suspension through a 40 µm aperture sieve and rinse the residue on the sieve with tap water. Collect the mixture of blended roots and nematodes from the sieve with distilled water in a beaker and put it on a Baermann funnel/dish (a 1 mm aperture sieve covered with tissue paper placed in a funnel or in a dish with distilled water). After an incubation period of 12–24 h, collect the nematodes at the base of the funnel or on the bottom of the dish in a beaker. Pour the suspension with the nematodes through a 25 µm aperture sieve and rinse the residue on the sieve with tap water (to eliminate bacteria, etc.). Finally, collect the nematodes on the sieve with distilled water in a beaker. Clean the blender, sieves and Baermann funnel/dish first with ethanol (to kill remaining nematodes) and then with soap and hot water.

Maceration–sieving method. Wash the roots with tap water and cut them in 1 cm pieces. Pour the root sample and 100 ml distilled water

into the blender. Macerate the root sample in distilled water for 30 s (three 10 s periods separated by 5 s intervals). Depending on the type of roots, macerate for a longer or shorter period. Pour the suspension of nematodes and root debris through nested 250, 106 and 40 µm aperture sieves and rinse with tap water (to eliminate bacteria, etc.). Collect the nematodes from the 40 µm sieve with distilled water in a beaker.

2. STERILIZATION OF ROOT-LESION NEMATODES (SEE DE WAELE, CHAPTER 6, FOR OTHER METHODS OF STERILIZATION).

Under laminar flow

Step A: selection of living nematodes from the suspension. Pour the nematodes in a counting dish and select the preferred nematodes. For a very dirty nematode suspension, the nematodes are hand-picked using a very thin needle. For a clean nematode suspension, the nematodes are aspirated with a micropipette, previously heated in a flame. The selected nematodes are then transferred to sterile water in a sterile Petri dish.

Step B: sterilization with $HgCl_2$. Start with a sterile Petri dish with nematodes in sterile water. Transfer the nematodes with a sterile pipette to a small sterile 20 µm aperture sieve and put the sieve with the nematodes in 0.01% $HgCl_2$ for 2 min. Rinse the sieve with the nematodes twice with sterile water. Finally, place the sieve with nematodes in sterile water and transfer the nematodes with a sterile pipette to sterile water in a sterile test tube

Step C: sterilization with streptomycin sulphate. Start with a sterile test tube with nematodes in sterile water (2 ml). Add 1 ml of 6000 µg ml^{-1} streptomycin sulphate with a sterile pipette. After an incubation period of 12 h, remove the streptomycin sulphate supernatant from the nematode pellet with a sterile pipette and add fresh sterile water. Wait until the nematodes have settled to the bottom of the tube and remove the supernatant again. Repeat two or three times and prepare new streptomycin sulphate each time.

3. SURFACE-STERILIZATION OF CARROT DISCS. Use thick carrots (e.g. cultivar Nantes) and fresh carrots with foliage. Be careful to use plastic tissue culture or glass Petri dishes so that nematodes do not adhere to dish walls. Cut off the foliage and wash the carrots with tap water and dry with tissue paper.

Under laminar flow. Dip into or spray the carrot with 95% ethanol and flame until the ethanol is burned off and epidermal tissues are blackened. Then, using a flame-sterilized vegetable peeler, peel several layers of epidermal tissue. Be sure to resterilize the peeler between

each layer of tissue. Cut the carrot in discs and put one or two discs in each Petri dish. Seal the Petri dishes with Parafilm and place the Petri dishes in a plastic box, to protect against mites, in an incubator at 25°C.

4. INOCULATION OF CARROT DISCS WITH NEMATODES.

Under laminar flow. Use the carrot discs immediately after preparation (before bacteria and fungi can develop). Be careful to use discs of different carrots for inoculation of a given nematode population (this spreads the risk of contamination due to bacteria and fungi in the carrot). To obtain high nematode densities, use > 75 nematodes as initial inoculum. For culture maintenance, use 20–50 nematodes. Transfer with a sterile pipette one drop of the sterilized nematodes from the sterilized test tube to a sterile Petri dish and aspirate females (especially thick, gravid females) with the micropipette from the drop on the sterile Petri dish. Be careful to inoculate the nematodes on the margin of the carrot disc, not in the middle. After the inoculation, seal the Petri dishes with Parafilm and incubate in a plastic box at 25°C.

The root-lesion nematodes can be extracted from the carrot discs either to start a new *in vitro* carrot disc culture following sterilization (with streptomycin sulphate, as described above) or as inoculum for screening experiments, for instance in pots in the glasshouse.

Loss of infectivity of *R. similis* and *Pratylenchus* species cultured on carrot discs has not been reported. The populations seem to maintain their reproductive fitness.

Protocol 2: Extraction of root-lesion nematodes from carrot discs

Use only carrot disc cultures where you can see many nematodes on the Petri dish and/or on the carrot disc. To remove nematodes from the dish, rinse the Petri dish with distilled water and pour the water through a 25 µm aperture sieve. Rinse the nematodes on the sieve with tap water (to eliminate bacteria, etc.) and collect the nematodes on the sieve with distilled water in a beaker. To collect the nematodes on or in the carrot disc use any of the extraction techniques as described above (see Protocol 1) to extract the nematodes from the carrot discs. Both fractions can be used for subculturing (i.e. inoculation of fresh carrot discs) or inoculation of plants for experimental purposes.

Nematode inoculation

Plants can be infected with root-lesion nematodes either by infesting the soil with nematodes from carrot disc cultures or other similar sources or by planting the plants in nematode-infested soil. Soil infestation with 1000–2500 vermiform (mixed life stage) nematodes per plant is usually used for glasshouse experiments. Inoculum can be from

nematodes obtained from cultures or nematodes extracted from roots collected from an infested field.

Protocol 3: Inoculation of plants

Extract the nematodes from the carrot discs, for example by using the maceration–sieving technique (see Protocol 1). Rinse the nematodes from the 25 μm aperture sieve with distilled water in a beaker and bring the nematode suspension to a known volume with water. Blow in the solution with a pipette or agitate by stirring to disperse the nematodes from the bottom and take a sample. Count the nematodes (eggs, juveniles and adults). Adjust the concentration of nematodes to approximately 300–1000 ml^{-1}. Make three or four holes around the base of the stem and add nematodes with a pipette. Close the holes with soil. Be sure that the soil in the pot is moist before infesting with nematodes and/or water lightly immediately after infesting.

Assessment of resistance and tolerance

In vitro *screening*

Usually, screening of crop germplasm for resistance or tolerance to *Pratylenchus* and *Radopholus* spp. is conducted either under glasshouse or field conditions. However, *in vitro* plant tissue cultures can also be used as an early, rapid and reliable method for determining resistance to these nematodes.

For *in vitro* screening aseptic nematodes are needed. *Pratylenchus* and *Radopholus* spp. reared on carrot discs are not free of contaminants since the carrot tissues are only surface-sterilized. Also surface sterilization with streptomycin sulphate of the nematodes extracted from carrot disc cultures is not sufficient. Callus tissue offers a good alternative to obtain aseptic nematodes. Lucerne callus has proved to be a good substrate for aseptic culturing of migratory endoparasitic nematodes, including *R. similis* and *Pratylenchus* species (Myers *et al.*, 1965; Mitsui *et al.*, 1975; Elsen *et al.*, 2001). The initiation of *in vitro* lucerne callus tissue cultures consists of three steps: (i) production of the callus; (ii) inoculation of the callus with nematodes; and (iii) extraction of the nematodes from the callus.

1. *Production of lucerne callus*. Lucerne seeds are sterilized by a 15 min soak in H_2SO_4 (95–97%) followed by four rinses with sterile, distilled water, a 15 min soak in $HgCl_2$ (1000 μg ml^{-1} in 30% ethanol), followed by four rinses with sterile, distilled water (Riedel and Foster, 1970). Sterile 4-day-old lucerne seedlings germinated from these seeds on plates of 1% agar with 10 g sucrose and 2 g yeast extract l^{-1} are placed on slants prepared from 14 ml aliquots of White's medium

(White, 1963), modified by adding 0.2 μg ml^{-1} α-naphthalene acetic acid (α-NAA) and 2 μg ml^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D). Seven to 10 days later, after allowing the callus to develop, then transfer the calluses to Petri dishes containing the same medium.

2. *Nematode inoculation of lucerne callus*. Root-lesion nematodes cultured on carrot discs are surface-sterilized for 2 min in 0.01% $HgCl_2$, followed by two rinses with sterile, distilled water. With a sterile micropipette, 25 females are transferred to each lucerne callus. The Petri dishes are incubated at 28°C in the dark. After 5 weeks, the nematodes start moving out of the callus. To maintain stock cultures of aseptic root-lesion nematode populations, subcultures can be made by aseptically transferring small pieces of infected callus to fresh callus.

3. *Nematode extraction from lucerne callus*. To extract the root-lesion nematodes, the callus is chopped and put on a sterile 70 μm aperture sieve. The sieve is placed on a sterile watch glass containing sterilized water. Within 48 h, living nematodes migrate through the sieve into the water. Prior to inoculation the nematodes are collected from the bottom of the watch glass. The extraction process is carried out under sterile conditions at room temperature.

In addition to lucerne callus, root-lesion nematodes have also been cultured on banana fruit callus (Brown and Vessey, 1985), callus from citrus leaves (Inserra and O'Bannon, 1975), okra callus (Feder, 1958) and on excised root culture (Huettel, 1990). Elsen *et al*. (2001) and Högger (1969) did not observe changes in infectivity of *R. similis* and *P. penetrans* on, respectively, banana and potato, after culturing on lucerne callus. In contrast, Stoffelen (2000) reported that after culturing on carrot callus a *R. similis* population lost its ability to reproduce on banana roots grown in soil in pots.

Developing an *in vitro* screening procedure for nematode resistance requires basically three steps. Firstly, establish *in vitro* propagation and aseptic culturing of the host plant and the nematode. Secondly, demonstrate the ability of the nematode to infect and reproduce on a susceptible variety of the host plant. Finally, validate the procedure by checking the host response of a known nematode-resistant variety of the host plant grown.

Significant differences in reproduction of *R. similis* were observed *in vitro* on resistant and susceptible *Anthurium* cultivars. Differences in plant damage between tolerant and sensitive cultivars were also found. Based on these results, this method was successfully used for evaluation of resistance and tolerance of 17 *Anthurium* cultivars (Wang *et al.*, 1997). Significant differences in reproduction of *R. similis* were also observed *in vitro* on resistant and susceptible *Musa* genotypes (De Waele, 1998).

The *in vitro* screening procedure has several advantages compared to glasshouse screening. The experiments can be performed under controlled conditions. Further, a small quantity of inoculum is sufficient and can be prepared precisely by manually picking nematodes. Less space and time is needed for plant maintenance and harvest. The experimental time is also reduced. A disadvantage is the extraction of nematodes from the medium by maceration and sieving since this is rather difficult and therefore time consuming. Also, the procedure can only be used when resistance to nematodes is expressed in host plants grown *in vitro*, which is not always the case (De Waele, 1998; Stoffelen, 2000).

Glasshouse screening

The size of the pots or bags in which to grow plants will depend on plant species and type of planting material (for instance in *Musa in vitro* tissue cultured plants or suckers) and the objective of the screening. When investigating nematode reproduction or early visual symptoms such as necrosis, data can be obtained after 2–3 months and 15 cm diameter pots are sufficient. The soil should be representative of the soil type in which the plants are cultivated in the region. Moisture and fertilization regimes should be optimal for plant growth.

Field screening

For field screening, a potential site should be sampled to determine the spectrum of nematodes present. Ideally, select a site either infested with only the nematode species of interest or a site where the nematode species composition is representative of the species community occurring in the region. The nematode infestation level at the site should be determined by examining soil samples and roots of host plants growing at the site. If the nematode population density present is large enough, the infested field can be used immediately. If the nematode population density present is too small, the site can be either planted with a good host of the species of interest to increase population densities or nematode-infected roots can be added when the plants are planted (using chopped or macerated infected roots).

Evaluation of resistance

There is no general agreement on the best parameter to estimate population densities of *Pratylenchus* and *Radopholus* in the roots. The

number of nematodes g^{-1} fresh roots has been broadly used, even when root growth rate can be variable among genotypes. Examining the whole root system appears to be a more accurate parameter but is not always possible, especially under field conditions. To avoid restrictions of one or other method the numbers of nematodes should be determined both per plant (that is per whole root system) and per g fresh roots.

Protocol 4: Estimation of migratory endoparasitic nematodes

1. Determination of root fresh weight. Take the plant out of the pot and wash the roots with tap water. Cut off the roots and weigh them. After chopping the roots in 1 cm pieces, take a sample. The sample size depends on/varies with the size of the root system (but for banana is 15 g). The roots can be stored in a refrigerator until time of analysis, if distilled water is added.
2. Nematode extraction (see Protocol 1, in which different extraction techniques are described).
3. Determination of final nematode population. Dilute the nematode solution with distilled water in a graduated cylinder to 200 ml. Blow in the suspension with a pipette or agitate by stirring and take a subsample of 6 ml. Count the nematodes in a counting dish and calculate the final nematode population per plant and nematodes per g of root.

In the screening experiments, replications should range between 8 and 15. To minimize variation in ambient conditions, the replications should be arranged in either a completely randomized design, a randomized complete block design or a split plot design.

Guidelines for screening of *Musa* germplasm for resistance and tolerance to root-lesion nematodes have been described in Speijer and De Waele (1997).

References

Afreh-Nuamah, K., Ahiekpor, E.K.S., Ortiz, R. and Ferris, R.S.B. (1996) Advanced *Musa* yield trial at the University of Ghana Agricultural Research station, Kade. 2. Banana weevil and nematode resistance. In: Ortiz, R. and Akoroda, M.O. (eds) *Plantain and Banana Production and Research in West and Central Africa. Proceedings of a Regional Workshop*, 23–27 September, 1995, Onne, Nigeria. IITA, Ibadan, Nigeria, pp. 133–136.

Alcañiz, E., Pinochet, J., Fernandez, C., Esmenjaud, D. and Felipe, A. (1996) Evaluation of *Prunus* rootstocks for root-lesion nematode resistance. *HortScience* 31, 1013–1016.

Anguiz, R. and Canto-Saenz, R. (1991) Reaction of sweet potato cultivars (*Ipomoea batatas* (L.) Lam.) to *Pratylenchus flakkensis* Seinhorst. *Nematropica* 21, 197–201.

Anon. (1994) Variety: Pelsart. Application no. 93/187. *Plant Varieties Journal* 7, 23.

Anon. (1997a) Variety: Kennedy syn QT6063. Application no. 96/209. *Plant Varieties Journa*l 10, 46–48.

Anon. (1997b) Variety: Sturt syn QT6285. Application no. 96/208. *Plant Varieties Journal* 10, 50.

Anon. (1997c) Variety: Baxter syn QT 625. Application no. 97/283. *Plant Varieties Journal* 10, 55–56.

Barnes, D.K., Thies, J.A., Rabas, D.L., Nelson, D.L. and Smith, D.M. (1990) Registration of two alfalfa germplasms with field resistance to the root-lesion nematode. *Crop Science* 30, 751–752.

Bernard, E.C. and Laughlin, C.W. (1976) Relative susceptibility of selected cultivars of potato to *Pratylenchus penetrans*. *Journal of Nematology* 8, 239–242.

Bernard, E.C. and Montgomery-Dee, M.E. (1993) Reproduction of plant-parasitic nematodes on winter rapeseed (*Brassica napus* ssp. *oleifera*). *Journal of Nematology* 25(S), 863–868.

Bolton, C. and De Waele, D. (1989) Host suitability of commercial sunflower hybrids to *Pratylenchus zeae*. *Journal of Nematology* 21(S), 682–685.

Bos, L. and Parlevliet, J. (1995) Concepts and terminology on plant/pest relationships: toward consensus in plant pathology and crop protection. *Annual Review of Phytopathology* 33, 69–102.

Brennan, P.S., Martin, D.J., Eisemann, R.L., Mason, L.R., Sheppard, J.A., Keys, P.J., Norris, R.G., Uebergang, R.W., The, D., Agius, P.J. and Fiske, M.L. (1994a) *Triticum aestivum* ssp. *vulgare* (bread wheat) cv. Tasman. *Australian Journal of Experimental Agriculture* 34, 872.

Brennan, P.S., Martin, D.J., Eisemann, R.L., Mason, L.R., Sheppard, J.A., Keys, P.J., Norris, R.G., Uebergang, R.W., The, D., Agius, P.J. and Fiske, M.L. (1994b) *Triticum aestivum* ssp. *vulgare* (bread wheat) cv. Houtman. *Australian Journal of Experimental Agriculture* 34, 859–860.

Brennan, P.S., Martin, D.J., Thompson, J.P., Mason, L.R., Sheppard, J.A., Keys, P.J., Uebergang, R.W., The, D., Agius, P.J., Fiske, M.L., Ross, J. and Clewett, T.G. (1994c) *Triticum aestivum* ssp. *vulgare* (bread wheat) cv. Pelsart. *Australian Journal of Experimental Agriculture* 34, 853–854.

Bridge, J., Fogain, R. and Speijer, P. (1997) The root lesion nematodes of banana. Musa *Pest Fact Sheet No. 2*. INIBAP, Montpellier, France, 4 pp.

Bristow, P.R., Barritt, B.H. and McElroy, F.D. (1980) Reaction of red raspberry clones to the root lesion nematode. *Acta Horticulturae* 112, 39–43.

Brodie, B.B. and Plaisted, R.L. (1993) Resistance in potato to *Pratylenchus penetrans*. *Journal of Nematology* 25, 466–471.

Brown, S.M. and Vessey, J.C. (1985) Rearing of *Radopholus similis* on banana fruit callus. *Revue de Nématologie* 8, 179–190.

Café Filho, A.C. and Huang, C.S. (1989) Description of *Pratylenchus pseudofallax* n.sp. with a key to species of the genus *Pratylenchus* Filipjev, 1936 (Nematoda Pratylenchidae). *Revue de Nématologie* 12, 7–15.

Castillo, P., Trapero-Casas, J.L. and Jimenez-Diaz, R.M. (1996) The effect of temperature on hatching and penetration of chickpea roots by *Pratylenchus thornei*. *Plant Pathology* 45, 310–315.

Castillo, P., Vovlas, N. and Jimenez-Diaz, R.M. (1998) Pathogenicity and histopathology of *Pratylenchus thornei* populations on selected chickpea genotypes. *Plant Pathology* 47, 370–376.

Christie, B.R. and Townshend, J.L. (1992) Selection for resistance to the root-lesion nematode in alfalfa. *Canadian Journal of Plant Science* 72, 593–598.

Cook, R. and Evans, K. (1987) Resistance and tolerance. In: Brown, R.H. and Kerry, B. (eds) *Principles and Practice of Nematode Control in Crops*. Academic Press, Orlando, Florida, pp. 179–231.

Coolen, W.A. and D'Herde, C.J. (1972) *A Method for the Quantitative Extraction of Nematodes from Plant Tissue*.

Ghent State Agriculture Research Centre, Merelbeke, Belgium, 77 pp.

Corbett, D.C.M. (1973) *Pratylenchus penetrans*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 2, No. 25, 4 pp.

Corbett, D.C.M. (1974) *Pratylenchus vulnus*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 3, No. 37, 3 pp.

Corbett, D.C.M. (1976) *Pratylenchus brachyurus*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 6, No. 89, 4 pp.

Cox, T.S., Raupp, W.J., Wilson, D.L., Gill, B.S., Leath, S., Bockus, W.W. and Browder, L.E. (1992) Resistance to foliar diseases in a collection of *Triticum tauschii* germ plasm. *Plant Disease* 76, 1061–1064.

Crossa-Raynaud, P. and Audergon, J.M. (1987) Apricot rootstocks. In: Rom, R.C. and Carlson, R.F. (eds) *Rootstocks for Fruit Crops*. John Wiley & Sons, New York, pp. 295–320.

Culver, D.J., Ramming, D.W. and McKenry, M.V. (1989) Procedures for field and greenhouse screening of *Prunus* genotypes for resistance and tolerance to root lesion nematode. *Journal of the American Society for Horticultural Science* 114, 30–35.

Dale, A. and Potter, J. (1998) Strawberry cultivars vary in their resistance to northern lesion nematode. *Journal of Nematology* 30(S), 577–580.

Da Silva, G.S., Ferraz, S., Dos Santos, J.M. and Da Silva Soares, G. (1989) Resistance of *Crotalaria* species to *Pratylenchus brachyurus* and *P. zeae*. *Nematologia Brasileira* 13, 81–86.

Davis, J.R., Hafez, S.L. and Soressen, L.H. (1992) Lesion nematode suppression with the Butte potato and relationships to Verticillium wilt. *American Potato Journal* 69, 371–383.

De Maeseneer, J. and D'Herde, C.J. (1963) Méthodes utilisées pour l'étude des anguillules libres du sol. *Revue Agriculture, Bruxelles* 16, 441–447.

De Waele, D. (1996) Plant resistance to nematodes in other crops: relevant research that may be applicable to *Musa*. In: Frison, E.A., Horry, J.P. and De Waele, D. (eds) *Proceedings of the Workshop on New Frontiers in Resistance Breeding for Nematode, Fusarium and Sigatoka*, 2–5 October, 1995, Kuala Lumpur, Malaysia. INIBAP, Montpellier, France, pp. 108–115.

De Waele, D. (1998) Identification of nematode resistance sources in banana and plantain by induced mutations. *Report of the Second Research Co-ordination Meeting of FAO/IAEA/BADC Co-ordinated Research Project on 'Cellular Biology and Biotechnology including Mutation Techniques for Creation of New Useful Banana Genotypes'*, 13–17 October, 1997, Kuala Lumpur, Malaysia. IAEA, Vienna, Austria, pp. 79–83.

Dinardo-Miranda, L.L. and Ferraz, L.C.C.B. (1991) Pathogenicity of *Pratylenchus brachyurus* and *P. zeae* to two sugarcane varieties (*Saccharum* sp.). *Nematologia Brasileira* 15, 9–16.

Dinardo-Miranda, L.L., Morelli, J.L., De Landell, M.G.A. and De Silva, M.A. (1996) Reaction of sugarcane genotypes in relation to *Pratylenchus zeae* parasitism. *Nematologia Brasileira* 20, 52–58.

Ducharme, E.P. and Birchfield, W. (1956) Physiological races of the burrowing nematode. *Phytopathology* 46, 615–616.

Duncan, L.W., Inserra, R.N., Thomas, K.H., Dunn, D., Mustika, I., Frisse, L.M., Mendes, M.L., Morris, K. and Kaplan, D.T. (1999) Molecular and morphological analysis of isolates of *Pratylenchus coffeae* and closely related species. *Nematropica* 29, 61–80.

Ellison, F.W., Brown, G.N., Mares, D.J., Moore, S.G. and Brien, L. (1995) Register of Australian winter cereal cultivars. *Triticum aestivum* ssp. *vulgare* (bread wheat) cv. Sunvale. *Australian Journal of Experimental Agriculture* 35, 416.

Elsen, A., Lens, K., Nguyet, D.T.M., Broos, S., Stoffelen, R. and De Waele, D. (2001) Development of aseptic culture systems of *Radopholus similis* for *in vitro* host–pathogen studies.

Journal of Nematology 33(2), (in press).

Fallas, G.A. and Sarah, J.L. (1994) Effect of storage temperature on the *in vitro* reproduction of *Radopholus similis*. *Nematropica* 24, 175–177.

Fallas, G.A. and Sarah, J.L. (1995) Effect of temperature on the *in vitro* multiplication of seven *Radopholus similis* isolates from different banana producing zones of the world. *Fundamental and Applied Nematology* 18, 445–449.

Fallas, G.A., Sarah, J.L. and Fargette, M. (1995) Reproductive fitness and pathogenicity of eight *Radopholus similis* isolates on banana plants (*Musa* AAA cv. Poyo). *Nematropica* 25, 135–141.

Fallas, G.A., Hahn, M.L., Fargette, M., Burrows, P.R. and Sarah, J.L. (1996) Molecular and biochemical diversity among isolates of *Radopholus* spp. from different areas of the world. *Journal of Nematology* 28, 422–430.

Farsi, M., Vanstone, V.A., Fisher, J.M. and Rathjen, A.J. (1995) Genetic variation in resistance to *Pratylenchus neglectus* in wheat and triticales. *Australian Journal of Experimental Agriculture* 35, 597–602.

Feder, W.A. (1958) Aseptic culture of the burrowing nematode, *Radopholus similis* (Cobb) Thorne on excised okra root tissue. *Phytopathology* 48, 392–393.

Ferraz, L.C.C.B. (1996) Reaction of soybean cultivars to *Pratylenchus brachyurus*. *Nematologia Brasileira* 20, 22–31.

Ferraz, L.C.C.B., Monteiro, A.R., Inomoto, M.M. and Barbosa-Ferraz, L.C.C. (1989) Reaction of Barbados cherry to seven phytonematode species in Brazil. *Nematologia Brasileira* 13, 39–49.

Florini, D.A., Loria, R. and Kotcon, J.B. (1987) Influence of edaphic factors and previous crop on *Pratylenchus* spp. population densities in potato. *Journal of Nematology* 19, 85–92.

Fogain, R. (1996) Screenhouse evaluation of *Musa* for susceptibility to *Radopholus similis*: evaluation of plantains AAB and diploid AA, AB and BB. In: Frison, E.A., Horry, J.P. and De Waele, D. (eds) *Proceedings of the Workshop on New Frontiers in Resistance Breeding for Nematode, Fusarium and Sigatoka*, 2–5 October, 1995, Kuala Lumpur, Malaysia. INIBAP, Montpellier, France, pp. 79–86.

Fogain, R. and Gowen, S.R. (1995) Pathogenicity on maize and banana among isolates of *Radopholus similis* from four producing countries of Africa and Asia. *Fruits* 50, 5–9.

Fogain, R. and Gowen, S.R. (1998) Yangambi km 5 (*Musa* AAA, Ibota subgroup) a possible source of resistance to *Radopholus similis* and *Pratylenchus coffeae*. *Fundamental and Applied Nematology* 21, 75–80.

Fortuner, R. (1976) *Pratylenchus zeae*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 6, No. 7, 3 pp.

Fortuner, R. (1977) *Pratylenchus thornei*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 7, No. 33, 3 pp.

France, R.A. and Brodie, B.B. (1995) Differentation of two New York isolates of *Pratylenchus penetrans* based on their reaction on potato. *Journal of Nematology* 27, 339–345.

France, R.A. and Brodie, B.B. (1996) Characterization of *Pratylenchus penetrans* from ten geographically isolated populations based on their reaction on potato. *Journal of Nematology* 28, 520–526.

Griffin, G.D. (1991) Differential pathogenicity of four *Pratylenchus neglectus* populations on alfalfa. *Journal of Nematology* 23, 380–385.

Griffin, G.D. and Gray, F.A. (1990) Biology and pathogenicity of *Pratylenchus neglectus* on alfalfa. *Journal of Nematology* 22, 546–551.

Hafez, S.L., Al-Rehiayani, S., Thornton, M. and Sundararaj, P. (1999) Differentiation of two geographically isolated populations of *Pratylenchus neglectus* based on their parasitism of potato and interaction with *Verticillium dahliae*. *Nematropica* 29, 25–36.

Hahn, M.L., Burrows, P.R., Gnanapragasam, N.C., Bridge, J., Vines, N.J. and Wright, D.J. (1994) Molecular

diversity amongst *Radopholus similis* populations from Sri Lanka detected by RAPD analysis. *Fundamental and Applied Nematology* 17, 275–281.

Hahn, M.L., Sarah, J.-L., Boisseau, M., Vines, N.J., Wright, D.J. and Burrows, P.R. (1996) Reproductive fitness and pathogenicity of selected *Radopholus* populations on two banana cultivars. *Plant Pathology* 45, 223–231.

Handoo, Z.A. and Golden, A.M. (1989) A key and diagnostic compendium to the species of the genus *Pratylenchus* Filipjev, 1936. *Journal of Nematology* 21, 202–218.

Högger, C.H. (1969) Comparison of penetration of potato roots by *Pratylenchus penetrans* grown in tissue cultures and from field populations. *Journal of Nematology* 1, 10.

Huettel, R.N. (1990) Monoxenic culturing of plant-parastic nematodes using carrot discs, callus tissue, and root-explants. In: Zuckerman, B.M., Mai, W.F. and Krusberg, L.R. (eds) *Plant Nematology Laboratory Manual*. University of Massachusetts Agricultural Experiment Station, Amherst, Massachusetts, pp. 163–172.

Huettel, R.N. and Dickson, D.W. (1981) Parthogenesis in the two races of *Radopholus similis* from Florida. *Journal of Nematology* 13, 13–15.

Huettel, R.N. and Yaegashi, T. (1988) Morphological differences between *Radopholus citrophilus* and *R. similis*. *Journal of Nematology* 20, 150–157.

Huettel, R.N., Dickson, D.W. and Kaplan, D.T. (1982) Sex attraction and behaviour in the two races of *Radopholus similis*. *Nematologica* 28, 360–369.

Huettel, R.N., Dickson, D.W. and Kaplan, D.T. (1983a) Biochemical identification of the two races of *Radopholus similis* by polyacrylamide gel electrophoresis. *Journal of Nematology* 15, 345–348.

Huettel, R.N., Dickson, D.W. and Kaplan, D.T. (1983b) Biochemical identification of the two races of *Radopholus similis* by starch gel electrophoresis. *Journal of Nematology* 15, 338–344.

Huettel, R.N., Dickson, D.W. and Kaplan, D.T. (1984a) Chromosome number of populations of *Radopholus similis* from North, Central and South America, Hawaii, and Indonesia. *Revue de Nématologie* 7, 113–116.

Huettel, R.N., Dickson, D.W. and Kaplan, D.T. (1984b) *Radopholus citrophilus* sp. n. (Nematoda), a sibling species of *Radopholus similis*. *Proceedings of the Helminthological Society* 51, 32–35.

Inomoto, M.M., Oliveira, C.M.G., Mazzafera, P. and Goncalves, W. (1998) Effects of *Pratylenchus brachyurus* and *P. coffeae* on seedlings of *Coffea arabica*. *Journal of Nematology* 30, 362–367.

Inserra, R.N. and O'Bannon, J.H. (1975) Rearing migratory endoparasitic nematodes in citrus callus and roots produced from citrus leaves. *Journal of Nematology* 7, 261–263.

Kaplan, D.T. (1990) Screening for resistance to *Tylenchulus semipenetrans* and *Radopholus* species. In: Starr, J.L. (ed.) *Methods for Evaluating Plant Species for Resistance to Plant-parasitic Nematodes*. The Society of Nematologists, Hyattsville, Maryland, pp. 51–57.

Kaplan, D.T. (1994) Molecular characterization of the burrowing nematode sibling species, *Radopholus citrophilus* and *R. similis*. In: Lamberti, F., De Georgi, G. and Bird, McK.D. (eds) *Advances in Molecular Plant Nematology*. Plenum Press, New York, pp. 77–83.

Kaplan, D.T. (1999) Molecular, genetic, and biological characterization of citrus parasitism and phylogeny in the burrowing nematode. *Nematropica* 29, 124.

Kaplan, D.T. and Opperman, C.H. (1997) Genome similarity implies that citrus-parasitic burrowing nematodes do not represent a unique species. *Journal of Nematology* 29, 430–440.

Kaplan, D.T., Vanderspool, M.C., Garrett, C., Chang, S. and Opperman, C.H. (1996) Molecular polymorphisms associated with host range in the highly conserved genomes of burrowing nematodes, *Radopholus* spp. *Molecular Plant–Microbe Interactions* 9, 32–38.

Kaplan, D.T., Vanderspool, M.C. and Opperman, C.H. (1997) Sequence Tag site and host range assays demonstrate that *Radopholus similis* and *R. citrophilus* are not reproductively isolated. *Journal of Nematology* 29, 421–429.

Layne, R.E. (1987) Peach rootstocks. In: Rom, R.C. and Carlson, R.F. (eds). *Rootstocks of Fruit Crops*. John Wiley & Sons, New York, pp. 385–416.

Ledbetter, C.L. (1994) Techniques for screening *Prunus* rootstocks for resistance to *Pratylenchus vulnus*. In: Nyczepir, A.P., Bertrand, P.F. and Beckman, T.G. (eds) *Stone Fruit Tree Decline. Sixth Workshop Proceedings. New Insights and Alternative Management Strategies*, 26–28 October, 1992, Fort Valley, Georgia. US Dept. Agr., Agr. Res. Serv, ARS-122, pp. 34–36.

Ledbetter, C.L. and Shonnard, R. (1991) Evaluation of *Prunus* germplasm for resistance to lesion nematode (*Pratylenchus vulnus*). *Hortscience* 26, 709.

Loof, P.A.A. (1978) *The Genus Pratylenchus filipjev, 1936 (Nematoda: Pratylenchidae): a Review of its Anatomy, Morphology, Distribution, Systematics and Identification*. Vaxskyddsrapporter, Jordbruk 5, Uppsala, Sweden.

Machon, J.E. and Hunt, D.J. (1985) *Pratylenchus goodeyi. C.I.H. Descriptions of Plant-parasitic Nematodes* Set 8, No. 120, 2 pp.

Mamiya, Y. (1971) Effect of temperature on the life cycle of *Pratylenchus penetrans* on *Cryptomeria* seedlings and observations on its reproduction. *Nematologica* 17, 82–92.

Marull, J. and Pinochet, J. (1991) Host suitability of *Prunus* rootstocks to four *Meloidogyne* species and *Pratylenchus vulnus* in Spain. *Nematropica* 21, 185–195.

Marull, J., Pinochet, J. and Verdejo, S. (1990) Respuesta de cinco cultivares de almendro a cuatro especies de nematodos lesionadores en Espana. *Nematropica* 20, 143–151.

Mateille, T. (1992) Comparative development of three banana-parasitic nematodes on *Musa acuminata* (AAA group) cvs Poyo and Gros Michel *vitro*-plants. *Nematologica* 38, 203–216.

Mateille, T. (1994) Comparative host tissue reactions of *Musa acuminata* (AAA group) cvs Poyo and Gros Michel roots to three banana-parasitic nematodes. *Annals of Applied Biology* 124, 65–73.

McFadden-Smith, W., Miles, N.W., Potter, J.W. and Monet, R. (1998) Greenhouse evaluation of *Prunus* rootstocks for resistance or tolerance to the root lesion nematode (*Pratylenchus penetrans*). *Proceedings of the Fourth International Peach Symposium*, 22–26 June, 1997, Bordeaux, France. *Acta Horticulturae* 465, 723–729.

Mehta, U.K., Natesan, N. and Sundararaj, P. (1994) Screening of sugarcane cultivars to *Pratylenchus zeae* for commercial release. *Afro-Asian Journal of Nematology* 4, 109–111.

Mitsui, Y., Yokozawa, R. and Ichinohe, M. (1975) Effect of temperature and pH on the propagation of *Pratylenchus* culturing with alfalfa callus tissues. *Japanese Journal of Nematology* 5, 48–55.

Mizukubo, T. (1992) Morphological and statistical differentiation of *Pratylenchus coffeae* complex in Japan (Nematoda: Pratylenchidae). *Applied Entomology and Zoology* 27, 213–224.

Mizukubo, T. (1995) Evidence for *Pratylenchus coffeae* races in differential reproduction on fifteen cultivars (Nematoda: Pratylenchidae). *Japanese Journal of Nematology* 25, 85–93.

Mizukubo, T. and Adachi, H. (1997) Effect of temperature on *Pratylenchus penetrans* development. *Journal of Nematology* 29, 306–314.

Moody, E.H., Lownsberry, B.F. and Ahmed, J.M. (1973) Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. *Journal of Nematology* 5, 225–226.

Myers, R.F., Feder, W.A. and Hutchins, P.C. (1965) The rearing of *Radopholus similis* (Cobb) Thorne on grapefruit, okra, and alfalfa root callus tissue.

Proceedings of the Helminthological Society of Washington 32, 94–95.

Nelson, D.L., Barnes, D.K. and MacDonald, D.H. (1985) Field and growth chamber evaluations for root-lesion nematode resistance in alfalfa. *Crop Science* 25, 35–39.

Nombela, G. and Romero, D. (1999) Host response to *Pratylenchus thornei* of a wheat line carrying the *Cre2* gene for resistance to *Heterodera avenae*. *Nematology* 1, 381–388.

Norton, D.C. (1989) Host efficiencies of *Zea diploperennis* and *Z. perennis* for *Pratylenchus* spp. *Journal of Nematology* 21, 547–548.

Novaretti, W.R.T., Carderan, J.O., Carpanezi, A. and Rodrigues, J.C.S. (1988) Reaction of three sugarcane varieties to the lesion nematode *Pratylenchus zeae* Graham, 1951. *Nematologia Brasileira* 12, 110–120.

O'Bannon, J.H. and Taylor, A.L. (1968) Migratory endoparasitic nematodes reared on carrot discs. *Phytopathology* 58, 385.

Okie, R.W. (1987) Plum rootstocks. In: Rom, R.C. and Carlson, R.F. (eds) *Rootstocks for Fruit Crops*. John Wiley & Sons, New York, pp. 321–360.

Oliveira, C.M.G., Monteiro, A.R., Antedomenico, S.R. and Inomoto, M.M. (1999) Host reaction of *Coffea* spp. to *Pratylenchus brachyurus*. *Nematropica* 29, 241–244.

Olowe, T. and Corbett, D.C.M. (1976) Aspects of the biology of *Pratylenchus brachyurus* and *P. zeae*. *Nematologica* 22, 202–211.

Olthof, T.H.A. (1968) Races of *Pratylenchus penetrans* and their effect on black root rot resistance. *Nematologica* 14, 482–488.

Olthof, T.H.A. (1986) Reaction of six *Solanum tuberosum* cultivars to *Pratylenchus penetrans*. *Journal of Nematology* 19, 424–430.

Orton Williams, K.J. and Siddiqi, M.R. (1973) *Radopholus similis*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 2, No. 27, 4 pp.

Payan, L.A. and Dickson, D.W. (1990) Comparison of populations of *Pratylenchus brachyurus* based on isozyme phenotypes. *Journal of Nematology* 22, 538–545.

Pinochet, J. (1979) Comparison of four isolates of *Radopholus similis* from Central America on Valery bananas. *Nematropica* 9, 40–43.

Pinochet, J. (1988a) Comments on the difficulty in breeding bananas and plantains for resistance to nematodes. *Revue de Nématologie* 11, 3–5.

Pinochet, J. (1988b) Nematode problems in *Musa* spp.: pathotypes of *R. similis* and breeding for resistance. *Proceedings of a Workshop on Nematodes and the Borer Weevil in Bananas. Present Status of Research and Outlook*. 7–11 December, 1987, Bujumbura, Burundi. INIBAP, Montpellier, France, pp. 66–70.

Pinochet, J. (1998c) A review of banana attacking nematodes in the subtropics with emphasis on *Pratylenchus goodeyi* in the Canary Islands. In: Galan Sauco, V. (ed.) *International Symposium on Banana in the Subtropics*, 10–14 November, 1997, Puerto de la Cruz, Tenerife, Spain. *Acta Horticulturae* 490, 353–359.

Pinochet, J. and Rowe, P.R. (1979) Progress in breeding for resistance to *Radopholus similis* on bananas. *Nematropica* 9, 76–78.

Pinochet, J., Verdejo, S., Soler, A. and Canals, J. (1992) Host range of the lesion nematode *Pratylenchus vulnus* in commercial fruit, nut tree, citrus and grape rootstocks in Spain. *Journal of Nematology* 24, 693–698.

Pinochet, J., Marull, J., Rodriguez-Kabana, R., Felipe, A. and Fernandez, C. (1993) Pathogenicity of *Pratylenchus vulnus* on plum rootstocks. *Fundamental and Applied Nematology* 16, 375–380.

Pinochet, J., Cenis, J.L., Fernandez, C., Doucet, M. and Marull, J. (1994) Reproductive fitness and random amplified polymorphic DNA variation among isolates of *Pratylenchus vulnus*. *Journal of Nematology* 26, 271–277.

Pinochet, J., Fernandez, C. and Sarah, J.L. (1995) Influence of temperature on *in*

vitro reproduction of *Pratylenchus coffeae*, *P. goodeyi* and *Radopholus similis*. *Fundamental and Applied Nematology* 18, 391–392.

Pinochet, J., Angles, M., Dalmau, E., Fernandez, C. and Felipe, A. (1996) *Prunus* rootstock evaluation to root-knot and lesion nematodes in Spain. *Journal of Nematology* 28(S), 616–623.

Pinochet, J., Jaizme, M.C., Fernandez, C., Jaumot, M. and De Waele, D. (1998) Screening bananas for root-knot nematode (*Meloidogyne* spp.) and lesion nematode (*Pratylenchus goodeyi*) resistance for the Canary Islands. *Fundamental and Applied Nematology* 21, 17–23.

Plowright, R.A., Matias, D., Aung, T. and Mew, T.-W. (1990) The effect of *Pratylenchus zeae* on the growth and yield of upland rice. *Revue de Nématologie* 13, 283–291.

Pohl, R.W. and Albertsen, M.C. (1981) Interspecific hybrids of *Zea mays* and *Z. diploperennis*. *Iowa State Journal of Research* 55, 257–259.

Potter, J.W. and Dale, A. (1994) Wild and cultivated strawberries can tolerate or resist root-lesion nematode. *HortScience* 29, 1074–1077.

Potter, J.W., Dirks, V.A., Johnson, P.W., Olthof, T.H.A., Layne, R.E.C. and McDonnell, M.M. (1984) Response of peach seedlings to infection by the root-lesion nematode *Pratylenchus penetrans* under controlled conditions. *Journal of Nematology* 16, 317–322.

Price, N.S. (1994a) Field trial evaluation of *Musa* varieties and of other crops as hosts of *Pratylenchus goodeyi* in Cameroon. *Afro-Asian Journal of Nematology* 4, 11–16.

Price, N.S. (1994b) Field trial evaluation of nematode susceptibility within *Musa*. *Fundamental and Applied Nematology* 17, 391–396.

Price, N.S. and McLaren, C.G. (1996) Techniques for field screening of *Musa* germplasm. In: Frison, E.A., Horry, J.P. and De Waele, D. (eds) *Proceedings of the Workshop on New Frontiers in Resistance Breeding for Nematode, Fusarium and Sigatoka*, 2–5 October, 1995, Kuala Lumpur, Malaysia. INIBAP, Montpellier, France, pp. 87–107.

Qing-Yu, Potter, J.W. and Qing-Y (1998) Population development of *Pratylenchus penetrans* in sweet corn cultivars under controlled conditions. *Canadian Journal of Plant Science* 78, 703–706.

Ramsdell, D.C., Bird, G.W., Warner, F.W., Davenport, J.F., Diamond, C.J. and Gillett, J.M. (1996) Field pathogenicity studies of four species of plant-pathogenic nematodes on French-American hybrid grapevine cultivars in Michigan. *Plant Disease* 80, 334–338.

Rich, J.R., Keen, N.T. and Thomason, I.J. (1977) Association of coumestans with the hypersensitivity of Lima bean roots to *Pratylenchus scribneri*. *Physiological Plant Pathology* 10, 105–116.

Riedel, R.M. and Foster, J.G. (1970) Monoxenic culture of *Ditylenchus dipsaci* and *Pratylenchus penetrans* with modified Krusberg's and White's medium. *Plant Disease Reporter* 54, 215–254.

Roberts, P.A. (1992) Current status of the availability, development and use of host plant resistance to nematodes. *Journal of Nematology* 24, 213–227.

Rowe, P. and Rosales, F. (1993) Diploid breeding at FHIA and the development of Goldfinger (FHIA-01). *InfoMusa* 2, 9–11.

Sarah, J.L., Blavignac, F., Sabatini, C. and Boisseau, M. (1992) Une méthode de laboratoire pour le criblage variétal des bananiers vis-à-vis de la résistance aux nématodes. *Fruits* 47, 559–564.

Sarah, J.L., Sabatini, C. and Boisseau, M. (1993) Differences in pathogenicity to banana (*Musa* sp., cv. Poyo) among isolates of *Radopholus similis* from different production areas of the world. *Nematropica* 23, 75–79.

Sarr, E. and Baujard, P. (1988) Sensibilité de trois variétés de nièbe (*Vigna unguiculata*) aux nematodes dans la zone Sahelienne du Senegal. *Revue de Nématologie* 11, 451–452.

Sato, H., Shimizu, N., Nakagawa, H. and Nakajima, K. (1990) A new registered cultivar 'Natsukaze' of guineagrass. *Japan Agricultural Research Quarterly* 23, 196–201.

Sawazaki, E., Lordello, A.I.L. and Lordello, R.R.A. (1987) Inheritance of corn resistance to *Pratylenchus* spp. *Bragantia* 46, 27–33.

Schneider, J.H.M., s'Jacob, J.J. and Van De Pol, P.A. (1995) *Rosa multiflora* Ludiek, a rootstock with resistant features to the root lesion nematode *Pratylenchus vulnus*. *Scientia Horticulturae* 63, 37–45.

Scotto La Massese (1975) Tests d'hôtes de quelques porte-greffe et variétés fruitières à l'égard de *Pratylenchus vulnus* Allen et Jensen. *Comptes Rendus de L'Academie Agronomique de France* 61, 1088–1095.

Seinhorst, J.W. (1950) De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaaltje *Ditylenchus dipsaci* (Kühn) Filipjev. *Tijdschrift voor Plantenziekten* 56, 289–348.

Seinhorst, J.W. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4, 67–69.

Seinhorst, J.W. (1966) Killing nematodes for taxonomic studies with hot F.A. 4:1. *Nematologica* 12, 178.

Siddiqi, M.R. (1972) *Pratylenchus coffeae*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 1, No. 6, 3 pp.

Sidhu, G.S. and Webster, J.M. (1981) The genetics of plant–nematode parasitic systems. *Botanical Review* 47, 387–419.

Simeone, A.M., Nicotra, A. and Di Vito, M. (1995) Susceptibility of progenies of *Actinidia deliciosa*, *A. chinensis* and *A. arguta* to four species of *Meloidogyne* and to *Pratylenchus vulnus*. *Nematologia Mediterranea* 23 (Suppl.), 85–89.

Smith, O.D., Boswell, T.E. and Thames, W.H. (1978) Lesion nematode resistance in peanuts. *Crop Science* 18, 1008–1011.

Sosamma, V.K., Koshy, P.K. and Bhaskara Rao, E.V.V. (1988) Response of coconut cultivars to the burrowing nematode, *Radopholus similis*. *Indian Journal of Nematology* 18, 136–137.

Speijer, P.R. and Bosch, C.H. (1996) Susceptibility of *Musa* cultivars to nematodes in Kagera Region, Tanzania. *Fruits* 51, 217–222.

Speijer, P.R. and De Waele, D. (1997) Screening of *Musa* germplasm for resistance and tolerance to nematodes. *INIBAP Technical Guidelines* 1. INIBAP, Montpellier, France, 47 pp.

Speijer, P.R., Boonen, E., Vuylsteke, D., Swennen, R.L. and De Waele, D. (1999) Nematode reproduction and damage to *Musa* sword suckers and sword sucker derived plants. *Nematropica* 29, 193–203.

Stalin, N., Esmenjaud, D., Minot, J.C., Voison, R. and Pinochet, J. (1994) Comparative host suitability to *Meloidogyne* spp. and *Pratylenchus vulnus* in Myrobalan plum genotypes (*Prunus cerasifera* Ehr.). *Nematologica* 41, 344.

Stalin, N., Salesses, G., Pinochet, J., Minot, J.C., Voisin, R. and Esmenjaud, D. (1998) Comparative host suitability to *Meloidogyne* spp. and *Pratylenchus vulnus* in Myrobalan plum (*Prunus cerasifera*). *Plant Pathology* 47, 211–215.

Stoffelen, R. (2000) Early screening of *Eumusa* and *Australimusa* bananas against root-lesion and root-knot nematodes. PhD thesis. Catholic University Leuven, Belgium.

Stoffelen, R., Jimenez, M.I., Dierckxsens, C., Tam, V.T.T., Swennen, R. and De Waele, D. (1999a) Effect of time and inoculum density on the reproductive fitness of *Pratylenchus coffeae* and *Radopholus similis* populations on carrot disks. *Nematology* 1, 243–250.

Stoffelen, R., Verlinden, R., Xuyen, N.T., Swennen, R. and De Waele, D. (1999b) Screening of Papua New Guinea bananas to root-lesion and root-knot nematodes. *InfoMusa* 8, 12–15.

Stoffelen, R., Verlinden, R., Pinochet, J., Swennen, R.L. and De Waele, D. (2000a) Host plant response of

Fusarium wilt resistant *Musa* genotypes to *Radopholus similis* and *Pratylenchus coffeae*. *International Journal of Pest Management* 46, 289–293.

Stoffelen, R., Verlinden, R., Xuyen, N.T., Swennen, R. and De Waele, D. (2000b) Host plant response of *Eumusa* and *Australimusa* bananas (*Musa* spp.) to migratory and root-knot nematodes. *Nematology* 2, 907–916.

Stover, R.H. (1972) *Banana, Plantain and Abaca Diseases*. Commonwealth Mycological Institute, Kew, UK.

Sundararaju, P. and Koshy, P.K. (1988) Screening of arecanut cultivars and hybrids for resistance to *Radopholus similis*. *Indian Journal of Nematology* 18, 376–378.

Tarte, R., Pinochet, J., Gabrielli, C. and Ventura, O. (1981) Differences in population increase, host preference and frequencies of morphological variants among isolates of the banana race of *Radopholus similis*. *Nematropica* 11, 43–52.

Tarumoto, I., Shiga, T., Sakamoto, S., Ando, T., Ishikawa, H., Kato, S., Takemata, T., Umehara, M. and Katayama, K. (1990) New sweet potato cultivar 'Fusabeni'. *Bulletin of the National Agriculture Research Centre* 19, 17–37.

Thies, J.A., Basigalup, D. and Barnes, D.K. (1994) Inheritance of resistance to *Pratylenchus penetrans* in alfalfa. *Journal of Nematology* 26, 452–459.

Thompson, J.P. and Haak, M.I. (1997) Resistance to root-lesion nematode (*Pratylenchus thornei*) in *Aegilops tauschii* Coss., the D-genome donor to wheat. *Australian Journal of Agricultural Research* 48, 553–559.

Tiwari, S.P., Vadhera, I., Shukla, B.N. and Bhatt, J. (1992) Studies on the pathogenicity and relative reactions of chickpea lines to *Pratylenchus thornei* (Filipjev, 1936) Sher and Allen, 1953. *Indian Journal of Mycology and Plant Pathology* 22, 255–259.

Toruan-Mathius, N., Pancoro, A., Sudarmadji, D., Mawardi, S. and Hutubarat, T. (1995) Root characteristics and molecular polymorphisms associated with resistance to *Pratylenchus coffeae* in Robusta coffee. *Menara-Perkebunan* 63, 43–51.

Townshend, J.L. (1989) Population densities of four species of root-lesion nematodes (*Pratylenchus*) in the oat cultivars, Saia and OAC Woodstock. *Canadian Journal of Plant Sciences* 69, 903–905.

Townshend, J.L. and Anderson, R.V. (1976) *Pratylenchus neglectus*. *C.I.H. Descriptions of Plant-parasitic Nematodes* Set 6, No. 82, 4 pp.

Townshend, J.L. and Baenziger, H. (1977) Evidence of resistance to root-knot and root-lesion nematodes in alfalfa clones. *Canadian Journal of Plant Science* 56, 977–979.

Valette, C., Nicole, M., Sarah, J.L., Boisseau, M., Boher, B., Fargette, M. and Geiger, J.P. (1997) Ultrastructure and cytochemistry of interactions between banana and the nematode *Radopholus similis*. *Fundamental and Applied Nematology* 20, 65–77.

Valette, C., Mounport, D., Nicole, M., Sarah, J.L. and Baujard, P. (1998) Scanning electron microscope study of two African populations of *Radopholus similis* (Nematoda: Pratylenchidae) and proposal of *R. citrophilus* as a junior synonym of *R. similis*. *Fundamental and Applied Nematology* 21, 139–146.

Vanstone, V.A., Rathjen, A.J., Ware, A.H. and Wheeler, R.D. (1998) Relationship between root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and performance of wheat varieties. *Australian Journal of Experimental Agriculture* 38, 181–188.

Verdejo-Lucas, S. and Pinochet, J. (1992) Population densities of five migratory endoparasitic nematodes in carrot disk cultures. *Journal of Nematology* 24, 96–98.

Viaene, N., Dueñas, J. and De Waele, D. (1997) Pot screening of *Musa* genotypes for resistance and tolerance to *Radopholus similis* and *Pratylenchus coffeae*. *Nematropica* 27, 123.

Viaene, N., Dueñas, J. and De Waele, D. (1998) Screening for resistance and tolerance to *Radopholus similis* and

Pratylenchus coffeae in banana and plantain. *Proceedings of the 24th International Nematology Symposium*, 4–9 August, 1998, Dundee, Scotland, p. 125.

Vrain, T.C. and Daubeny, H.A. (1986) Relative resistance of red raspberry and related genotypes to the root lesion nematode. *HortScience* 21, 1435–1437.

Vrain, R.C., Daubeny, H.A., Hall, J.W., De Young, R.M. and Anderson, A.K. (1994) Inheritance of resistance to root lesion nematode in red raspberry. *HortScience* 29, 1340–1341.

Waeyenberghe, L., Rys, A., Moens, M., Pinochet, J. and Vrain, T.C. (2000) Molecular characterisation of 18 *Pratylenchus* species using rDNA Restriction Fragment Length Polymorphism. *Nematology* 2, 135–142.

Wang, K., Kuehnle, A.R. and Sipes, B.S. (1997) *In vitro* screening for burrowing nematode, *Radopholus citrophilus*, tolerance and resistance in commercial *Anthurium* hybrids. *In Vitro Cellular and Developmental Biology–Plant* 33, 205–208.

Webb, R.M. (1996) *In vitro* studies of six species of *Pratylenchus* (Nematoda: Pratylenchidae) on four cultivars of oilseed rape (*Brassica napus* var. *oleifera*). *Nematologica* 42, 89–95.

Wehunt, E.J., Hutchinson, D.J. and Edwards, D.I. (1978) Reaction of banana cultivars to the burrowing nematode (*Radopholus similis*). *Journal of Nematology* 10, 368–370.

White, P.R. (1963) *The Cultivation of Animal and Plant Cells*, 2nd edn. Ronald Press, New York.

Wicks, Z.W., Smolik, J.D., Carson, M.L. and Scholten, G.G. (1990a) Registration of SD101 parental line of maize. *Crop Science* 30, 242.

Wicks, Z.W., Smolik, J.D., Carson, M.L. and Scholten, G.G. (1990b) Registration of SD102 and SD103 parental lines of maize. *Crop Science* 30, 242–243.

Wiryadiputra, S. (1996) Resistance of Robusta coffee to coffee root lesion nematode, *Pratylenchus coffeae*. *Pelita-Perkebunan* 12, 137–148.

Yamakawa, O., Kukimura, H., Komaki, K., Hidaka, M., Yoshinaga, M., Yoshida, T., Tabuchi, S. and Kumagai, T. (1995) Joy White: a new sweet potato cultivar. *Bulletin of the Kyushu National Agricultural Experiment Station* 28, 297–316.

Yamakawa, O., Yoshinaga, M., Kumagai, T., Hidaka, M., Komaki, K., Kukimura, H. and Ishiguro, K. (1998) 'J-Red': a new sweet potato cultivar. *Bulletin of the Kyushu National Agricultural Experiment Station* 33, 49–72.

Yamakawa, O., Kumagai, T., Yoshinaga, M., Ishiguro, K., Hidaka, M., Komaki, K. and Kukimura, H. (1999) 'Sunny Red': a new sweet potato cultivar for powder. *Bulletin of the Kyushu National Agricultural Experiment Station* 35, 19–40.

Young, T.W. (1954) An incubation method for collecting migratory endoparasitic nematodes. *Plant Disease Reporter* 38, 794–795.

9

The Citrus Nematode: *Tylenchulus semipenetrans*

S. Verdejo-Lucas[1] and D.T. Kaplan[2]

[1]Departamento de Protección Vegetal, Institut de Recerca i Tecnologia Agroalimentarias, Crta. de Cabrils s/n, 08348 Cabrils, Barcelona, Spain; [2]US Department of Agriculture, Agriculture Research Service, 2120 Camden Road, Orlando, FL 32803, USA

The citrus nematode, *Tylenchulus semipenetrans* Cobb, infects citrus worldwide (Van Gundy and Meagher, 1977; Heald and O'Bannon, 1987) and is the most abundant and frequent plant-parasitic nematode in citrus groves. Yield losses are estimated at about 10% worldwide. The citrus nematode is associated with poor growth of young citrus trees planted in infested groves and with poor performance of mature citrus trees (Duncan and Cohn, 1990). The host range of *T. semipenetrans* includes all *Citrus* species and most hybrids of citrus with other members of the rutaceous family such as trifoliate orange (*Poncirus trifoliata* L. Raf). Non-rutaceous plants such as grape (*Vitis vinifera*, L.), olive (*Olea europea*, L.) and persimmon (*Diospyrus* spp.) are also hosts.

Citrus nematode females become semi-endoparasitic and sedentary following infection of fibrous roots of susceptible rootstocks. They establish feeding sites within the root cortex composed of nurse cells that surround the female nematode head. The nurse cell cytoplasm becomes dense and granular as feeding sites mature. The posterior portion of the adult female protrudes from the root and is surrounded by a gelatinous matrix in which eggs are deposited (Cohn, 1965). The citrus nematode male appears to complete its life cycle without feeding (Van Gundy, 1958). One generation of the nematode normally occurs in 6–8 weeks at a soil temperature of 24–26°C. After hatching, the infective second-stage juvenile may spend more than 2 weeks in the soil before infecting fibrous roots. Juveniles can persist in soils for more than a year without a host (Baines *et al.*, 1962); they prefer either finely

 Plant Resistance to Parasitic Nematodes (eds J.L. Starr, R. Cook and J. Bridge)

textured or loamy sand soil types. They will infect citrus growing in coarsely textured sandy soils, but require longer periods of time to become established (Van Gundy, 1958; Van Gundy *et al.*, 1964; O'Bannon, 1968; Bello *et al.*, 1986). Citrus nematodes are more prevalent in soils where temperatures are 20–35°C (O'Bannon *et al.*, 1966), and can survive in arid soils but are not capable of assuming an anhydrobiotic state as readily as some other nematodes (Tsai and Van Gundy, 1989). Damage thresholds, nematode population densities that suppress tree growth and yield, are influenced by several factors, including aggressiveness of the nematode population, soil type, rootstock, other diseases and grove management practices. Action thresholds have been set at about 5000 juveniles 250 cm^{-3} soil and 1200 females g^{-1} root in spring samples (Garabedian *et al.*, 1984). The application of management strategies will be most effective during periods of active root development since conditions that favour root growth are conducive to *T. semipenetrans* (Duncan and Noling, 1987).

The genus *Tylenchulus* Cobb contains four species: *T. semipenetrans* Cobb, 1913; *T. furcus* Van den Berg and Spaull, 1982; *T. graminis* Inserra *et al.*, 1988; and *T. palustris* Inserra *et al.*, 1988. Of these, only *T. semipenetrans* is a parasite of citrus and morphological and morphometric characters of the second-stage juveniles, males and females are used for identification (Siddiqi, 1974). Inserra *et al.* (1988) provide diagnostic characters to differentiate *T. semipenetrans* from *T. graminis* and *T. palustris* (Table 9.1). *Tylenchulus graminis* and *T. palustris* were previously considered 'wild' races of *T. semipenetrans.* A furcate tail in the second-stage juvenile differentiates *T. furcus* from the other three species (Van den Berg and Spaull, 1982). Biochemical or serological methods for identification of the citrus nematode have not been developed.

Physiological races or biotypes of *T. semipenetrans* that differ in their host preference were first described in California (Baines *et al.*, 1969, 1974). Three biotypes are recognized: citrus, mediterranean and poncirus (Inserra *et al.*, 1980, 1994; Gottlieb *et al.*, 1987; Verdejo-Lucas *et al.*, 1997a). The citrus biotype infects many genera in the *Rutaceae*, including *Citrus* spp., Troyer and Carrizo citrange (*Citrus sinensis* L. × *P. trifoliata*), as well as olive, grape and persimmon, but reproduces poorly on *P. trifoliata* and some hybrids of this genus. The host range of the mediterranean biotype is similar to the citrus biotype, but olive is a non-host. The poncirus biotype reproduces on *Citrus* spp., *P. trifoliata* and hybrids of *P. trifoliata*, as well as grape, but not olive.

Sources of Resistance

The only source of citrus nematode resistance that has been incorporated into commercially acceptable citrus rootstocks is derived from

Table 9.1. Major characters for identification of *Tylenchulus semipenetrans*, *T. graminis* and *T. palustris*.

Nematode life stage	Differential morphological characters	*T. semipenetrans*		*T. graminis*		*T. palustris*	
		Range	Mean	Range	Mean	Range	Mean
Mature adult females	Body shape expressed as % of the total body that is swollen	34.4–60.0	47.4	66.0–85.1	72.7	32.3–53.8	40.0
	Cuticle thickness	2.9–5.65	3.7	1.0–4.0	2.1	2.5–4.4	3.5
	Postvulval section cavity	1.8–7.1	4.3	5.1–11.2	7.5	5.1–12.2	7.1
	Postvulval section length	26.5–52.0	40.0	34.6–45.9	40.1	20.4–33.6	27.5
	Postvulval section width	9.1–13.2	10.9	12.2–14.2	12.9	11.2–17.3	14.0
	Rectum and anus	Imperceptible		Perceptible		Imperceptible	
Adult males	Basal bulb width	5.1–8.0	6.4	8.1–12.2	9.2	8.1–11.2	9.1
	Body width	10.2–12.2	11.2	13.2–16.2	14.6	12.2–14.2	13.3
	Stylet knob width	0.9–1.2	1.0	1.6–2.1	2.0	1.7–2.1	1.9
	Tail length	34.6–44.8	39.9	48.9–65.9	55.6	33.6–43.8	37.1
Second-stage juveniles	Length of the posterior body section without large fat globules	48.9–60.1	55.3	58.1–76.6	69.6	28.5–59.1	49.8
	Rectum and anus	Imperceptible		Discernible		Discernible occasionally	
	Tail length (from anus to body posterior end)	Cannot be calculated		59.1–72.4	65.0	Cannot be calculated	
	Genital primordium excretory pore distance	6.1–24.4	16.5	22.4–43.8	33.5	2.0–25.5	12.8

After Inserra *et al.* (1988). Values are expressed in μm.

P. trifoliata. This rootstock is also the source of resistance to citrus tristeza virus and of tolerance to *Phytophthora*. Some selections of *P. trifoliata* support very low levels of nematode reproduction ($P_f/P_i < 1$) and they are considered to be highly resistant to many populations of *T. semipenetrans*, whereas others are moderately susceptible ($P_f/P_i > 1$) but less than the reference susceptible rootstock (Ducharme, 1948; Cameron *et al.*, 1954; Feder, 1968; Baines *et al.*, 1969; O'Bannon and Ford, 1977; McCarty *et al.*, 1979; Reddy and Agarwal, 1987; Crozzoli and González, 1989). Trifoliate orange hybridizes readily with most *Citrus* spp. but most of the resulting hybrids are susceptible to *T. semipenetrans* (Hutchinson and O'Bannon, 1972), and only a few of them inherit resistance to the citrus nematode. Of these, the hybrid rootstock Swingle citrumelo (*Citrus paradisi* Macf × *P. trifoliata*) is highly resistant to the citrus nematode in Florida (Kaplan and O'Bannon, 1981) and in Italy (Lo Giudice and Inserra, 1980). Duncan *et al.* (1994) have reported a population of *T. semipenetrans* capable of

circumventing resistance in Swingle citrumelo in Florida but this population appears to be confined to the nursery site where it was detected. Other potential sources of citrus nematode resistance among non-cultivated rutaceous plants have been reported (Baines *et al.*, 1960; Kaplan and O'Bannon, 1981). However, these genera are not horticulturally acceptable as rootstocks and their hybridization with *Citrus* spp. has had limited success.

General Considerations for Screening for Citrus Nematode Resistance

Citrus breeding programmes have a regional scope and their objectives are to find rootstocks that are easily propagated, horticulturally acceptable, adapted to climatic and edaphic conditions and resistant to, or tolerant of, important citrus diseases. Although resistance to *T. semipenetrans* is available in *P. trifoliata* and Swingle citrumelo, both rootstocks have an important limitation: they perform poorly in calcareous and alkaline soils. Therefore, there is still a need for new rootstocks to satisfy regional demands. Screening for resistance to *T. semipenetrans* has not been considered a top priority in many breeding programmes since the citrus nematodes have not been fully accepted or recognized as serious pests of citrus in many citrus-growing areas of the world. However, the resistance of citrus relatives to *T. semipenetrans* has been assessed in many regions of the world (Cameron *et al.*, 1954; Baines *et al.*, 1960; Hutchinson and O'Bannon, 1972; Chhabra and Bindra, 1974; O'Bannon and Ford, 1977; McCarty *et al.*, 1979; Lo Giudice and Inserra, 1980; Geraci *et al.*, 1981; Reddy and Agarwal, 1987; Reddy *et al.*, 1987; Spiegel-Roy *et al.*, 1988; Crozzoli and González, 1989; Zhu *et al.*, 1992; Niles *et al.*, 1995; Verdejo-Lucas *et al.*, 1997b, 2000). The methods and procedures described here are those reported in the literature and they should be taken as a guide. The protocols have been taken from previously published work as indicated.

Inoculum

To evaluate plant resistance, enough nematodes should be introduced into the soil to enable the population to become established without causing an artificial level of damage. Rearing large quantities of citrus nematode inoculum for screening tests is difficult because rutaceous plants grow slowly and because *T. semipenetrans* requires at least 6–12 months to build up high population densities. The citrus nematode can be reared in pot cultures in a glasshouse or collected directly from field-infected citrus roots (Hutchinson and O'Bannon, 1972; Niles

et al., 1995; Verdejo-Lucas *et al.*, 1997b). Stock cultures of the nematode can be maintained in the glasshouse in large bins that minimize temperature and moisture fluctuations. However, if bins are maintained for extended periods of time, citrus nematode populations may decline due to predators and parasites (Walter *et al.*, 1993). Populations of the biotypes of *T. semipenetrans* most frequently found in the region should be included in the screening tests. For biotype identification, trifoliate orange, *Citrus* spp. and olive should be used as the differential host plant species (Inserra *et al.*, 1980, 1994).

Nematode eggs used as inoculum may be obtained by maceration of infected citrus roots (McSorley *et al.*, 1984). Roots are washed free of soil and fibrous roots (< 2 mm diameter) are cut into 1–2 cm sections. They are then placed in a blender, covered with water, and macerated at the highest speed for 15 s. The sides of the blender jar are rinsed, and roots macerated for another 15 s. Alternatively, 0.05% NaOCl can be used to extract eggs. The slurry of macerated roots is poured over a 74 μm aperture sieve nested over a 25 μm aperture sieve. The debris on the upper sieve is rinsed with water and eggs are collected on the lower sieve. After eggs are rinsed from the 25 μm aperture sieve into a beaker, the eggs may be quantified using a compound microscope.

Juveniles obtained from *T. semipenetrans*-infected citrus roots may also be used as inoculum (Kaplan, 1990). Fibrous roots are gently rinsed with water and then placed in buckets of water at room temperature. Roots are aerated continuously by bubbling air through the water. Alternatively, juveniles can be obtained using a mist chamber (Hutchinson and O'Bannon, 1972). Juveniles emerging during the first 24 h extraction period are discarded. Juveniles are collected daily thereafter for 5–7 days, combined, concentrated and stored at 15°C for later use as inoculum. Baermann funnels or variations thereof may also be used to concentrate the juveniles actively emerging from eggs. It is advisable to rinse the nematodes just before use by repeated centrifugation in sterile water to reduce possible fungal contaminants. The Baermann funnel technique can also be used to obtain juveniles from eggs extracted by maceration.

Inoculation

Citrus seedlings may be grown in seedling trays of 50×40 cm, and inoculated by pipetting a suspension of juveniles or eggs into the soil around the roots. Seedlings are grown for 4–5 weeks after inoculation to ensure adequate nematode infection before being transplanted to larger containers (Van Gundy and Martin, 1961). Alternatively, seedlings grown in trays of sterile soil for 2–6 months can be transplanted into citrus nematode-infested soil. Plants may be grown together in

these bins of various sizes for approximately 6 months to ensure that seedling roots are infected by nematodes. Seedlings are often transplanted into individual 20 cm pots containing the same soil mix. Plants are grown for an additional 6 months before assessing nematode reproduction (O'Bannon and Ford, 1977). Van Gundy and Tsao (1963) compared two methods of inoculating plants and found that infesting seedlings in the trays was more effective than at transplanting. Transplanting stress may account for such differences. Also, nematode-infested soil can be mixed in volumetric parts with the potting medium in such a proportion as to give the required nematode inoculum level (Lo Giudice and Inserra, 1980). When nematode-infected roots are used as the source of inoculum (Crozzoli and González, 1989), weighed subsamples of roots are macerated in a blender to determine the number of nematodes per g of root. The amount of roots needed to provide the required inoculum is then estimated and introduced into the potting medium. Alternatively, citrus seedlings grown individually may be inoculated by pipetting a water suspension of eggs or juveniles into potting media (Cameron *et al.*, 1954; Kaplan, 1990; Verdejo-Lucas *et al.*, 1997b). Potting media may be infested with citrus nematodes by adding infective juveniles or eggs all at once or at intervals over a period of weeks (McCarty *et al.*, 1979; Niles *et al.*, 1995). Optimal citrus nematode inoculum densities have been established in several studies but experimental conditions, including the method of inoculation, pot size and potting medium can affect the rate of nematode increase (Van Gundy and Tsao, 1963; O'Bannon *et al.*, 1966; Niles *et al.*, 1995; Verdejo-Lucas *et al.*, 1997b). In general, large numbers of nematodes are added per plant. Usually 5000 juveniles or 10,000 eggs of *T. semipenetrans* have been used to inoculate individual plants. The inoculum is introduced into at least three holes made in the potting medium near the base of the plant to ensure a uniform infestation. Excessive watering of test plants following inoculation should be avoided to prevent inoculum from being washed from the potting media.

Screening Protocols

Because there is differential susceptibility to *T. semipenetrans* among selections of *P. trifoliata*, *Citrus* spp. and hybrids, rootstocks selected for screening may include rootstocks with or without *P. trifoliata* in their parentage. Identifying intermediate levels of resistance to *T. semipenetrans* could be useful for field application if rootstocks possess desirable horticultural characteristics or specific resistance to other pathogens. The initial evaluation is usually done under glasshouse conditions because 1–2 years are required for evaluating plant

material due to the perennial nature of citrus and the slow life cycle of the citrus nematode.

Citrus and their hybrids are normally propagated from seeds but plant material may be propagated vegetatively through the use of rooted branches or leaves (Gottlieb *et al.*, 1987), although this may not be easily accomplished for all selections on a routine basis. However, for polyembryonic species, vegetative reproduction should be considered (Kaplan, 1990). Seeds are germinated in seedbeds, individually transplanted to containers after 2–3 months, and grown for 6 months before infestation of soil with nematodes or eggs. Plant material should be as genetically uniform and as representative of the germplasm source as possible. Test plants should be grown under stringent sanitary conditions to avoid introduction of other root pathogens. Containers should be adequate in size to allow good plant performance and to minimize temperature and moisture fluctuation of the root system. Clay or plastic pots or black plastic bags have been used in screening tests and containers of about 3 dm^3 capacity are suggested. Large clay pots help to minimize soil temperature fluctuations and keep soil cool, but are heavy to handle. Plastic pots or bags are light weight and plants are easily removed from them but changes in moisture and temperature can be greater. Potting media with a high content of organic matter should be used because soil high in organic matter favours root penetration by citrus nematodes (O'Bannon *et al.*, 1966). Soil mixtures of sphagnum peat moss (50–60%) and fine sand (50–40%) mixed in volumetric parts are used with the addition of macro- and microelements. The pH of the potting media should be around 6.5 and it can be adjusted with the addition of $CaCO_3$ (Verdejo-Lucas *et al.*, 1997a). Potting media should be pasteurized with steam due to the high content of organic matter of the mixtures.

In addition to test materials, known susceptible and resistant rootstocks should be included as references to verify the pathogenicity of the nematode isolate and to verify that experimental conditions were conducive to nematode development. Both susceptible and resistant rootstocks should be included. Because nematode population densities may be highly variable, six to ten plant replicates should be tested for each accession. A randomized complete block design is appropriate for screening rootstocks but a randomized, split block design with replicates extending from one side of the glasshouse to the other may be used if variation in ambient conditions is known to occur. Routinely, tests should remain in the glasshouse for a minimum of 6 months following infestation of potting media. Maximum citrus nematode activity in the region should be considered when planning the experiments, so that planting is adjusted to enable harvesting and nematode extraction to coincide with this period. In general, tests that run from spring to autumn provide better conditions for nematode development than

other seasons because this is also the time most suitable for plant development.

Soil temperature should be monitored closely during the experiments since temperature fluctuations can significantly influence nematode population densities. Temperatures should be maintained between 19 and 32°C. A nematode-free source of water should be used to irrigate test plants, which should be fertilized periodically. Tests should be routinely surveyed for insect pests and diseases. Care should be taken to avoid the use of systemic insecticides, which may influence nematode development.

Nematode population densities may be monitored during the test period by using soil augers to collect roots at regular intervals throughout the experiment. At the end of the test period, plants are removed from containers and soil gently shaken from roots. Roots are carefully dipped in a container of water so as to avoid washing off egg masses while removing excess soil and other debris. Fibrous roots are weighed after being removed from primary and secondary roots. The roots can be processed immediately or they can be frozen at –20°C (Verdejo-Lucas *et al.*, 1997b) or preserved in 0.5% formalin (Baines *et al.*, 1969) until processed at a later date. Weights of the entire root system are also useful in comparing rootstock vigour and growth in relation to nematode population densities. Nematodes are best extracted from all fibrous roots from each plant in order to estimate reproduction, but this may also be estimated by randomly collecting 3–5 g of fibrous roots from each test plant. Nematodes are extracted from fibrous roots by cutting roots into small pieces and then incubating them in 250 ml glass jars with about 10 ml of water for 2–5 days (Young, 1954). Roots can be incubated in sealed polyethylene plastic bags (*c.* 500 ml capacity) with the addition of 3% H_2O_2 at 21°C for 2 days (Tarjan, 1972). Blender maceration (McSorley *et al.*, 1984) may also be used to extract nematodes from roots followed by centrifugal flotation using 1 M sucrose, $MgSO_4.7H_2O$ at a specific gravity of 1.10 (Kaplan, 1990) or colloidal silica at 1.16 specific gravity (Greco and D'Addabbo, 1990) as a flotation medium. To facilitate counting, nematodes in the water suspension can be stained using acid fuchsin (Baines *et al.*, 1969). Data are generally expressed as nematodes per g fresh root weight. If nematodes have been extracted by the incubation method, then roots can be dried in an oven at 76°C for 24 h, and nematodes expressed per g dry weight. Data can also be expressed per cm of root (Baines *et al.*, 1960), although this procedure may be too laborious when testing numerous selections (Hutchinson and O'Bannon, 1972).

A reliable, time- and resource-efficient assay system developed to estimate resistance to burrowing nematode (*Radopholus similis* Cobb) (Kaplan, 1994) has also been used for *T. semipenetrans*. This assay is performed in autoclaved glass culture tubes (24 × 150 mm) filled with

c. 55 cm^3 of air-dried fine sand amended with 15% shredded peat moss, pH 5.8 (adjusted with $CaCO_3$). Citrus seeds, peeled from the hilum-end downward to remove the seed coat, are surface disinfected in 0.2% NaOCl for 20 min and rinsed four times with sterile distilled water. Sand in each tube is moistened with 3 ml of sterile distilled water before transferring a single seed into a depression made in the surface of the sand. The seed is then covered with sterile sand. Tubes are incubated at 25 ± 1.5°C and soil moisture is maintained at *c.* 0.3% w/w of soil through the addition of sterile distilled water. Light is supplied with a 400 W metal halide lamp (350 µmol m^{-2} s^{-1} at canopy level). The soil in each tube is infested 45 days after planting by adding 1 ml of an aqueous suspension of citrus nematode juveniles (*c.* 325 juveniles ml^{-1} of sterile distilled water). The inoculation procedure is repeated 7 days later. Root systems are harvested individually, 90 days after initial nematode infestation, by using a 6 mm diameter copper tube that is tapered down to 3 mm in diameter, and connected to a water supply. At low water pressure, the tube is inserted into the culture tube and used to gently wash the roots free of soil. The root system is returned to the tube and each tube is covered and incubated at 25°C for 5 days. Then 2 ml of water is added to the tube which is shaken for 20 s at 250 r.p.m. The resulting suspension is transferred to a counting dish and the number of nematodes and eggs determined. Data can be expressed as nematodes per root system or per g root fresh weight.

Tolerance to *T. semipenetrans,* defined in relation to the amount of damage caused by the nematode to the plant (see Roberts, Chapter 2), has been assessed in some studies by determining shoot and fresh root weight (O'Bannon and Ford, 1977; McCarty *et al.*, 1979; Crozzoli and González, 1989). Differences in growth of root systems are not always associated with citrus nematode infection. Citrus plants are tolerant of citrus nematodes, which are well adapted to their hosts (Cohn *et al.*, 1965). Therefore, evaluating rootstock tolerance of citrus nematodes in glasshouse potted plants may not be meaningful. Additionally, shoot growth can not be used to estimate rootstock tolerance of citrus nematodes in glasshouse trials as growth is highly dependent on the availability of water; glasshouse care enables young citrus trees to produce lush shoots with minimal root systems.

Infection and development (females g^{-1} root) and reproductive potential (eggs + juveniles g^{-1} root) of *T. semipenetrans* are indicators of the response of the rootstocks. The number of females per g root provides an estimate of the nematode population that has parasitized existing root systems, whereas counts of eggs and juveniles provide information on the influence of plant germplasm on nematode reproduction. Since citrus is a perennial, it is more meaningful to have an indication of the potential for population growth than simply numbers of existing females. Some plants may not impair female development,

but they may adversely influence nematode reproduction (Kaplan, 1990). To establish the host status of the rootstocks tested, the number of females and reproductive potential on test rootstocks are compared with those on a susceptible stock. To identify rootstocks expressing resistance to *T. semipenetrans*, host status of individual rootstocks is compared with that of the reference resistant stock. Resistance ratings have been used when numerous rootstocks showing different levels of susceptibility were compared (Baines *et al.*, 1960; Hutchinson and O'Bannon, 1972; O'Bannon and Ford, 1977; Reddy and Agarwal, 1987). The resistance identified in the initial screening tests in glasshouse conditions should be verified under field conditions.

Genetics of Resistance

Resistance to *T. semipenetrans* seems to be dominant and oligogenic (Hutchinson, 1985). This conclusion is supported by the results reported by Ling *et al.* (2000). Histopathological studies have shown that the resistance is characterized by a hypersensitive response to nematode feeding and subsequent formation of wound periderm (Van Gundy and Kirkpatrick, 1964; Kaplan, 1981; Kaplan and O'Bannon, 1981).

Rootstock resistance can play an important role in limiting losses attributed to the citrus nematode but these rootstocks should provide resistance to all major citrus diseases. Genes conferring resistance to the citrus nematode have been identified but their mechanisms of resistance need to be determined (Kaplan, 1988). Marker-assisted selection may speed up the screen process. Ling *et al.* (2000) identified 11 random amplified polymerase DNA (RAPD) markers linked to a locus conferring resistance to *T. semipenetrans*. Quantitative trait loci (QTL) analysis revealed that 54% of the phenotypic variation was explained by this locus.

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References

Baines, R.C., Bitters, W.P. and Clarke, O.F. (1960) Susceptibility of some species and varieties of citrus and some other rutaceous plants to the citrus nematode. *Plant Disease Reporter* 44, 281–285.

Baines, R.C., Martin, J.P., De Wolfe, T.A., Boswell, S.B. and Garber, M.J. (1962) Effect of high doses of D-D on soil organisms and the growth and yield of lemon trees. *Phytopathology* 52, 723 (Abstract).

Baines, R.C., Miyakawa, T., Cameron, J.W. and Small, R.H. (1969) Infectivity of two biotypes of the citrus nematode on citrus and on some other hosts. *Journal of Nematology* 1, 150–159.

Baines, R.C., Cameron, J.W. and Soot, R.K. (1974) Four biotypes of *Tylenchulus semipenetrans* in California, and their importance to the development of resistant citrus rootstocks. *Journal of Nematology* 6, 63–66.

Bello, A., Navas, A. and Belart, C. (1986) Nematodes of citrus groves in the Spanish Levante. Ecological study focused to their control. In: Cavalloro, R. and Di Martino, E. (eds) *Integrated Pest Control in Citrus Groves*. Balkema, A.A., Rotterdam, Boston, pp. 211–226.

Cameron, J.W., Baines, R.C. and Clarke, O.F. (1954) Resistance of hybrid seedlings of the trifoliate orange to infestation by the citrus nematode. *Phytopathology* 44, 456–458.

Chhabra, H.K. and Bindra, O.S. (1974) Screening of citrus rootstocks against the citrus nematode, *Tylenchulus semipenetrans* Cobb, 1913. *Indian Journal of Horticulture* 31, 194–195.

Cohn, E. (1965) On the feeding and histopathology of the citrus nematode. *Nematologica* 11, 47–54.

Cohn, E., Minz, G. and Monselise, S.P. (1965) The distribution, ecology and pathogenicity of the citrus nematode in Israel. *Israel Journal of Agricultural Research* 15, 187–200.

Crozzoli, R. and González, A. (1989) Reacción de once patrones de cítricos al nematodo *Tylenchulus semipenetrans*. *Agronomía Tropical* 39, 4–6.

Ducharme, E. (1948) Resistance of *Poncirus trifoliata* rootstock to nematode infestation in Argentina. *The Citrus Industry* 29, 9–15.

Duncan, L.W. and Cohn, E. (1990) Nematode diseases of citrus. In: Bridge, J., Luc, M. and Sikora, R. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, UK, pp. 321–346.

Duncan, L.W. and Noling. J.W. (1987) The relationship between development of the citrus root system and infestation by *Tylenchulus semipenetrans*. *Revue de Nématology* 10, 61–66.

Duncan, L.W., Inserra, R.N., O'Bannon, J.H. and El-Morshedy, M.M. (1994) Reproduction of a Florida population of *Tylenchulus semipenetrans* on resistant citrus rootstocks. *Plant Disease* 78, 1067–1071.

Feder, W.A. (1968) Differential susceptibility of selections of *Poncirus trifoliata* to attack by the citrus nematode, *Tylenchulus semipenetrans*. *Israel Journal of Agricultural Research* 18, 175–179.

Garabedian, S., Van Gundy, S.D., Mankau, R. and Radewald, J.D. (1984) Nematodes. *Integrated Pest Management for Citrus*. Division of Agriculture and Natural Resources Publications, University of California, Berkeley, California, pp. 129–131.

Geraci, G., Lo Giudice, V. and Inserra, R.N. (1981) Response of *Citrus* spp. and hybrid rootstocks to *Tylenchulus semipenetrans*. *Rivista Ortoflorofrutticola Italiana* 65, 169–172.

Gottlieb, Y., Cohen, E. and Spiegel-Roy, P. (1987) Assessing resistance of citrus rootstocks to *Tylenchulus semipenetrans* with rooted leaves. *Revue de Nematologie* 10, 119–121.

Greco, N. and D'Addabbo, T. (1990) Efficient procedure for extracting *Tylenchulus semipenetrans* from citrus roots. *Journal of Nematology* 22, 590–593.

Heald, C.M. and O'Bannon, J.H. (1987) Citrus declines caused by nematodes. V. Slow decline. *Nematology Circular No. 143*. Florida Department of Agriculture and Consumer Services, Division of Plant Industry, 4 pp.

Hutchinson, D.J. (1985) Rootstock development, screening and selection for disease tolerance and horticultural characteristics. *Fruit Varieties Journal* 39, 21–25.

Hutchinson, D.J. and O'Bannon, J.H. (1972) Evaluating the reaction of citrus selections to *Tylenchulus semipenetrans*. *Plant Disease Reporter* 56, 747–751.

Inserra, R.N., Vovlas, N. and O'Bannon, J.H. (1980) A classification of *Tylenchulus semipenetrans* biotypes. *Journal of Nematology* 12, 283–287.

Inserra, R.N., Esser, R.P. and O'Bannon, J.H. (1988) Identification of *Tylenchulus* species from Florida. *Nematology Circular No. 153*. Florida Department of Agriculture and Consumer Services, Division of Plant Industry, 4 pp.

Inserra, R.N., Duncan, L.W., O'Bannon, J.H. and Fuller, S.A. (1994) Citrus nematode biotypes and resistant citrus rootstocks in Florida. *Nematology Circular No 205*. Florida Department of Agriculture and Consumer Services, Division of Plant Industry, 4 pp.

Kaplan, D.T. (1981) Characterisation of citrus rootstock responses to *Tylenchulus semipenetrans* (Cobb). *Journal of Nematology* 13, 492–498.

Kaplan, D.T. (1988) Future considerations for nematode management in citrus. In: Goren, R and Mendel, K. (eds) *Proceedings of the Sixth International Citrus Congress*, Balaban Publishers, Tel Aviv, Israel, pp. 969–975.

Kaplan, D.T. (1990) Screening for resistance to *Tylenchulus semipenetrans* and *Radopholus* species. In: Starr, J. L. (ed.) *Methods for Evaluating Plant Species for Resistance to Plant-parasitic Nematodes*. Society of Nematologists, Hyattsville, Maryland, pp. 51–57.

Kaplan, D.T. (1994) Measuring burrowing nematode virulence to select rootstocks for Florida citrus groves. *Florida State Horticultural Society* 107, 84–89.

Kaplan, D.T. and O'Bannon, J.H. (1981) Evaluation and nature of citrus nematode resistance in Swingle citrumelo. *Proceeding of Florida State Horticultural Society* 94, 33–36.

Ling, P., Duncan, L.W., Zeng, Z., Dunn, D., Hu, S. and Gmitter, F.G., Jr (2000) Inheritance of citrus nematode resistance and its linkage with molecular markers. *Theoretical and Applied Genetics* 100, 1010–1017.

Lo Giudice, V. and Inserra, R.N. (1980) Reaction of citrus and noncitrus rootstocks to *Tylenchulus semipenetrans*. *Nematologia Mediterranea* 8, 103–105.

McCarty, C.D., Bitters, W.P. and Van Gundy, S.D. (1979) Susceptibility of 25 citrus rootstocks to the citrus nematode. *Hortscience* 14, 54–55.

McSorley, R., Parrado, J.L. and Dankers, W.H. (1984) A comparative method for the extraction of nematodes from roots. *Nematropica* 14, 72–84.

Niles, R.K., Freckman, D.W. and Roose, M.L. (1995) Use of trifoliate orange as a standard for assessing the resistance of citrus rootstocks to citrus nematode. *Plant Disease* 79, 813–818.

O'Bannon, J.H. (1968) The influence of an organic soil amendment on infectivity and reproduction of *Tylenchulus semipenetrans* on two citrus rootstocks. *Phytopathology* 58, 597–601.

O'Bannon, J.H. and Ford, H.W. (1977) Resistance in citrus rootstock to *Radopholus similis* and *Tylenchulus semipenetrans* (Nematoda). *Proceedings International Society Citriculture* 2, 544–549.

O'Bannon, J.H., Reynolds, H.W. and Leathers, C.R. (1966) Effect of temperature on penetration, development and reproduction of *Tylenchulus semipenetrans*. *Nematologica* 12, 483–487.

Reddy, P.P. and Agarwal, P.K. (1987) Resistance in citrus rootstocks to the citrus nematode *Tylenchulus semipenetrans*. *Indian Journal Horticulture* 44, 111–114.

Reddy, P.P., Khan, R.M. and Agarwal, P.K. (1987) Selection of citrus rootstocks and hybrids resistant to the citrus nematode, *Tylenchulus semipenetrans*. *Pakistan Journal of Nematology* 5, 69–72.

Siddiqi, M.R. (1974) *Tylenchulus semipenetrans*. *C.I.H. Descriptions of plant-parasitic Nematodes,* Set, 3, No. 34. CAB International, Wallingford, UK, 4 pp.

Spiegel-Roy, P., Vardi, A., Elhanati, A., Solel, Z. and Bar-Joseph, M. (1988) Rootstock selections from a poorman orange × *Poncirus trifoliata* cross. In: Goren, R. and Mendel, K. (eds) *Proceedings of the Sixth International Citrus Congress*, Balaban Publishers, Tel Aviv, Israel, pp. 195–200.

Tarjan, A.C. (1972) Observations on extracting citrus nematodes, *Tylenchulus semipenetrans*, from citrus roots. *Plant Disease Reporter* 56, 186–188.

Tsai, B.Y. and Van Gundy, S.D. (1989) Tolerance of proto-anhydrobiotic citrus nematodes to adverse conditions. *Revue de Nématologie* 12, 107–112.

Van den Berg, E. and Spaull, V.W. (1982) Two new species of Tylenchuloidea (Nematoda) on sugar-cane in South Africa. *Phytophylactica* 14, 131–144.

Van Gundy, S.D. (1958) The life history of the citrus nematode *Tylenchulus semipenetrans* Cobb. *Nematologica* 3, 283–294.

Van Gundy, S.D. and Kirkpatrick, J.D. (1964) Nature of resistance in certain citrus rootstocks to citrus nematode. *Phytopathology* 54, 419–427.

Van Gundy, S.D. and Martin, J.P. (1961) Influence of *Tylenchulus semipenetrans* on the growth and chemical composition of sweet orange seedlings in soils of various exchangeable cation ratios. *Phytopathology* 51, 146–151.

Van Gundy, S.D. and Meagher, J.W. (1977) Citrus nematode (*Tylenchulus semipenetrans)* problems worldwide. *Proceedings of International Society of Citriculture* 3, 823–826.

Van Gundy, S.D. and Tsao, P.H. (1963) Infesting citrus seedlings with the citrus nematode, *Tylenchulus semipenetrans*. *Phytopathology* 53, 228–229.

Van Gundy, S.D., Martin, J.P. and Tsao, P.H. (1964) Some soil factors influencing reproduction of the citrus nematode and growth reduction of sweet orange seedlings. *Phytopathology* 54, 294–299.

Verdejo-Lucas, S., Sorribas, F.J., Pons, J., Forner, J.B. and Alcaide, A. (1997a) The mediterranean biotypes of *Tylenchulus semipenetrans* in Spanish citrus orchards. *Fundamental and Applied Nematology* 20, 399–404.

Verdejo-Lucas, S., Sorribas, F.J., Forner, J.B. and Alcaide, A. (1997b) Screening hybrid citrus rootstocks for resistance to *Tylenchulus semipenetrans* Cobb. *Hortscience* 32, 1116–1119.

Verdejo-Lucas, S., Sorribas, F.J., Forner, J.B. and Alcaide, A. (2000) Resistance of hybrid citrus rootstocks to a mediterranean biotype of *Tylenchulus semipenetrans* Cobb. *Hortscience* 35, 269–273.

Walter, D.E., Kaplan, D.T. and Davis, E.L. (1993) Colonization of greenhouse nematode cultures by nematophagous mites and fungi. *Journal of Nematology* 25 (4S), 789–794.

Young, T.W. (1954) An incubation method for collecting migratory endoparasitic nematodes. *Plant Disease Reporter* 38, 794–795.

Zhu, W.S., Chen, H., Lan, X.Y., Chen, Q.Y. and Chian, K.M. (1992) Observation on the population of citrus root nematode in the soil and the resistance of different rootstocks. *China-Citrus* 22, 17–18.

10

The Yam Nematode: *Scutellonema bradys*

C. Kwoseh*, R.A. Plowright and J. Bridge

CABI Bioscience UK Centre, Bakeham Lane, Egham, Surrey TW20 9TY, UK

Yams, *Dioscorea* spp., are probably one of the oldest carbohydrate food crops known to man (Alexander and Coursey, 1969) and are grown for their tubers. They are one of the only tropical root and tuber crops capable of long-term storage (2–5 months) after harvest, thus supplying palatable carbohydrates to consumers the year round (IITA, 1985) and are relied on for food security. The earliest domesticated yams in West and Central Africa included *D. rotundata* Poir, *D. cayenensis* Lam. and *D. dumetorum* (Knuth) Pax, whereas in Southeast Asia, *D. alata* L. was the first yam species cultivated (Orkwor, 1998). Their large-scale cultivation as food crops is restricted largely to West Africa, the Pacific area including Japan, and the Caribbean (Jatala and Bridge, 1990). They are also of importance in parts of eastern Africa and tropical America. *D. rotundata* and *D. cayenensis* account for most of the yam produced in Africa whereas *D. alata* is more common in Asia and has the widest geographical distribution (Ng, 1990). *D. alata* spread from Southeast Asia to India and the Pacific Ocean more than 2000 years ago (Coursey and Martin, 1970).

Yams are attacked by many plant parasitic nematodes, but the most important, particularly in West Africa, is the yam nematode, *Scutellonema bradys* (Steiner and LeHew) Andrassy (Bridge, 1972; Jatala and Bridge, 1990). *S. bradys* is the cause of a decay of yam tubers known as 'dry rot disease'. This disease is widely distributed in the

* Present address: c/o Crop Science Department, University of Science and Technology, Kumasi, Ghana.

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tropics, especially in areas where yams are grown and has been reported from the West African countries of Nigeria, Côte d'Ivoire, Senegal, The Gambia, Ghana, Togo and Cameroon, from Cuba, Jamaica, Guatemala, Puerto Rico, Guadeloupe, Haiti, Jamaica, Martinique in the Caribbean and also from India, Venezuela and Brazil (Jatala and Bridge, 1990; Crozzoli and Parra, 1991; Plowright and Kwoseh, 1998; Park *et al.*, 1998). *S. bradys* belongs to the family *Hoplolaimidae* and, like other members of this group, is a relatively large, vermiform nematode measuring about 1 mm in length with a well-developed, robust stylet. Reproduction is amphimictic with separate sexes. Eggs of the nematode are laid in the soil or in roots and tubers and juveniles develop into mature adults within 21 days. *S. bradys* is a migratory endoparasite, invading and feeding on tissues in the outer periderm and subperiderm of yam tubers resulting in dry rot disease (Colour Plate 10). All active stages are infective. *S. bradys* invades the young, developing tubers through the tissues of the tuber growing point, along lateral emerging roots and shoots, through roots and also through cracks or damaged areas of the tuber skin (Bridge, 1972).

Infested yam tubers are greatly damaged in storage as a result of the continued reproduction of the nematode in stored tubers causing dry rot symptoms (Bridge, 1973; Jatala and Bridge, 1990; Kwoseh, 2000). Initial symptoms are yellowing of the outer tissues of the tuber, which then turn brown to black as the dry rot disease progresses, cracks also occur in the skin (Colour Plate 10) and, in severe cases, total decay can occur during storage. The damage caused to cells by the nematode is confined to the outer subepidermal, peridermal and parenchymatous tissues of the tuber to a depth of 1–2 cm, although occasionally deeper. *S. bradys* can cause a reduction of 20–30% in tuber weight at harvest (Smit in Bridge, 1982). However, the main losses occur during storage of tubers with at least 25% of the fresh weight of the nematode-infected tubers being lost or rendered inedible; the final cumulative losses caused by *S. bradys* can be 'staggering' (Adesiyan and Odihirin, 1975; Adesiyan *et al.*, 1975).

The most commonly grown food yams of the genus *Dioscorea* are all hosts of *S. bradys* and are susceptible to dry rot disease. Other crop hosts of *S. bradys* occur but these tend to be relatively poor hosts compared to yams except for cowpea (*Vigna unguiculata*), sesame or beniseed (*Sesamum indicum*) and melon (Adesiyan, 1976; Jatala and Bridge, 1990).

Resistance to *S. bradys* has not yet been found in any of the landraces or accessions examined in two of the main yam species, *D. alata* and *D. rotundata*. One cultivar of the yellow yam, *D. cayenensis,* and cultivars and accessions of two other food yam species, *D. esculenta* and *D. dumentorum*, have shown resistance (Bridge, 1982; Kwoseh, 2000). Resistance in crops is not as common against the migratory

endoparasitic nematodes, such as *S. bradys*, as it is against the sedentary endoparasites with specialized feeding sites, such as *Meloidogyne*, *Globodera*, *Heterodera*, *Rotylenchulus* and *Tylenchulus*. It is possible to achieve control of *S. bradys* in yams by hot water treatment of seed tubers (Bridge, 1975) although this method is very rarely used by farmers throughout the yam growing areas and resistance would be a better means of managing these nematodes.

Germplasm and Sources of Resistance

As mentioned for other nematodes, the most likely source of resistance to *S. bradys* will be found in the germplasm collections and the search for resistance is best handled as part of an existing breeding programme. A good example is the Yam Breeding Programme using the Yam Parental Germplasm Collection at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Plant material for evaluation of nematode resistance can be whole or portions of tubers, or propagated from true botanic seeds, tissue culture plants, or yam tuber minisetts (Otoo *et al.*, 1987). The minisett technique is recommended as it is less costly. In this technique, the head region of the yam tuber is removed and then the remaining tuber is sliced into sections (approx. 1–2 cm thick). The outer portions of these sections with the epidermis intact are cut into small flat pieces (setts) weighing 40–50 g for planting in pots and 100 g for field trials. The cut surfaces of the setts should be treated with a contact fungicide mixed with wood ash, fungicide alone or wood ash alone. The treated setts are allowed to sprout in a quantity of sterilized medium such as moist cocopeat (shredded coconut husk), sand, rice straw or sawdust in boxes or baskets in the glasshouse. This growing medium is moistened with Benlate (benomyl, Du Pont) (2.3 g l^{-1} water) or another fungicide. The treated setts are spread on top of the moistened medium in a box or basket and then covered with another layer of moist medium (Fig. 10.1). This method gives a more uniform plant establishment, tuber size and tuber maturity. Uniform plantlets are ready for transplanting 4–5 weeks after planting the setts.

Potting Medium and Field Plots

Heat pasteurized 1 : 1 mix or 1 : 2 cocopeat–top soil mix may be used as potting media. Pot sizes of 600–1000 cm^3 containing *c.* 500–900 cm^3 soil mix are adequate for evaluation. About 4-month-old yam plantlets should be potted in the soil mix and the plants allowed about 2 weeks after potting to establish in the glasshouse at a temperature of at least

25°C. Each plant should be provided with about equal volume of water and a nematode-free source of water should be used for watering. In field experiments, yam plantlets (or tubers) can be planted in mounds or ridges at 1 × 1 m spacing.

Nematode Inoculum

Inoculum used to evaluate plant resistance to the yam nematode can be produced by monoxenic culturing on callus yam slices in Petri dishes (Kwoseh *et al.*, 1998; Kwoseh, 2000). A nematode-free tuber of *D. rotundata* is washed, peeled, cut into slices and treated with a fungicide (e.g. Bio-Supercarb, carbedazin, Pan Britannica Industries). The fungicide should not be toxic to nematodes and it is worth first testing to determine if the available fungicide is safe to use with the nematodes. The washed yam tuber is cut into pieces with skin intact as for minisetts and then dipped in the fungicide for 15 min. The pieces are then air dried before the skin of each piece is peeled off by hand and the remaining tissue sterilized in sodium hypochlorite (1% available chlorine) for 15 min. The yam pieces are rinsed six times with sterilized distilled water and then blotted dry on sterile filter paper. Each slice, weighing 3–6 g is plated on to 1% water agar medium in Petri dishes and kept for 3 weeks to produce callus (shown by a whitening of the outer tissues). The nematodes are sterilized in 0.1% malachite green (technical grade) for 5 min and then rinsed 10 times with sterilized distilled water. The plates are then each inoculated with 20–30

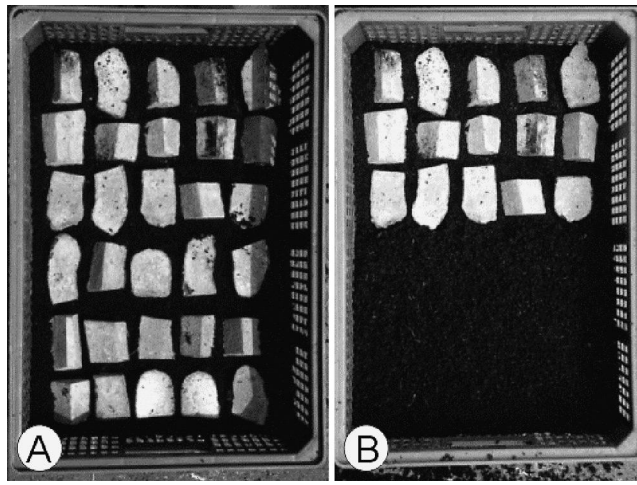

Fig. 10.1. Yam minisetts arranged on cocopeat in plastic baskets: (A) yam minisetts arranged on moistened cocopeat, (B) yam minisetts partly covered with moistened cocopeat.

active stages of *S. bradys* by picking them singly from a watch glass. The inoculated plates are sealed with tape and kept at *c.* 25°C in the dark. Substantial nematode production is achieved by 5 months after inoculation. *S. bradys* is extracted from the chopped yam pieces and agar of the plates on a modified Baermann tray over a period of days.

S. bradys can also be obtained from infested yam tubers if these are freely available from the field or storage. Infected tubers, i.e. those showing symptoms of dry rot, are peeled and the peelings bulked and chopped to about 3–4 mm width and 1–2 cm length (Fig. 10.2). Peelings are thoroughly mixed and populations of *S. bradys* present in the tuber peelings are estimated by extracting a sample (5–10 g) on a modified Baermann tray for a minimum of 48 h. Peelings are then used for pot inoculation or field infestation.

Inoculation Methods

To assess the reaction of yams, the initial inoculum is of crucial importance because susceptible varieties may be misclassified at very low levels. Known numbers of nematodes in water suspension can be inoculated into a small hole in the soil made at the base of each yam stem in pots (about the same distance from each stem). The nematode suspension should be homogenized by bubbling air through using a pipette or by stirring before aliquots are taken for inoculation. Inoculum homogeneity should be determined by taking two separate 1 ml subsamples of the inoculum at the beginning and end of the inoculation. All the nematodes in the subsample should be counted. The inoculated plants can be left for about 9 weeks and all entries in the screen harvested.

The plants in pots can also be infested with *S. bradys* in chopped infected yam tuber peelings (Fig. 10.2). This is done by removing soil

Fig. 10.2. Chopped yam tuber peelings used for inoculations.

near each plant to expose the yam roots and the peelings placed around the plant and re-covered with soil. This works as well for both pot and field experiments.

Inoculum level has been found to influence the reaction of yam to *S. bradys*. Initial population densities between 120 and 2800 active *S. bradys* (equivalent infected yam tuber peelings) can be used in 600–1000 cm^3 size pots containing about 500–800 cm^3 sterile soil mix to demonstrate clear resistance or susceptibility in yams. Naturally or artificially infested soil in field, glasshouse or screenhouse studies containing 1 to 2 nematodes cm^{-3} of soil mix can also be used for resistance evaluation.

Experimental Design

For efficiency and simplicity, a Latin square is the best field experimental design for nematode-resistance studies, but a randomized complete block design (RCBD) is also good. Naturally infested soil plus artificial infestation with *S. bradys* in tuber peelings give consistent results and are best used for field trials. Continuous cropping of yams on the same piece of land builds up *S. bradys* populations. A simple line design where accessions are blocks or RCBD can be used for pot trials. For precision in estimating quantitative differences in susceptibility of yams to *S. bradys*, 5–10 replicates (a plant representing a replication) are ideal. The costs of the experiment in terms of land space, time, labour and availability of inoculum need to be considered.

Data Collection

S. bradys reproduction in yam roots does not correlate with that which occurs in tubers. Generally, roots have more nematodes per gram of tissue than tubers. This may be due to the physiological differences between the two organs, the functions of roots and tubers, and possible different infection processes of *S. bradys* on roots and tubers. As a result of this difference, a root protocol, which would have been easier to manage, cannot be used to evaluate resistance or susceptibility against *S. bradys* in yams. This is because a variety may have susceptible roots, but resistant tubers and such valuable material could be lost due to misrepresentation. Therefore, tubers, which are the part of the plant of economic importance or of interest, should be used in all *S. bradys* resistance studies.

Accuracy and precision of nematode estimates for potted plants or stored yam tubers are dependent on sampling methods. In both, destructive sampling is done. Potted plants are harvested according to

Table 10.1. Description of infection types and scores for damage on tubers.

Infection score	% soft tuber tissue	Dry or wet rot	Surface cracking
0	0	None	None
1	< 25	Light	Light
2	26–50	Moderate	Moderate
3	> 50	Severe	Severe

replicates. Plants are uprooted and the tubers of each test plant placed on a sieve and washed under running tap water and then blotted dry with tissue paper. The tubers are examined for symptoms of nematode damage and infection types on yam tubers are scored on a 0–3 scale (Table 10.1). Each infection type is scored independently and fresh weights of tubers are recorded separately. For yam tubers from field plots, fresh weights are taken at harvest and after at least 4 weeks of storage under ambient conditions. Tuber damage is rated as described for tubers from pot tests.

After surface symptom scores, internal rot and nematode populations are estimated for each tuber in the screen. Three peelings are taken from each tuber in strips of 1.5–2 cm width and 3–5 mm thickness from the proximal to the distal end at about equal distance from each other. However, smaller samples of peels taken randomly over the tuber would probably work just as well. Dry rot is further assessed on the exposed tissues of the tuber. After nematode injury assessment, peelings are chopped and the yam tissues are thoroughly hand-mixed. Samples of 3–5 g chopped peelings are randomly taken for extraction of nematodes on a modified Baermann tray for at least 2 days at 25°C.

The nematode extract is then homogenized by bubbling air through a pipette or by stirring and 1–2 ml aliquots are used for counting. For consistency, two or three separate aliquots should be taken per sample and the number of nematodes counted separately.

There is a strong linear relationship between dry rot symptoms and *S. bradys* population densities in yam tubers, therefore dry rot symptoms can be used effectively to select for resistance or susceptibility in yam tubers at harvest and after a period of storage under ambient conditions. Using these protocols, a considerable amount of yam germplasm material can be screened and susceptible varieties discarded in a relatively short period of time.

Dry rot of yam tubers is also caused by other nematodes, particularly *Pratylenchus coffeae*, in different parts of the world, such as the Pacific and the Caribbean. Similar protocols to those described for *S. bradys* can be used to assess resistance of yams to *Pratylenchus*.

References

Adesiyan, S.O. (1976) Host range studies of the yam nematode, *Scutellonema bradys*. *Nematropica* 6, 60–63.

Adesiyan, S.O. and Odihirin, R.A. (1975) Plant parasitic nematodes associated with yam tubers in mid-West State, Nigeria. *Nigerian Journal of Plant Protection* 3, 171–179.

Adesiyan, S.O., Odihirin, R.A. and Adeniji, M.O. (1975) Economic losses caused by the yam nematode *Scutellonema bradys* in Nigeria. *Plant Disease Reporter* 59, 477–480.

Alexander, J. and Coursey, D.G. (1969) The origin of yam cultivation. In: Ucko, P.J. and Dimpleby, G.W. (eds) *The Domestication and Exploitation of Plants and Animals*. Gerald Duckworth Press, London, pp. 405–425.

Bridge, J. (1972) Nematode problems with yams (*Dioscorea* spp.) in Nigeria. *PANS* 18, 89–91.

Bridge, J. (1973) Nematodes as pests of yams in Nigeria. *Mededelingen Fakulteit Ladbouwetenschappen Universitat Gent* 38, 841–852.

Bridge, J. (1975) Hot water treatment to control plant parasitic nematodes of tropical crops. *Mededelingen Fakulteit Ladbouwetenschappen Universitat Gent* 40, 249–259.

Bridge, J. (1982) Nematodes of yams. In: Miege, J. and Lyonga, S.N. (eds) *Yams. Igname*. Clarendon Press, Oxford, pp. 253–264.

Coursey, D.G. and Martin, R.W. (1970) The past and future of yams as crop plants. In: *Proceedings of the Second Symposium of Tropical Root Crops*, Hawaii, pp. 87–90, 99–101.

Crozzoli, P.R. and Parra, M.D.M. (1991) Detection of the nematode *Scutellonema bradys* causing dry rot of yams in Venezuela. *Fitopatologia Venezolana* 4, 26.

International Institute of Tropical Agriculture (IITA) (1985) *Research Highlights and Annual Report for 1984*. Ibadan, Nigeria.

Jatala, P. and Bridge, J. (1990) Nematode parasites of root and tuber crops. In: Luc, M., Sikora, R.A. and Bridge, J. (eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, UK, pp. 137–180.

Kwoseh, C.K. (2000) Identification of resistance to major nematode pests of yams (*Dioscorea* spp.) in West Africa. PhD thesis, Department of Agriculture, Reading University, 175 pp.

Kwoseh, C.K., Plowright, R.A., Stanfield, J. and Asiedu, R. (1998) Culturing *Scutellonema bradys* on yam tuber slices. *Proceeding of the 7th Triennial Symposium of the International Society for Tropical Root Crops – Africa Branch, Cotonou, Benin*, 11–17 October 1998. Poster abstract.

Ng, S.Y.C. (1990) *In vitro* tuberisation in white yam (*Dioscorea rotundata* Poir). *IITA Research Highlight 1990*, pp. 11–13.

Orkwor, G.C. (1998) The importance of yams. In: Orkwor, G.C., Asiedu, R. and Ekanayake, I.J. (eds) *Food Yams. Advances in Research*. International Institute of Tropical Agriculture (IITA) and National Root Crops Research Institute, Nigeria, p. 249.

Otoo, J.A., Osiru, D.S.O., Ng, S.Y.C. and Hahn, S.K. (1987) *Improved Technology for Seed Yam Production*. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 56 pp.

Park, S.D., Khan, Z., Kim, S., Kim, K., Min, K., Kim, S.J., Kim, K.J. and Min, K.K. (1998) Occurrence and distribution of plant parasitic nematodes in yam (*Dioscorea batatas*) field in Korea. *International Journal of Nematology* 8, 141–144.

Plowright, R.A. and Kwoseh, C.K. (1998) Farmers perceptions of nematode disease in yams in Ghana and the prevalence of endoparasitic nematodes in stored tubers. *Nematologica* 44, 558–559 (Abstract).

Ectoparasitic Nematodes

11

J.L. Starr and I.F. Bendezu

Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132, USA

Those plant-parasitic nematodes that are typically characterized as having an ectoparasitic relationship with their hosts are a large and diverse group of genera, parasitizing most important crop species (Table 11.1). The distinction between migratory ectoparasites and migratory endoparasites is not always clear, species of *Hoplolaimus* and *Helicotylenchus* may exhibit both types of parasitic behaviour. Although it is widely accepted that the root-knot and cyst nematodes are responsible for most of the crop losses due to nematodes, several of the ectoparasites are major pathogens of a given crop in localized regions (e.g. *Criconemella xenoplax* on peach in the southeastern USA). In many cases, especially in crops of low cash value, little effort has been made to document yield losses due to parasitism by ectoparasitic species. In limited surveys of sorghum in Botswana and Zimbawe, relatively large population densities of *Longidorus*, *Tylenchorhynchus* and *Xiphinema* were frequently associated with unthrifty growth of sorghum (Starr, unpublished data), but evidence for causal relationships is lacking. As a group, the ectoparasites are generally viewed as not being particularly aggressive pathogens, typically requiring rather large initial population densities to suppress crop growth. A notable exception is *Belonolaimus longicaudatus*, which can cause measurable losses to cotton, maize or soybean even if initial population densities are near the lower limits of detection.

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Table 11.1. Ectoparasitic nematodes associated with economic losses of crop plants (Luc *et al*., 1990; Evans *et al*., 1993).

Genus	Crop
Aphelenchus	Tomato, mushroom
Belonolaimus longicaudatus	Maize, cotton, potato, groundnut, sugarcane, bean, pepper, celery, turf grasses, strawberry, soybean, sorghum, clover
Cacopaurus	Walnut
Criconemella	Peach, plum, walnut, grape, groundnut, tobacco
Dolichodorus heterocephalus	Sweet corn, bean, celery
Helicotylenchus dihystera	Sugarcane
Hemicriconemoides kanayaensis	Tea
Hemicycliophora arenaria	Citrus
Hemicycliophora nudata	Maize, bean, sugarcane, carrot
Hoplolaimus	Maize, cotton, sugarcane, banana, pine, turf grasses
Longidorus elongatus	Sugarcane, sugarbeet, potato, tomato, lettuce, cane berries, strawberry, peach, cherry, mint, conifers, clovers, turf grasses
Paratylenchus	Celery, plum, pineapple, cane berries, clover
Scutellonema siamense	Black pepper
S. brachyurus	Cocoa, sugarcane
Trichodorus/Paratrichodorus	Beets, potato, pea, onion, maize, tomato, pepper, cotton, tobacco, citrus, grape, sugarcane, lucerne, clover, turf grasses
Tylenchus	Spruce
Xiphinema	Grape, almond, stone fruits, apple, pear, hops, strawberry, raspberry, bean, tomato, tobacco, sorghum, sugarcane, turf grasses, clover, citrus, conifers

Host–Parasite Relations

Ectoparasitic nematodes are often perceived to have brief and non-invasive relationships with their hosts, and to have a primitive mode of parasitism that typically results in cell death (Wyss, 1981). However, even when the interaction between the nematode stylet and the host cell is short compared to sedentary nematodes (Hussey *et al*., 1992), their feeding activity, when it does not cause cell death (Wyss, 1997), can induce galling (i.e. *Xiphinema* in grapevine and *Longidorus* in strawberry and ryegrass) and other elaborate cellular modifications in host tissues (Bleve-Zacheo *et al*., 1987; Hussey *et al*., 1992). The host cell death associated with feeding seems likely to have its origins in a high number of nematodes simultaneously feeding from the same area (Streu *et al*., 1961; Klinger, 1975). Galling by *Xiphinema* spp. is produced by synchronous mitosis without cytokinesis (Rumpenhost and Weischer, 1978; Wyss *et al*., 1980), whereas *Longidorus* spp. produce galling by the continuous development (enlargement) of a cluster of empty cells surrounded by modified uninucleate cells (Griffiths and

Robertson, 1984). Secretions from the nematode dorsal oesophageal gland appear to be involved in degradation of host cell walls so that cytoplasm from neighbouring cells is contiguous (Robertson *et al.*, 1984). Production (Smant *et al.*, 1998) and secretion (Wang *et al.*, 1999) of cellulase enzymes has been demonstrated for sedentary endoparasitic nematodes. In the case of *Criconemella xenoplax*, the plasmodesmata between the food cell and the surrounding cells are modified in a manner that is likely to facilitate transport of solutes into the food cell (Hussey *et al.*, 1992). The modified plasmodesmata had increased diameters, lacked desmotubules and were lengthened by callose-like deposits. Feeding by *Hemicycliophora typica* on emerging rice root tips produces cavities that are surrounded by intact cells with partially dissolved cell walls similar to those of syncytia (Bleve-Zacheo *et al.*, 1987). Additionally, nematode oesophageal gland secretions may liquefy the cytoplasm to facilitate ingestion.

Nematode feeding activity at a single feeding cell can last from a few minutes (*X. index*) or hours (*Tylenchorhynchus dubius*) (Wyss, 1987) to 8 days (*C. xenoplax*) (Hussey *et al.*, 1992). Most ectoparasitic nematode species feed on a group of cells for several hours until the transport of cytoplasm becomes difficult because of cell death or most likely when the nematode has ingested enough cytoplasm. During feeding activities, *C. xenoplax* produces a feeding tube and the appearance of host cell cytoplasm and organelles near the feeding tube becomes different from that of cytoplasm more distant from the feeding tube and stylet (Hussey *et al.*, 1992). Feeding activity by ectoparasites has been shown to affect gene expression in host cells. Barthels *et al.* (1997) have shown expression of a promoterless β-glucuronidase (GUS) reporter gene in the multinucleate feeding cells induced by *X. diversicaudatum*.

Although it is evident that the host cell modifications induced by ectoparasitic species are not as elaborate as those induced by the sedentary endoparasites, they are sufficiently complex to lead to the conclusion that disruption of these modifications would likely result in the plant being a less favourable host. Similarly, any alteration in the plant response to nematode feeding activities that reduces the period of feeding at a single site is likely to result in that plant being a less favourable host. All of the available evidence suggests that the relationship of ectoparasites with their hosts is sufficiently complex such that resistant host genotypes that do not allow establishment of the normal host–parasite relationship are likely to occur within the available germplasm resources.

Resistance to Ectoparasitic Nematodes

One result of the limited attention given to ectoparasites, especially relative to the sedentary and migratory endoparasites, is that there have

been few attempts to identify useful levels of resistance or tolerance and even less effort to develop resistant or tolerant crop cultivars. Table 11.2 summarizes some of the recent efforts to identify resistance and tolerance in a variety of crops. In the majority of these reports only a few readily available accessions or cultivars were tested. Only with groundnut and *Prunus* spp. have relatively large numbers of accessions been examined. Despite these limited efforts, resistance or tolerance was reported for most of the nematode/crop systems examined. These data would suggest that additional sources of resistance and tolerance will be identified if a greater effort is made to screen germplasm resources.

In some crops, because of the importance of nematodes as components of a complex disease syndrome, greater effort has been placed on resistance or tolerance to the syndrome than directly to the nematode. In the case of peach tree short life syndrome, even though resistance to *C. xenoplax* has not been detected (Westcott and Zehr, 1991; Westcott *et al.*, 1994), peach genotypes with improved resistance to the syndrome have been identified based on increased longevity of trees in fields infested with the nematode and other pathogens involved in the complex (Beckman *et al.*, 1997).

In the majority of the reports summarized in Table 11.2, resistance was measured based on nematode reproduction. Exceptions were the reports of resistance to *T. brevilineatus* in groundnut (Siva Rao *et al.*, 1986; Mehan *et al.*, 1993). Because of the distinct lesions that develop on the pods in response to nematode parasitism, resistance in this interaction was measured using a subjective lesion severity index (0–5 scale) based on percentage of the pod surface covered with necrotic lesions. Harris (1983) used both nematode reproduction and root symptoms to characterize the resistance in *Vitis* spp. to *X. index*, using a root damage index of 0–3 to rate the severity of root-tip swelling caused by nematode parasitism. The susceptible *V. vinifera* cultivar had a root damage index of 2 and a P_f/P_i ratio of 9.9, whereas several resistant *Vitis* accessions had root damage indices of 0 with $P_f/P_i < 1$.

Westcott and Zehr (1991) attempted to distinguish fecundity from the possible confounding influence of carrying capacity when screening *Prunus* spp. for resistance to *C. xenoplax* in pots where root growth would be restricted. They used the model of

$$P_f/P_i = 1 + \forall\ (2^{[d-\gamma]/\beta} - 1)$$

where d = degree days with a base of 9°C, γ is a correction factor based on pot volume with small pots having a value of 0, β is the number of degree days required for a doubling of the nematode population, and $\forall$ is the proportionate doubling increment. Values of β were used to compare peach accessions under the assumption that resistance to reproduction of *C. xenoplax* would increase the value of β. In contrast

Table 11.2. Recent evaluation of plants for resistance or tolerance to ectoparasitic nematode species.

Plant	Nematode	Accessions tested	Resistance	Tolerance	Source
Bermudagrass	*Belonolaimus longicaudatus*	41	yes	yes	Giblin-Davis *et al*. (1992)
Cauliflower	*Tylenchorhynchus brassicae*	10	yes	–	Pasha and Tiyagi (1997)
Cotton	*Hoplolaimus columbus*	84	–	yes	Bowman and Schmitt (1994)
Grape	*Xiphinema index*	12	yes	–	Coiro *et al*. (1990)
Groundnut	*T. brevilineatus*	1599	yes[a]	–	Mehan *et al*. (1993)
Groundnut	*T. brevilineatus*	48	yes[a]	–	Siva Rao *et al*. (1986)
Maize	*Mesocriconema* spp.	4	no	–	McSorley and Gallaher (1997)
Maize	*Helicotylenchus pseudodigonicus*	8	yes	–	Ismail *et al*. (1994)
Nicotiana spp.	*T. vulgaris*	17	yes	yes	Patel and Patel (1995)
Prunus spp.	*Criconemella xenoplax*	369	no	–	Westcott and Zehr (1991)
Prunus spp.	*C. xenoplax*	410	no	–	Westcott *et al*. (1994)
Rice	*Paralongidorus australis*	14	–	no	Stirling *et al*. (1989)
St Augustine grass	*B. longicaudatus*	8	yes	–	Busey *et al*. (1993)
St Augustine grass	*Hoplolaimus galeatus*	7	no	no	Henn and Dunn (1989)
Sunflower	*X. basiri*	6	no	yes	Eldin and Siddiqui (1995)
Vitis spp.	*X. index*	37	yes	–	Harris (1983)

[a]Resistance evaluation based on severity of symptoms on pods, not on nematode reproduction.

to fecundity, Westcott and Zehr (1991) defined carrying capacity (*C*) as being equal to $(P_f/P_i)/W$, where *W* is the root weight. They reported that β and *C* were not related to each other and neither was β correlated with tree survival (a measure of tolerance) in infested fields. Because no resistance to *C. xenoplax* was identified in this study, this approach to the identification of resistance requires further testing in a system where resistant accessions can be compared with susceptible ones.

Little is known of the inheritance of resistance to ectoparasitic species. Meredith *et al*. (1982) examined the segregation ratios for resistance (which they called tolerance but resistance was the more appropriate term) to *X. index* in progeny of 33 crosses among seven *Vitis* spp. They used a damage index (1–4 scale) based on swollen root tips rather than nematode reproduction to distinguish susceptible and resistant individuals. All plants with a rating of ≤ 1 were classified as resistant, and those with a rating of ≥ 2 were classified as susceptible. Although there were few F_2 plants from each cross (ranging from 2 to 84 individuals), chi square analysis indicated that in all but two of the crosses the segregation ratios fit either a one gene or a two gene model.

Tolerance to Ectoparasites

Tolerance as defined by Roberts and others (see Chapter 2) may be as useful as resistance in terms of alleviating crop losses due to ectoparasites. It is possible that tolerance may have wider utility, depending on the mechanisms involved, than resistance because it may lack the specificity of resistance. Because tolerance involves a plant's ability to sustain growth and yield potential under conditions of stress, if the stress caused by different species of ectoparasites is similar, then the plant's response (and tolerance to different nematodes) may be similar. This hypothesis needs rigorous testing.

Testing for tolerance is probably more difficult than testing for resistance because it is unlikely to be a monogenic trait and, hence, variability in response is likely to be great. Bowman and Schmitt (1994) used a pooled error variance from the analysis of variance to determine the minimum number of replications needed to measure specific difference in tolerance in cotton to *Hoplolaimus columbus*. In their studies in glasshouse tests, 231 replicates of each cotton accession were needed to distinguish a 10% difference in tolerance at the 5% probability level whereas only five replications were needed to distinguish a 50% difference in tolerance at a 20% probability level (Fig. 11.1). In the field plots, 57 replicates of each accession were needed for a 10% difference at the 5% probability level and two replications were needed for a 30% difference at the 20% probability level. Although these values will likely vary greatly with different nematode species/crop combinations

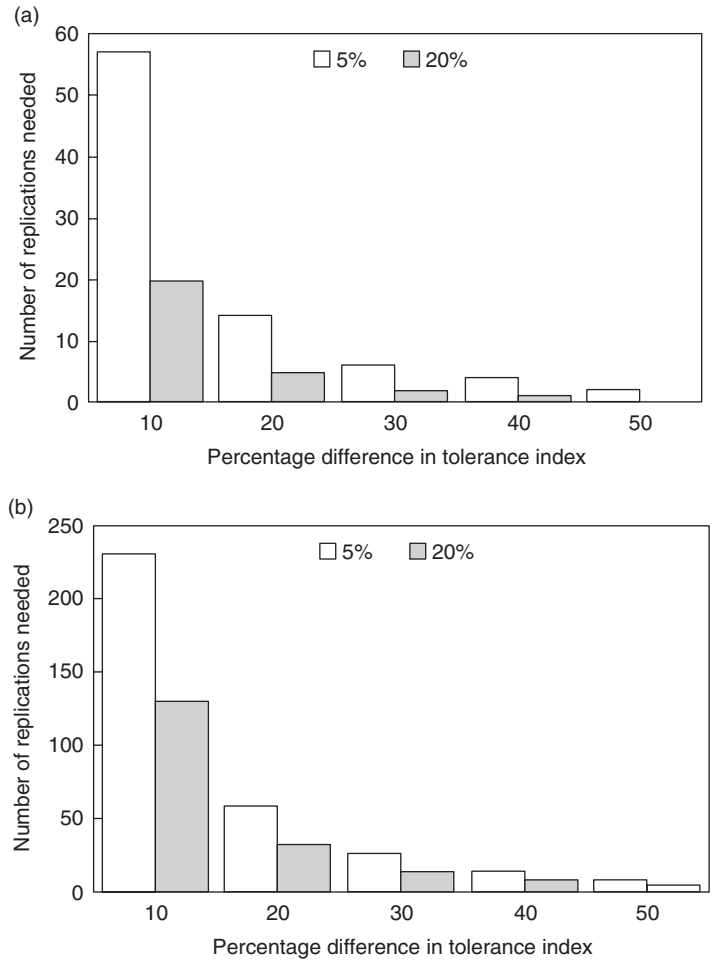

Fig. 11.1. Estimation of number of replications needed to distinguish cotton accessions differing in tolerance to *Hoplolaimus columbus*, based on selected magnitude of difference at 5% and 20% probability levels. (a) Field tests; (b) glasshouse tests. (From Bowman and Schmitt, 1994.)

and with environment, they serve to illustrate the care needed to identify tolerance. It is also apparent that to screen a large number of accessions, initially one will have to use a higher probability level than the usual 5% level and test for relatively large differences among accessions. One can impose more rigorous standards in subsequent tests to confirm initial observations.

Another question to be considered in testing for tolerance is the value of tests conducted in small pots in a glasshouse or similar environment relative to field tests. Plant growth response to nematode parasitism in a confined space is likely to differ greatly from the

response in the natural field environment. However, in field plots it is difficult to ensure uniform initial nematode population densities, and this variability in nematode population densities will lead to increased variability in host response. Field microplots such as those described by Barker *et al*. (1979) are an intermediate approach in that plant growth is more typical of that in field plots but with a much higher degree of control over initial nematode population densities. The cost of installing a large number of field microplots can be an impediment to their use in screening large numbers of accessions for tolerance.

Species Identification, Inoculum Production and Other Considerations

As with all other investigations of resistance to plant-parasitic nematodes, because of the specificity of resistance, it is essential to have a correctly identified nematode population when attempting to identify resistant germplasm. Because of the limited deployment of resistance to ectoparasites, there are as yet no reports of the existence of races among these species based on differences in virulence to resistant host genotypes. Little is known of the specificity between nematode genotype and crop genotype with regard to tolerance, and such specificity may be lacking among tolerant crop genotypes. As discussed above, tolerance to the stress imposed by the parasitic activity of one nematode species may also result in tolerance to other nematodes with similar parasitic activities. The taxonomy of many of the genera of ectoparasites has undergone extensive revision during the recent past. In the case of *Xiphinema*, many more species are recognized today than there were 20 years ago. Persons lacking specific expertise in nematode taxonomy should always have the identification of the nematode species with which they are working confirmed by others with such expertise.

Maintenance of cultures for inoculum production for most ectoparasites is usually more difficult than for the root-knot and cyst species because they frequently have lower fecundity and (or) longer life cycles. The life cycles of *Longidorus* and *Xiphinema* spp. are typically longer than 1 year as compared to 4 weeks for *Meloidogyne* spp. A single *Meloidogyne* female on a good host may produce as many as 2000 eggs whereas many ectoparasites will produce fewer than 100 eggs per female. The typical measurement of reproduction, the P_f/P_i ratio, can exceed 1000 for *Meloidogyne* spp. on a susceptible annual crop, whereas Meredith *et al*. (1982) reported after 8 months incubation a maximum P_f/P_i of only 9.9 for *X. index* on a susceptible *Vitis* sp. Additionally, large quantities of mixed life stages for inoculum of the ectoparasites can be more difficult to extract from soil than are cysts of

Globodera or *Heterodera* spp. or the eggs of *Meloidogyne* spp. from host roots. In many of the studies listed in Table 11.2, the authors used infested soil as the source of inoculum, which was obtained from either glasshouse cultures or from infested fields. The use of field soil has the limitation that it is likely to be also infested with other nematode species and fungal and bacterial pathogens.

For successful screening of germplasm for either resistance or tolerance, it is important to have a thorough knowledge and understanding of the biology of the nematode in question. Are there unique restrictions with respect to temperature, soil type or soil moisture? *Belonolaimus longicaudatus* requires a coarsely textured soil, typically requiring > 80% sand, for reproduction and survival. Some nematode and host species can be relatively sensitive to excessive fluctuations in soil temperature and moisture. Lownsbery *et al*. (1978) used 12-l containers of soil sunk in a bed of wood chips to reduce such fluctuations during a study of the pathogenicity of *C. xenoplax* on walnut.

Summary

The previous limited efforts to identify and develop resistance to ectoparasitic nematodes have yielded sufficient success to justify greater efforts. Very little of the available germplasm resources of most crops has been examined for resistance or tolerance to these diverse nematodes. Although the losses caused by individual species are minor relative to the total losses attributed to the sedentary endoparasites, many ectoparasites are of sufficient importance in localized regions to warrant additional effort in developing resistant cultivars.

References

Barker, K.R., Daughtry, B.I. and Corbett, D.W. (1979) Equipment and techniques for establishing field microplots for the study of soilborne pathogens. *Journal of Nematology* 11, 106–108.

Barthels, N., van der Lee, F.M., Klap, J., Goddijn, O.J.M., Karimi, M., Puzio, P., Grundler, F.M.W., Ohl, S.A., Lindsey, K., Robertson, L., Robertson, W.M., Montagu, M.V., Gheysen, G. and Sijmons, P.C. (1997) Regulatory sequences of *Arabidopsis* drive reporter gene expression in nematode feeding structures. *Plant Cell* 9, 2119–2134.

Beckman, T.G., Reighard, G.L., Okie, W.R., Nyczepir, A.P., Zehr, E.I. and Newall, W.C. (1997) History, current status, and future potenial of Guardian™ (BY520–9) peach rootstock. *Acta Horticulturae* 451, 251–258.

Bleve-Zacheo, T., Lamberti, F. and Chinappen, M. (1987) Root cell response in rice attacked by *Hemicycliophora typica*. *Nematologia Mediterranea* 5, 129–138.

Bowman, D.T. and Schmitt, D.P. (1994) Screening cotton for tolerance to *Hoplolaimus columbus*. *Plant Disease* 78, 695–697.

Busey, P., Giblin-Davis, R.M. and Center, B.J. (1993) Resistance in *Stenotaphrum* to the sting nematode. *Crop Science* 33, 1066–1070.

Coiro, M.I., Taylor, C.E., Borgo, M. and Lamberti, F. (1990) Resistance of grape rootstocks to *Xiphinema index*. *Nematologia Mediterranea* 18, 119–121.

Eldin, E.M. and Siddiqui, M.A. (1995) Plant-parasitic nematodes associated with sunflower at Abu-Naama, Sudan and observations on host suitability of sunflower cultivars to *Xiphinema basiri*. *Afro-Asian Journal of Nematology* 5, 148–150.

Evans, K., Trudgill, D.L. and Webster, J.M. (eds) (1993) *Plant Parasitic Nematodes in Temperate Agriculture*. CAB International, Wallingford, UK, 648 pp.

Giblin-Davis, R.M., Cisar, J.L., Bilz, F.G. and Williams, K.E. (1992) Host status of different bermudagrasses (*Cynodon* spp.) for the sting nematode, *Belonolaimus longicaudatus*. *Supplement to Journal of Nematology* 24, 749–756.

Griffiths, B.S. and Robertson, W.M. (1984) Morphological and histochemical changes occurring during the life-span of root-tip galls on *Lolium perenne* induced by *Longidorus elongatus*. *Journal of Nematology* 16, 223–229.

Harris, A.R. (1983) Resistance of some *Vitis* rootstocks to *Xiphinema index*. *Journal of Nematology* 15, 405–409.

Henn, R.A. and Dunn, R.A. (1989) Reproduction of *Hoplolaimus galeatus* and growth of seven St Augustine grass (*Stenotaphrum secundatana*) cultivars. *Nematropica* 19, 81–87.

Hussey, R.S., Mims, C.W. and Westcott, S.W., III (1992) Ultrastructure of root cortical cell parasitized by the ring nematode *Criconemella xenoplax*. *Protoplasma* 167, 55–65.

Ismail, A.E., Hasabo, S.A. and Abdel-Massih, M.I. (1994) Reaction of some corn cultivars to the infection of *Helicotylenchus pseudodigonicus*. *Afro-Asian Journal of Nematology* 4, 161–164.

Klinger, J. (1975) Beobachtungen uber die parasitische Aktivitat des nematoden *Macrophstonia xenoplax* an Rebenwurzeln. *Zeitschrift fuer Pflanzenkrankheiten Pflanzenpathologie und Pflanzenschutz* 82, 722–728.

Lownsbery, B.F., Moody, E.H., Moretto, A., Noel, G.R. and Burlando, T.M. (1978) Pathogenicity of *Macropostonia xenoplax* to walnut. *Journal of Nematology* 10, 232–236.

Luc, M., Sikora, R.A. and Bridge, J. (1990) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, UK, 629 pp.

McSorley, R. and Gallaher, R.N. (1997) Effect of compost and maize cultivars on plant-parasitic nematodes. *Supplement to the Journal of Nematology* 29, 731–736.

Mehan, V.K., Reddy, D.D.R. and McDonald, D. (1993) Resistance in groundnut genotypes to Kalahasti malady caused by the stunt nematode, *Tylenchorhynchus brevilineatus*. *International Journal of Pest Management* 39, 201–203.

Meredith, C.P., Lider, L.A., Raski, D.J. and Ferrari, N.L. (1982) Inheritance of tolerance to *Xiphinema index* in *Vitis* species. *American Journal of Enology and Viticulture* 33, 154–158.

Pasha, M.J. and Tiyagi, S.T. (1997) Response of cauliflower cultivars/accessions to stunt nematode *Tylenchorhynchus brassicae*. *Tests of Agrochemicals and Cultivars* 13, 92–93.

Patel, S. and Patel, H.R. (1995) Screening of *Nicotiana* spp against stunt nematodes. *Tobacco Research* 21, 86–87.

Robertson, W.M., Trudgill, D.L. and Griffiths, B.S. (1984) Feeding of *Longidorus elongatus* and *L. leptocephalus* on root-tip galls of perennial ryegrass (*Lolium perenne*). *Nematologica* 30, 222–229.

Rumpenhost, H.J. and Weischer, B. (1978) Histopathological and histochemical studies on grapevine roots damaged by *Xiphinema index*. *Revue de Nématologie* 1, 217–225.

Siva Rao, D.V., Srinivasan, S. and Raja Reddy, C. (1986) Reaction of selected groundnut cultivars to nematode infection (*Tylenchorhynchus brevilineatus*) under field conditions. *Tropical Pest Management* 32, 168–169.

Smant, G., Stokkermans, J.P.W.G., Yan, Y., de Boer, J.M., Baum, T.J., Wang, X., Hussey, R.S., Gommers, F.J., Henrissat, B., Davis, E.L., Helder, J., Schjots, A. and Bakker, J. (1998) Endogenous cellulases in animals: Isolation of β-1,4-endoglucanse genes from two species of plant-parasitic nematodes. *Proceedings of the National Academy of Sciences, USA* 95, 4906–4911.

Stirling, G.R., Vawdrey, L.L. and Shannon, E.L. (1989) Options for controlling needle nematode (*Paralongidorus australis*) and preventing damage to rice in northern Queensland. *Australian Journal of Experimental Agriculture* 29, 223–232.

Streu, H.T., Jenkins, W.R. and Hutchinson, M.T. (1961) Nematodes associated with carnations. *Dianthus caryophyllus*. L. with special reference to the parasitism and biology of *Criconemoides curvatum* Raski. *New Jersey Agricultural Experimental Station Bulletin* 800.

Wang, X., Meyers, D., Yan, T., Baum, T.J., Smant, G., Hussey, R.S. and Davis, E.L. (1999) In planta localization of a beta-1,4-endoglucanase secreted by *Heterodera glycines*. *Molecular Plant–Microbe Interactions* 12, 64–67.

Westcott, S.W., III and Zehr, E.I. (1991) Evaluation of host suitability in *Prunus* for *Criconemella xenoplax*. *Journal of Nematology* 23, 393–401.

Westcott, S.W, III, Zehr, E.I., Newhall, W.C., Jr and Cain, D.W. (1994) Suitability of *Prunus* selections for the ring nematode (*Criconemella xenoplax*). *American Society for Horticultural Science* 119, 920–924.

Wyss, U. (1981) Ectoparasitic root nematodes: feeding behavior and plant cell responses. In: Zuckerman, B.M. and Rohde, R.A. (eds) *Plant Parasitic Nematodes*, Vol. III. Academic Press, New York, pp. 325–351.

Wyss, U. (1987) Video assessment of root responses to Dorylamid and Tylenchid nematodes. In: Veech, J.A. and Dickson, D.W. (eds) *Vistas on Nematology: A Commemoration to the Twenty-fifth Anniversary of the Society of Nematologists*. Society of Nematologists, Hyattsville, Maryland, pp. 211–220.

Wyss, U. (1997) Root parasitic nematodes: an overview. In: Fenoll, C., Grundler, F.M.W. and Ohl, S.A. (eds) *Cellular and Molecular Aspects of Plant–Nematode Interactions*. Kluwer Academic Publishers, Dordrecht, pp. 5–22.

Wyss, U., Lehmann, H. and Jank-Ladwig, R. (1980) Ultrastructure of modified root-tip cells in *Ficus carica*, induced by the ectoparasitic nematode *Xiphinema index*. *Journal of Cell Science* 41, 193–208.

12 Marker-assisted Selection for Soybean Cyst Nematode Resistance

N.D. Young and J. Mudge

Departments of Plant Pathology and Plant Biology, University of Minnesota, St Paul, MN 55108, USA

Because the soybean cyst nematode (SCN, *Heterodera glycines*) is such a destructive pathogen, researchers have devoted a great deal of effort to developing new resistant varieties. Traditionally, selection relies on glasshouse assays, in which progeny of crosses between a resistant donor line and a susceptible, but agronomically superior, cultivar are evaluated for response to SCN (Thomas *et al.*, 1975; Concibido *et al.*, 1994). Unfortunately, glasshouse assay systems are time-consuming, taking 35–40 days to complete. Moreover, response to SCN is strongly affected by experimental conditions, especially temperature, water status and ambient light (Anand *et al.*, 1983). Not surprisingly, there tends to be significant variation among different SCN trials, no matter how much effort is made to standardize conditions.

Recently, scientists have developed selectable genetic markers based on defined DNA sequences. DNA markers are valuable in plant breeding because they can be used to monitor the inheritance of economically important genes (Tanksley *et al.*, 1989). In the past 10 years, nearly 100 plant disease resistance loci have been mapped in relation to DNA markers, including loci that control resistance to SCN (Concibido *et al.*, 1994, 1996a,b, 1997; Mahalingham and Skorupska, 1995; Webb *et al.*, 1995; Vierling *et al.*, 1996; Chang *et al.*, 1997; Qiu *et al.*, 1997). Through the use of these DNA markers, it is rapidly becoming possible to select SCN-resistant lines based on genotype rather than phenotype. Because genotype is unaffected by environment, this approach to selection overcomes problems due to variation in environmental conditions. It also means that only a single sample

 Plant Resistance to Parasitic Nematodes (eds J.L. Starr, R. Cook and J. Bridge)

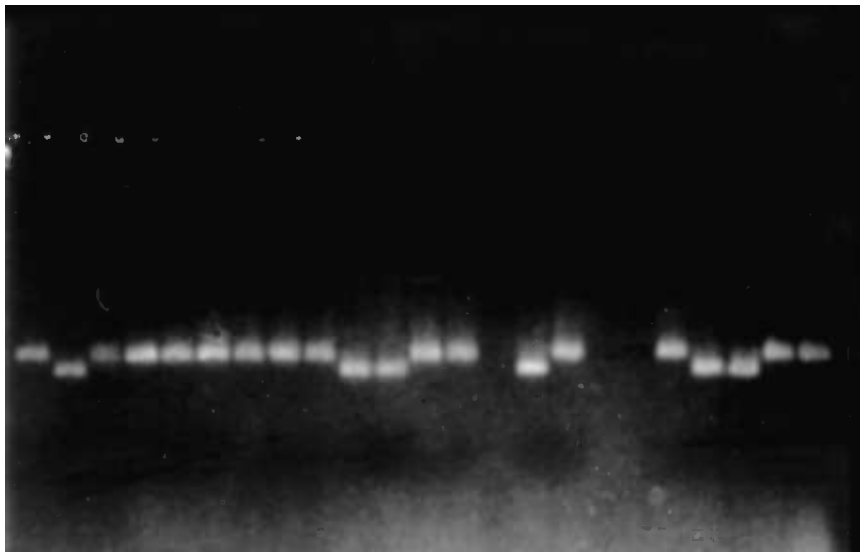

Fig. 12.1. BARC-Satt309 amplification products of selected $F_{4:5}$ progeny derived from a cross between Lambert and M92–1631. M92–1631 carries PI 209.332-derived resistance. DNA products were electrophoresed on a 3% Agarose SFR ® gel system (Amresco, Solon, Ohio) and stained with ethidium bromide. Lane 1, Lambert; lane 2, M92–1631; lanes 3–23, $F_{4:5}$ progeny. (Photo courtesy of Eric Boehlke.)

needs to be assayed per line (assuming the assay is performed on advanced generation material), rather than the multiple replicates typically required for glasshouse assays. Finally, the results of DNA analysis can be completed in a matter of days, dramatically speeding up the overall process.

Introduction to DNA Markers

With the advent of modern molecular techniques, including gel electrophoresis, DNA–DNA hybridization (Southern, 1975) and the polymerase chain reaction (PCR) (Saiki *et al.*, 1988), it has become feasible to isolate and analyse defined plant DNA segments with ease. When two soybean genotypes differ in a particular DNA sequence, molecular techniques can often be used to uncover the difference. In fact, as molecular technology has become more powerful, it has even become possible to monitor hundreds or even thousands of DNA polymorphisms between individuals using DNA markers. This means that very dense genetic maps, with markers spaced as close as 1 centimorgan (cM) apart, can be constructed (Keim *et al.*, 1997; Cregan *et al.*, 1999a).

There are four primary types of DNA markers: restriction fragment length polymorphisms (RFLPs) (Botstein *et al.*, 1980), random amplified polymorphic DNAs (RAPDs) (Williams *et al.*, 1990), amplified fragment length polymorphisms (AFLPs) (Vos *et al.*, 1995) and simple sequence repeats (SSRs or microsatellites) (Tautz, 1989; Weber and May, 1989) (Fig. 12.1). Recently, another powerful type of DNA marker has been added to this list, the single nucleotide polymorphism (SNP) (Cho *et al.*, 1999). However, since this type of DNA marker has not yet been optimized for marker-assisted selection (MAS), it will not be discussed further.

RFLPs were the first type of DNA marker and they are still especially useful in comparative genomics, the study of similarities and differences in genome organization among related taxa (Boutin *et al.*, 1995). However, RFLPs also rely on DNA hybridization and radionucleotides, which are time-consuming and tedious. For this reason, RFLP markers are generally not favoured for MAS applications.

RAPDs and AFLPs both enable quick and efficient monitoring of multiple loci. RAPDs rely on arbitrary decamer nucleotide primers – leading to the amplification of a small number of loci throughout the genome (Williams *et al.*, 1990). Because the technique is so simple, many RAPD reactions can easily be accomplished in a short time and many marker loci examined. Unfortunately, RAPD markers can also be unreliable and produce artefactual products. This generally makes them unsuitable for marker-assisted selection. However, marker systems derived from RAPDs, such as sequence characterized amplified regions (SCARs) (Paran and Michelmore, 1993), can be made very reliable and suitable for molecular breeding.

AFLPs are a more sophisticated technique that involve a combination of restriction digestion, ligation of specific oligonucleotide primer/adapter sequences to the DNA fragment ends and selective PCR (Vos *et al.*, 1995). Despite the relatively complicated technology, AFLP is still considered a 'high-throughput' technique because so many polymorphic loci can be examined in a single reaction. Even in soybean, where DNA sequence polymorphism is relatively low, ten or more informative marker loci can be analysed from a single AFLP reaction. Indeed, a genetic map composed of 600 AFLPs was constructed for soybean in less than a year (Keim *et al.*, 1997).

The marker technology of choice for MAS, especially SCN resistance, are SSRs. SSR markers tend to be highly polymorphic (Tautz, 1989; Weber and May, 1989) as well as highly reproducible. SSRs also generally define unique (rather than duplicated) positions in the genome (Cregan *et al.*, 1994). The map positions of major SCN resistance loci have been determined (Concibido *et al.*, 1994, 1996a,b; Mahalingham and Skorupska, 1995; Webb *et al.*, 1995; Vierling *et al.*, 1996; Chang *et al.*, 1997; Qiu *et al.*, 1997) and SSRs near most of these

loci are known (Mudge *et al.*, 1997; Cregan *et al.*, 1999b,c; Lightfoot, personal communication). Because of these desirable features, the MAS strategies for SCN resistance described later in this chapter are all based on SSR markers.

Marker-assisted Breeding Strategies

The use of DNA markers in plant breeding has many potential advantages over traditional breeding. Environmental conditions have little or no effect on DNA isolation and samples can be taken from very young plants, even seedlings. Moreover, strategically placed markers can potentially select for the desired genomic segment(s), while simultaneously selecting against other genomic regions with undesirable genes (Tanksley *et al.*, 1989; Young and Tanksley, 1989). This may be especially relevant in the case of SCN resistance breeding, since linkage drag to a yield depression locus has previously been reported (Mudge *et al.*, 1996).

Still, DNA marker-based selection is clearly not appropriate for all breeding and selection applications. Costs for DNA marker analysis are in the range of US$1.00–2.00 per datapoint (Denny *et al.*, 1996; Lange *et al.*, 1998). DNA marker techniques are frequently more complicated than corresponding phenotypic assays. Lastly, marker analysis takes at least a few days, whereas many phenotypic assays take only as long as visual evaluation and note-taking.

In the case of SCN resistance breeding, however, these concerns do not apply (Young, 1999). MAS is no more expensive or complicated than the glasshouse assay, and DNA markers are certainly much faster as glasshouse tests require up to 7 weeks to complete. In combination with the need for only a single sample for each (homozygous) line and the independence from environmental effects, MAS makes for a very attractive option for soybean breeders working to develop SCN resistant varieties.

DNA Marker Mapping of SCN Resistance Loci

Classical genetic studies have indicated that resistance to SCN is complex and controlled by several genes, some of which are shared by different sources of resistance. (Caldwell *et al.*, 1960; Matson and Williams, 1965; Thomas *et al.*, 1975; Hartwig, 1985; Hancock *et al.*, 1987; Anand and Rao-Arelli, 1989; Rao-Arelli *et al.*, 1989, 1992; Myers and Anand, 1991; Mansur *et al.*, 1993; Rao-Arelli, 1994). Although much has been learned about SCN resistance through classical genetic studies, segregation ratios are complicated by heterogeneity in the host

and pathogen, environmental variation, and possible effects of linkage (Luedders, 1989). Recently, molecular studies have been conducted that have greatly increased our understanding of the genetic control of SCN resistance.

One major partial resistance locus, *rhg1*, is present in most sources of resistance examined to date, including Peking (Concibido *et al.*, 1996b, 1997; Chang *et al.*, 1997), PI 209.332, PI 88.788, PI 90.763, (Concibido *et al.*, 1994, 1996a,b, 1997) and PI 437.654 (Webb *et al.*, 1995). This locus, located on molecular linkage group (MLG) 'G' (Shoemaker and Olson, 1993), generally explains more than 50% of total variation in SCN resistance. It is effective against several races of SCN, including races 1, 3 and 6 (Concibido *et al.*, 1997). Interestingly, neither Vierling *et al.* (1996), working with a population with PI 437.654- and Peking-derived resistance, nor Qiu *et al.* (1997) working with Peking-derived resistance, uncovered *rhg1* in their populations. Vierling *et al.* (1996) did, however, find a major locus for SCN resistance linked to the RFLP, A006, thought to be on MLG-B. The basis of this discrepancy is unknown.

Another SCN resistance locus, *Rhg4*, has been found to be significant for SCN resistance in several crosses (Concibido *et al.*, 1994; Webb *et al.*, 1995; Chang *et al.*, 1997; Qiu *et al.*, 1997). It was originally uncovered in classical genetic studies and was shown to be linked to the *I* locus, which controls seed coat colour (Matson and Williams, 1965; Weiss, 1970). Both *Rhg4* and the *I* locus have been placed on MLG-A (Keim *et al.*, 1990). In a cross of PI 437.654 × BSR101, Webb *et al.* (1995) found that the MLG-G and MLG-A loci together conferred complete resistance to race 3, but individually conferred only modest gains in resistance over the susceptible parent. In addition to loci on MLG-G and MLG-A, several minor loci for SCN resistance have also been reported (Concibido *et al.*, 1994, 1996a, 1997; Mahalingham and Skorupska, 1995; Webb *et al.*, 1995; Vierling *et al.*, 1996; Qiu *et al.*, 1997).

Methods for Marker-assisted Selection for SCN Resistance

Most methods of MAS for resistance to SCN have focused on *rhg1* because of its large and generally consistent effects across SCN races and resistance sources (Concibido *et al.*, 1997). SSRs have been the marker system of choice for MAS at *rhg1*. The region surrounding this locus has been saturated with SSRs both by random and targeted methods (Mudge *et al.*, 1997; Cregan *et al.*, 1999b,c). Currently, there are 16 SSR markers within a 20 cM stretch surrounding *rhg1* on MLG-G and more are being developed (Cregan, personal communication).

Because the SSRs are so tightly linked to *rhg1*, MAS for *rhg1* can be performed with relatively low risk of crossover. This reduces the need to assay flanking markers, which increases the cost and labour. Several SSRs have been placed on either side of the locus, so selection to reduce linkage drag can be performed with the next closest markers (Tanksley *et al.*, 1989; Young and Tanksley, 1989). This is especially important in light of the apparent existence of a linked yield depression locus (Mudge *et al.*, 1996). SSRs are now available that differentiate alleles from resistant genotypes from most, if not all, commonly used susceptible genotypes (Cregan *et al.*, 1999b).

Several SSRs have been used for MAS and *rhg1*. The most tightly linked markers, and therefore those used most predominantly, have all been on the distal side of *rhg1* and include BARC-Satt038 (Mudge *et al.*, 1997), BARC-Satt309 (Fig. 12.1) and BARC-Sat_168 (Cregan *et al.*, 1999b). Between BARC-Satt309 and BARC-Sat_168, which are within 1 cM of *rhg1*, all the resistant sources typically used in breeding programmes can be differentiated from most susceptible genotypes (Cregan *et al.*, 1999b). In many crosses, these SSRs can be assayed on agarose, rather than polyacrylamide (Table 12.1; Fig. 12.1). Agarose gel electrophoresis requires less labour and eliminates the need to work with harmful chemicals such as polyacrylamide, formamide and radioactivity. Polyacrylamide gels do, however, allow better separation of DNA bands and require less product for loading. Still, for the high-throughput needs of MAS, agarose gels are the system of choice.

All three of these SSRs have shown greater than 95% accuracy in predicting SCN resistance in mapping populations based on a qualitative score (Mudge *et al.*, 1997; Cregan *et al.*, 1999b). In these studies, lines that showed greater than 30% of the number of cysts on the susceptible check were classified as susceptible and below 30% as resistant. However, MAS using BARC-Satt309 on breeding populations that had gone through some selection for agronomic characters seemed to be less efficient (Mudge *et al.*, personal observation). In this case, BARC-Satt309 efficiently selected against lines that were *not* resistant, based on the 30% cyst index cutoff, but was less efficient at selecting for resistant lines. This may have been due to the fact that *rhg1* alone was not sufficient for resistance. Even with these limitations, MAS is still very valuable in reducing population sizes, though lines kept in a programme must still be tested phenotypically for resistance. A reduced number of lines with the resistant parent's allele at markers near *rhg1* was also observed by Lightfoot *et al.* (1998). The use of MAS for SCN resistance in early generations and before extensive agronomic selection can potentially prevent discarding large numbers of resistant lines.

Markers tightly linked to *Rhg4* on MLG-A have also been described and used for MAS in combination with *rhg1*. Webb *et al.* (1995)

examined the efficiency of MAS using markers linked to both *rhg1* and *Rhg4* to test lines in a mapping population with PI 437654-derived resistance. All lines with the resistant parent allele at both loci were resistant, although a small number of resistant lines were missed with this method (Webb *et al.*, 1995). Weisemann *et al.* (1992) uncovered

Table 12.1. A sample protocol for performing MAS for SCN resistance. The example uses the DNA disc extraction, SSR amplification with BARC-Satt309 and agarose gel electrophoresis based on Lange *et al.* (1998) and Cregan *et al.* (1999b).

DNA disc extraction	**1.** Collect fresh leaves. (The age of the leaf seems to make little difference.) **2.** Cut leaves to desired size (1–2 cm^2). With larger leaf cuttings, fewer samples can be placed on each card, but more discs can be obtained from each sample. **3.** Place miracloth (Cal Biochem, La Jolla, California) over the collection surface of the DNA extraction card (Gentra Systems, Minneapolis, Minnesota). Arrange leaf samples on miracloth and cover with a plastic transparency. **4.** Rub leaves on to collection surface using a pestle. Discard miracloth, leaves, and plastic. **5.** Punch discs from the leaf rubbings into a 96-well microtitre plate using a 1/8 in. hole punch. **6.** Add 100 μl of Generation DNA Purification Solution ® (Gentra Systems) to each well. Remove solution after 15 min. (In order to remove as much solution as possible, we prefer to centrifuge the solution out through small holes in the bottom of each well. Liquid is collected in a lid for a 96-well plate placed under the samples.) Alternatively, liquid can be removed using a vacuum manifold or a pipetter. Repeat three times. **7.** Add 100 μl of ethanol. Remove after 1 min. **8.** Dry discs at 60°C for 30 min or at room temperature overnight. **9.** If microtitre plates have holes in the bottom for centrifuging, discs must be transferred into a new plate or strip tubes for SSR amplification. This can be done either one at a time using forceps or by placing the new plate or tubes upside down over the other plate, inverting the plates and gently tapping the discs into the new wells.
SSR amplification	**1.** Prepare cocktail with the following concentrations of reagents: 1 mM $MgCl_2$; 100 μM each dNTP; 0.2 μM each forward and reverse primers for BARC-Satt309 primer sequences (see Cregan *et al.*, 1999b);1X buffer; 0.1 U μl^{-1} of reaction of Taq. **2.** Add 21 μl of cocktail to each disc. **3.** Place samples in thermocycler. Complete 32 cycles of the following: 94°C for 25 s, 47°C for 25 s and 68°C for 25 s.
Agarose gel electrophoresis	**1.** Separate on a 3% agarose gel in TBE (see Sambrook *et al.*, 1989) according to the manufacturer's instructions. Gels should be made of a high resolution agarose such as Agarose SFR ® (Amresco, Solon, Ohio) or Metaphor ® (FMC, Rockland, Maine). **2.** Stain with ethidium bromide. Figure 12.1 shows an example of BARC-Satt309 electrophoresed on an agarose gel.

markers linked to *Rhg4* by finding markers linked to the *I* locus and these have subsequently been used for MAS (Lightfoot *et al.*, 1998).

Other regions of the genome thought to contain loci that directly or indirectly affect SCN resistance may ultimately be used for MAS as well. Because the effects of these loci are small, methods such as Perkin-Elmer Taqman ® or DNA 'chips', where several loci can be assayed quickly, will be necessary to justify the added effort.

In any MAS programme, it is necessary to screen a large number of lines. DNA extraction can be very labour-intensive. Traditional methods of extraction require a large amount of plant material for extraction, especially when markers such as RFLPs are used. Extracting DNA from thousands of soybean lines in an SCN breeding programme can be especially daunting. Several methods have been developed recently by researchers working on SCN resistance to speed up the process of DNA extraction and use less plant material (Lange *et al.*, 1998; Lightfoot *et al.*, 1998). These methods even allow researchers to collect samples for DNA extraction from seedlings. Thus, lines can be screened with molecular markers and decisions about which to harvest can be made while the plants are still growing, greatly reducing the number of plots that need to be individually harvested.

One of these DNA extraction methods has been described by Lange *et al.* (1998) (Table 12.1). In this method, leaf imprints are rubbed on to filter cards (Gentra Systems, Minneapolis, Minnesota), which can be easily catalogued or bar-coded. The cards can be stored for at least a year at 4° or 20°C. Obtaining DNA from winter nurseries or remote stations is easily accomplished because leaf imprints can be rubbed on site without the need for laboratory equipment or chemicals and then the cards can be shipped at room temperature to a central laboratory facility. To purify the DNA, small discs (< 6 mm diameter) are punched from the card with a hole punch followed by three 15 min washes with an extraction solution (Gentra Systems) and an ethanol wash in a 96-well plate. Dried discs can be used directly in a PCR reaction or stored at room temperature. The main drawback of this method is the inability to bulk samples, which would allow detection of segregating lines in a single reaction. Still, the ease of storing and tracking samples, along with the ability to extract DNA quickly and easily from seedlings, makes this an attractive alternative to traditional DNA extraction methods.

Other methods of DNA extraction have also been used for MAS for SCN resistance. Lightfoot *et al.* (1998) used a method that yields DNA in the traditional liquid form. The method starts with a hole-punch of fresh or frozen leaf material that is extracted 96 samples at a time using

a so-called 'matrix mill' that grinds tissue in NaOH. This method allows bulking of DNA and the DNA is stored in tubes in liquid form. The initial cost of the matrix mill makes this method initially more expensive than discs, although the cost per sample is eventually less. Methods of extraction from seeds that do not affect germination ability are also being developed (Lightfoot *et al.*, 1998).

Perspectives on Marker-assisted Selection

In the future, marker-assisted selection for SCN resistance is likely to become much more efficient. Two key advances can be expected: (i) improved mapping of relevant SCN resistance loci in relation to DNA markers and (ii) improved DNA marker technologies. Of special interest are those marker systems that do not require gel electrophoresis, including the method known as 'Taqman' ® (Perkin-Elmer) (Lee *et al.*, 1993; Livak *et al.*, 1995). Using this technology, researchers are starting to optimize detection of SNPs and other PCR-based markers. In this technique, a segment of genomic DNA surrounding the SNP is amplified by PCR in a thermocycler. Two probes, reflecting the nucleotide difference, are included in the reaction. Each probe has a unique reporter dye plus a quencher dye that suppresses the fluorescence of the reporter dye. If the sequence for one of the probes is present in the amplification product, the probe will hybridize and its 3′ quencher dye will be cleaved off by a second enzyme, leaving the reporter dye to fluoresce without interference. The results of amplification can be read by computer and a fluorescence detection system, thereby avoiding the tedious work of gel electrophoresis. SNP markers linked to the major SCN resistance are currently being sought (Grimm *et al.*, 1998). Indeed, it may not be too long before 'DNA chips' capable of genotyping individuals throughout the entire genome in a single step may become available. Already DNA chips that genotype the genome of yeast have been described (Winzeler *et al.*, 1998).

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References

Anand, S., Brar, G. and Gallo, K. (1983) Screening for cyst nematode resistance in soybean breeding *Heterodera glycines*. *Soybean Genetics Newsletter* 10, 63–66.

Anand, S.C. and Rao-Arelli, A.P. (1989) Genetic analyses of soybean genotypes resistant to soybean cyst nematode race 5. *Crop Science* 29, 1181–1184.

Botstein, D., White, R.L., Skolnick, M.H. and Davis, R.W. (1980) Construction of a genetic map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32, 314–331.

Boutin, S.R., Young, N.D., Olson, T., Yu, Z.-H., Shoemaker, R. and Vallejos, C. (1995) Genome conservation among three legume genera detected with DNA markers. *Genome* 38, 928–937.

Caldwell, B.E., Brim, C.A. and Ross, J.P. (1960) Inheritance of resistance of soybeans to cyst nematode, *Heterodera glycines*. *Agronomy Journal* 52, 635–636.

Chang, S., Doubler, T., Kilo, V., Abuthredeih, J., Prabhu, R., Freire, V., Suttner, R., Klein, J., Schmidt, M., Gibson, P. and Lightfoot, D. (1997) Association of loci underlying field resistance to soybean sudden death syndrome (SDS) and cyst nematode (SCN) race 3. *Crop Science* 37, 965–971.

Cho, R.J., Mindrinos, M., Richards, D.R., Sapolsky, R.J., Anderson, M., Drenkard, E., Dewdney, L., Reuber, T.L., Stammers, M., Federspiel, N., Theologis, A., Yang, W.H., Hubbell, E., Au, M., Chung, E.Y., Lashkari, D., Lemieux, B., Dean, C., Lipshutz, R.J., Ausubel, F.M., Davis, R.W. and Oefner, P.J. (1999) Genome-wide mapping with biallelic markers in *Arabidopsis thaliana*. *Nature Genetics* 23, 203–207.

Concibido, V.C., Denny, R.L., Boutin, S.R., Hautea, R., Orf, J.H. and Young, N.D. (1994) DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). *Crop Science* 34, 240–246.

Concibido, V., Denny, R., Lange, D., Orf, J. and Young, N.D. (1996a) RFLP mapping and marker-assisted selection of soybean cyst nematode resistance in PI 209332. *Crop Science* 36, 1643–1650.

Concibido, V., Young, N., Lange, D., Denny, R., Danesh, D. and Orf, J. (1996b) Targeted comparative genome analysis and qualitative mapping of a major partial-resistance gene to the soybean cyst nematode. *Theoretical and Applied Genetics* 93, 234–241.

Concibido, V., Lange, D., Denny, R., Orf, J. and Young, N.D. (1997) Genome mapping of soybean cyst nematode resistance genes in Peking, PI 90763, and PI 88788 using DNA markers. *Crop Science* 37, 258–264.

Cregan, P.B., Bhagwat, A.A., Akkaya, M.S. and Rongwen, J. (1994) Microsatellite fingerprinting and mapping of soybean. *Methods in Molecular and Cellular Biology* 5, 49–61.

Cregan, P., Jarvik, T., Bush, A., Shoemaker, R., Lark, K., Kahler, A., Kaya, N., VanToai, T., Lohnes, D., Chung, J. and Specht, J. (1999a) An integrated genetic linkage map of the soybean genome. *Crop Science* 39, 1464–1490.

Cregan, P.B., Mudge, J., Fickus, E.W., Danesh, D., Denny, R. and Young, N.D. (1999b) Two simple sequence repeat markers to select for soybean cyst nematode resistance Conditioned by the *rhg1* locus. *Theoretical and Applied Genetics* 99, 811–818.

Cregan, P.B., Mudge, J., Fickus, E.W., Marek, L.F., Danesh, D., Denny, R.L., Shoemaker, R.C., Matthews, B.F., Jarvik, T. and Young, N.D. (1999c) Targeted isolation of simple sequence repeat markers through the use of bacterial artificial chromosomes. *Theoretical and Applied Genetics* 98, 919–928.

Denny, R.L., Lange, D.L., Penuela, S., Mudge, J., Orf, J.H. and Young, N.D.

(1996) Marker-assisted selection for soybean cyst nematode resistance. *Soybean Genetics Newsletter* 23, 179–182.

Grimm, D.R., Danesh, D., Mudge, J., Young, N.D. and Cregan, P.B. (1998) Assessment of single nucleotide polymorphisms (SNPs) in soybean. (Abstract) *7th Biennial Conference on the Molecular and Cellular Biology of the Soybean*, 26–29 July, 1998. Knoxville, Tennessee.

Hancock, J.A., Hancock, F.G., Caviness, C.E. and Riggs, R.D. (1987) Genetics of resistance in soybean to 'race X' of soybean cyst nematode. *Crop Science* 27, 704–707.

Hartwig, E.E. (1985) Breeding productive soybeans with resistance to soybean cyst nematode. In: Shibles, R.A. (ed.) *World Soybean Research Conference III*. Westview Press, Boulder, Colorado, pp. 394–399.

Keim, P., Diers, B.W., Olson, T.C. and Shoemaker, R.C. (1990) RFLP mapping in soybean, association between marker loci and variation in quantitative traits. *Genetics* 126, 735–742.

Keim, P., Schupp, J., Travis, S., Clayton, K., Zhu, T., Shi, L., Ferreira, A. and Webb, D. (1997) A high-density soybean genetic map based on AFLP markers. *Crop Science* 37, 537–543.

Lange, D., Penuela, S., Denny, R., Mudge, J., Concibido, V., Orf, J. and Young, N.D. (1998) A plant DNA isolation protocol suitable for polymerase chain reaction based marker-assisted breeding. *Crop Science* 38, 217–220.

Lee, L., Connell, C. and Bloch, W. (1993) Allelic discrimination by nick-translation PCR with fluorogenic probes. *Nucleic Acids Research* 21, 3761–3766.

Lightfoot, D.A., Meksem, K., Garvey, G. and Bell-Johnson, B. (1998) *Workshop on Biotechnology Approaches to Improve Resistance to SCN*. United Soybean Board, St. Louis, Missouri.

Livak, K., Flood, S.J.A., Marmaro, J., Giusti, W. and Deetz, K. (1995) Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods and Applications* 4, 357–362.

Luedders, V. (1989) Inheritance of genes for resistance to soybean cyst nematode populations. *Crop Science* 29, 667–671.

Mahalingham, R. and Skorupska, H. (1995) DNA markers for resistance to *Heterodera glycines* Race 3 in soybean cultivar Peking. *Breeding Science* 45, 435–443.

Mansur, L.M., Carriquiry, A.L. and Rao-Arelli, A.P. (1993) Generation mean analysis of resistance to race 3 of soybean cyst nematode. *Crop Science* 33, 1249–1253.

Matson, A.L. and Williams, L.F. (1965) Evidence of a fourth gene for resistance to soybean cyst nematode. *Crop Science* 5, 477.

Mudge, J., Concibido, V.C., Denny, R.L., Young, N.D. and Orf, J.H. (1996) Genetic mapping of a yield depression locus near a major gene for soybean cyst nematode resistance. *Soybean Genetics Newsletter* 23, 173–178.

Mudge, J., Cregan, P.B., Kenworthy, J.P., Kenworthy, W.J., Orf, J.H. and Young, N.D. (1997) Two microsatellite markers that flank the major soybean cyst nematode resistance locus. *Crop Science* 37, 1611–1615.

Myers, G. and Anand, S. (1991) Inheritance of resistance and genetic relationships among soybean plant introductions to races of soybean cyst nematode. *Euphytica* 55, 197–201.

Paran, I. and Michelmore, R. (1993) Development of reliable PCR based markers linked to downy mildew resistance genes in lettuce. *Theoretical and Applied Genetics* 85, 985–993.

Qiu, B., Sleper, D. and Arelli, A. (1997) Genetic and molecular characterization of resistance to *Heterodera glycines* race isolates 1, 3, and 5 in Peking. *Euphytica* 96, 225–231.

Rao-Arelli, A. (1994) Inheritance of resistance to *Heterodera glycines* race 3 in soybean accessions. *Plant Disease* 78, 898–900.

Rao-Arelli, A.P., Anand, S.C. and Myers, G.O. (1989) Partial dominance of susceptibility in soybean to soybean cyst nematode races 3, 4, and 5. *Crop Science* 29, 1562–1564.

Rao-Arelli, A.P., Anand, S.C. and Wrather, J.A. (1992) Soybean resistance to soybean cyst nematode race 3 is conditioned by an additional dominant gene. *Crop Science* 32, 862–864.

Saiki, R., Gelfand, D., Stoffel, S., Scharf, S., Higuchi, R., Horn, G., Mullis, K. and Erlich, H. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239, 487–491.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning, a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, p. 6.12.

Shoemaker, R.C. and Olson, T.C. (1993) Molecular linkage map of soybean (*Glycine max* L. Merr.). In: O'Brien, S.J. (ed.) *Genetic Maps: Locus Maps of Complex Genomes*. Cold Spring Harbor Press, Cold Spring Harbor, New York, pp. 6.131–6.138.

Southern, E. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* 98, 503–517.

Tanksley, S.D., Young, N.D., Patterson, A.H. and Bonierbale, M.W. (1989) RFLP mapping in plant breeding: new tools for an old science. *Biotechnology* 7, 257–264.

Tautz, D. (1989) Hypervariability of simple sequences as general for polymorphic DNA markers. *Nucleic Acids Research* 17, 6463–6472.

Thomas, J.D., Caviness, C.E., Riggs, R.D. and Hartwig, E.E. (1975) Inheritance of reaction to a race 4 of soybean-cyst nematode resistance gene, *Rhg4*. *Crop Science* 15, 208–210.

Vierling, R., Faghihi, J., Ferris, V. and Ferris, J. (1996) Association of RFLP markers with loci conferring broad-based resistance to the soybean cyst nematode (*Heterodera glycines*). *Theoretical and Applied Genetics* 92, 83–86.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995) AFLP, a new technique for DNA fingerprinting. *Nucleic Acids Research* 23, 4407–4414.

Webb, D.M., Baltazar, B.M., Rao-Arelli, A.P., Schupp, J., Clayton, K., Keim, P. and Beavis, W.D. (1995) Genetic mapping of soybean cyst nematode race-3 resistance loci in the soybean PI 437.654. *Theoretical and Applied Genetics* 91, 574–581.

Weber, J. and May, P. (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* 44, 388–396.

Weisemann, J.M., Matthews, B.F. and Devine, T.E. (1992) Molecular markers located proximal to the soybean cyst nematode resistance gene, *Rhg4*. *Theoretical and Applied Genetics* 85, 136–138.

Weiss, M.G. (1970) Genetic linkage in soybeans, linkage group VII. *Crop Science* 10, 627–629.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18, 7213–7218.

Winzeler, E.A., Richards, D.R., Conway, A.R., Goldstein, A.L., Kalman, S., McCullough, M.J., McCusker, J.H., Stevens, D.A., Wodicka, L., Lockhart, D.J. and Davis, R.W. (1998) Direct allelic variation scanning of the yeast genome. *Science* 281, 1194–1197.

Young, N.D. (1999) A cautiously optimistic vision for marker-assisted breeding. *Molecular Breeding* 5, 505–510.

Young, N.D. and Tanksley, S.D. (1989) RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. *Theoretical and Applied Genetics* 77, 353–359.

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