# Management of plant parasitic nematodes

D.L. Trudgill

The research of the SCRI Nematology Group has a high international profile. In 1998, it organized the 24th International Nematology Symposium which attracted 400 delegates from all over the world. The SCRI has a world-wide reputation for research on nematode vectors of plant viruses, on potato cyst nematodes (PCN, *Globodera pallida* and *G. rostochiensis*) and for fundamental studies on nematode genetics and secretions. In the past 5 years, we have been involved in seven EU-funded shared cost projects (value to SCRI >£1 million), two grants from the British Potato Council (£250,000), two Link project (£300,000), and various other grants and commercial contracts (>£100,000). Currently, there are eight Ph.D. students in Nematology.

**Nematode damage is increasing - nematologists are decreasing** Nematodes are hidden in the soil and, consequentially, the damage they cause as crop pests, and their beneficial role in helping cycle soil nutrients, is not widely appreciated. The importance of nematodes to agriculture is continually increasing. As agriculture has become progressively more intensive throughout the world, so the area of nematode infested land, and levels of infestation have increased. Nematicide use has also increased, with all the associated costs, including increased damage to the environment. This has already led to some nematicides being banned, the use of others being restricted, and a search for more acceptable methods of controlling nematodes. In contrast to the increasing importance of nematodes, the number of applied nematologists is decreasing, and there is a swing to more fundamental studies.

Figure 1 *Caenorhabditis elegans* - the first metazoan to have its complete genome sequenced.

Molecular approaches have attracted considerable funding in recent years. However, rapid advances in molecular techniques, particularly in the rate at which DNA can be sequenced, are creating new demands. Sequencing the entire expressed genome of the 'model' nematode *Caenorhabditis elegans* (Fig. 1) could now be done in 3 months, and sequencing of almost the entire human genome was achieved almost 2 years sooner than initially anticipated. But, as almost half of the genes sequenced so far have no known analogues, their functional analysis is now an increasing priority. Progress will be greatest if disciplines collaborate (especially molecular and biological/ecological), and if increased priority is given to biologists and ecologists. As SCRI is a multi-disciplinary Institute, it is well placed to be at the forefront of such research.

**Three reasons for nematological research** Soil nematodes are of research interest because :

1) nematodes are also a major component of the soil microfauna, and differences in their occurrence, abundance and community structure have the potential to provide unique information regarding the soil environment;

2) they are of practical interest because of the damage caused by plant parasitic species;

3) they are of fundamental interest because
   i) *C. elegans* was the first animal to have its genome sequenced,
   ii) many of the most damaging plant parasitic nematodes have evolved complex interactions with their hosts.

Additionally, the infective stages of many of the nematodes which parasitise mammals, arthropods and molluscs, live in the soil.

Ecology/soil processes

Nematode are the most abundant animals in the world. Soil populations are typically *c.* 100 billion (1 x $10^{11}$) per ha. Their communities typically comprise more than 50 species, including plant parasites, the juvenile stages of parasites of animals and insects, fungal and bacterial feeders, omnivores and predators. They are potentially holistic indicators of soil processes as they are active within the soil throughout the whole of the year and are much easier to extract, count and study than bacteria or fungi. The different trophic groups can usually be recognised from differences in the structure of their feeding apparatus. Soil nematodes also comprise different ecological groups. Some have short life cycles and potentially rapid rates of population increase (*r* strategists), others have long generation times and reproduce slowly (*K* strategists). We have shown that the majority of soil nematodes are beneficial as they help mineralise and cycle nitrogen and other plant nutrients, or parasitise fungal, insect and mollusc pests. Only plant and animal parasites are harmful. Two examples from our research of the wider value of studying nematode ecology are given below.

Temperature and development (thermal-time) We showed that there is a linear relationship between temperature and rates of nematode development (Fig. 2). This enabled us to determine the specific thermal-time requirements of nematodes for different developmental processes (Table 1). The cardinal values for development are the base temperature ($T_b$ - below which there is no development) and the heat sum (*S*) (expressed in $^{o}$C days above $T_b$). Differences between nematode species, some derived from the literature, are given in Table 1. It is apparent that values for $T_b$ vary and that some nematodes have a much higher rates of development (smaller value of *S*) than others, *e.g.* the free-living, bacterial-feeding species *C. elegans* can have four generations (43$^{o}$C days per generation) in the time required for an egg of the plant-parasitic *Longidorus elongatus* to hatch (154$^{o}$C days).

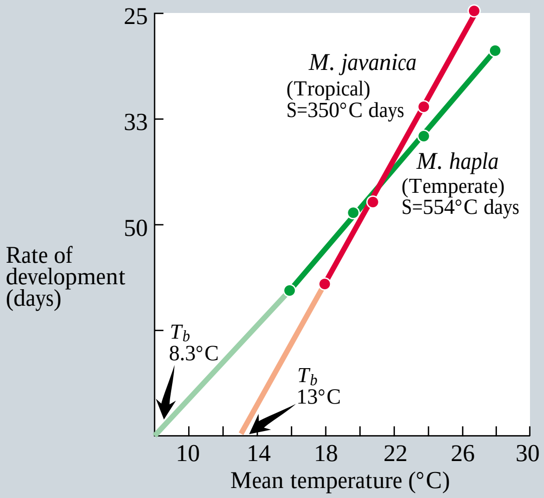


**Figure 2** Relation between temperature and rates of development of a tropical *(Meloidogyne javanica)* and temperate *(M. hapla)* nematode.

| Species (food source) | *Tb* (°C) | *S* (°C days) |
|---|---|---|
| | Embryogenesis | |
| *Haemonchus contortus* (animal parasite) | 6.6 | 15 |
| *Aphelenchus averne* (fungal feeder) | 8.3 | 31 |
| *Meloidogyne javanica* (plant parasite) | 13.0 | 138 |
| *Longidorus elongatus* (plant parasite) | 8.5 | 154 |
| | Complete life cycle | |
| *Caenorhabditis elegans* (bacterial feeder) | 5.3 | 43 |
| *Goodeyus ulmis* (bacterial/fungal feeder) | 0.9 | 114 |
| *Meloidogyne hapla* (plant parasite) | 8.3 | 554 |
| *Meloidogyne javanica* (plant parasite) | 13.1 | 343 |

**Table 1** Base temperature *(Tb)* and thermal requirements (*S*; °C days) for embryogenesis and one generation of different nematodes.

Thermal time has been applied to show that root-knot nematodes can have several generations on crops growing in tropical conditions, that potato cyst nematode can be controlled by trap crops lifted at the correct time, and that the northern root-knot nematode (RKN; *Meloidogyne hapla*) does not pose a threat in Scotland. But its most important contribution has been the general hypothesis that differences in *S* within many poikilothermic groups (nematodes, insects, plants) often reflect differences in their ecological strategies and, more widely, that differences in $T_b$ reflect the thermal environment to which each species is adapted. A comparison of the requirements of two species of RKN, temperate *M. hapla* and tropical *M. javanica*, demonstrated that there was an inverse relationship between $T_b$ and *S*, so that as one increases, the other decreases (Table 1). This ensures that each species develops faster than its relatives in the environ-

| | Temperature (°C) 10 | 15 | 20 | 25 |
|---|---|---|---|---|
| | No of days | | | |
| *Meloidogyne hapla* (temperate) | 316 | 82 | 47 | 33 |
| *Meloidogyne javanica* (tropical) | No development | 171 | 49 | 29 |

**Table 2** Effect of temperature on minimum duration of one generation for a temperate and tropical species of nematode.

ment to which it is adapted, *e.g.* temperate *M. hapla* develops fastest than tropical *M. javanica* below 21°C, but the converse occurs above 21°C (Table 2).

Nematode communities and populations Agricultural soils are one of our most valuable natural resources, and their long-term, sustainable management is crucial. However, modern agriculture makes increasing demands on soil, including expecting it to cope with a range of 'pollutants'. These include deposition of 'diffuse' pollutants from the atmosphere and a wide variety of pesticides. British soils receive about 30,000 t of pesticides per annum, but we have an incomplete understanding of their long-term, wider effects. Whilst soil nematodes are potentially a holistic indicator of soil conditions, we lack a good understanding of the factors that regulate their community structure and population densities. In 1997 and 1998, large patches of poor growth were observed in some Scottish cereals (Fig. 3). One area of damage was investigated and found to be heavily infested with a migratory ectoparasitic nematode (*Tylenchorhynchus* spp.) whose numbers had increased to 20,000 per kg soil (60 billion per ha), many times greater than is usual. Why this should have happened is unknown, but other plant parasitic nematodes were scarce, suggesting that the community was unbalanced. The

Figure 3 Damage by *Tylenchorhynchus* spp. to spring barley.

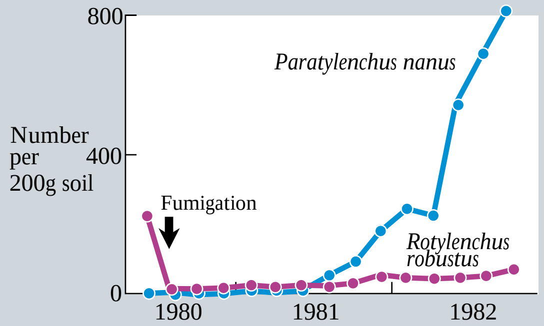


**Figure 4** Rapid increase of *Paratylenchus nanus* (r strategist) after fumigation to control *Rotylenchus robustus* (K strategist).

results from some trials with soil fumigants indicate that the numbers of some *r* strategy species of nematodes (e.g. *Paratylenchus nanus*) can increase rapidly after treatment to levels much greater than previously (Fig. 4), suggesting that the *K* strategy species (e.g. *Rotylenchus robustus*) suppress the *r* strategists and maintain a balance.

## Nematodes as crop pests

Nematode problems are increasing Modern agricultural practices actively spread nematodes in soil moved by machinery and irrigation water, and by the movement of infected plant material. In the 1970s and 1980s, the two species of potato cyst nematode (PCN) were estimated to infest less than 40% of potato fields in the UK, and the white species (wPCN; *G. pallida*) was much less common than the yellow species (yPCN; *G. rostochiensis*). The widespread and repeated growing of potato cultivars resistant only to yPCN has progressively changed the distribution and importance of the two species. A recent survey of 500 potato fields in England and Wales showed that 64% are now infested compared with only 40% a decade ago, and the white species was now present in 92% of infestations[2].

Similar changes involving other nematodes are occurring throughout the world. Examples include the cropping of virgin land in Burkina Faso, where damage due to RKN, *Meloidogyne* spp. typically appears in the second or third crop (Sawadago, pers. comm.). In Ecuador, a survey coordinated by SCRI of 207 horticultural crops showed that 99% were RKN infested. In the Netherlands, a serious pest of potato in northwest America, the Columbian RKN (*M. chitwoodi*), has recently been identified. The pine wilt nematode (*Bursaphelenchus xylophilus*), which has killed millions of trees in Japan and China, has just been reported from Portugal. A Chinese Ph.D. student in Nematology, Miss Qing Chen, has just returned from China and reports that the pine forests around her hometown have been devastated since her last visit home (Fig. 5).

Figure 5 Scots pine showing damage caused by *Bursaphelenchus xylophilus* (Figure courtesy of Nemapix).

Once a damaging nematode has been introduced, intensive agriculture, often involving the frequent cropping of good hosts, generally increases the problem. Improved husbandry, which increases plant growth and yields, also provides more food for nematodes, further increasing nematode populations and damage. The difficulties of managing such problems are discussed below.

**Nematicides and the problems of controlling nematodes in soil** Chemical nematicides are the only means available for most farmers to actively protect their crops from nematode damage. Their use can be viewed from several perspectives. The perspective presented here is that, because nematodes are in soil, they are exceptionally difficult to control with chemicals and, consequently, rates of nematicide application are much higher than for other pesticides. Nematode populations are often huge; populations of PCN have to be >3,000 million eggs per ha before they cause damage, and may exceed 300,000 million per ha. To control such large populations, nematicides have to be mixed thoroughly in the soil. This is a considerable technical challenge because the cultivated soil-layer in each ha weighs *c.* 3000 t and contains up to 500 t of water, 100 t of organic matter, 800 $m^3$ of air, but <10 kg of nematodes (Fig. 6).

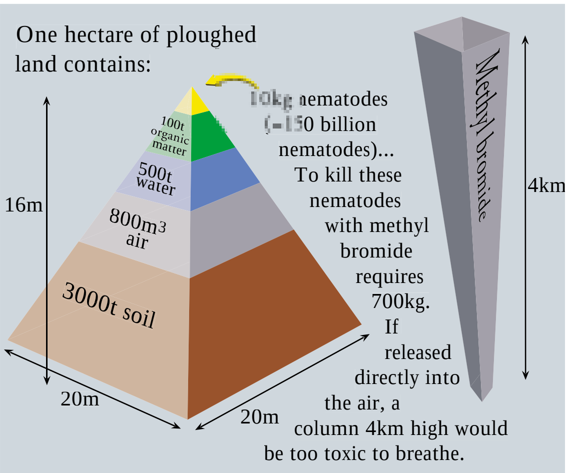


**Figure 6** Schematic representation of the relative components of 1 ha of soil, and the volume of air that would exceed 17ppm methyl bromide following a standard rate of methyl bromide.

**Nematicides – toxicity** There are of two types of nematicide; fumigants that release toxic gases into the soil, and non-fumigants whose active ingredients dissolve and move in the soil water. Nematicides must remain active in the soil for sufficient time to exert the control required. For potato crops infested with wPCN, granular nematicides have to be active for >6 weeks to cover the extended hatching period. To try and over-come problems of incorporation, movement and persistence, nematicides are applied at much greater rates than pesticides applied to control foliar pathogens. Also, some nematicides are exceptionally toxic – only 1 mg of aldicarb (Temik 10G, widely used in the UK on carrots, potatoes and sugar beet) is needed to kill a rat weighing 1 kg (recommended rates of application are up to 3.3 kg a.i. per ha). The fumigant methyl bromide (used to control a range of soil-borne pathogens, particularly RKN, *Meloidogyne* spp.) is both very toxic and applied at a high rate (up to 700 kg per ha). If the recommended rate of methyl bromide was mixed into the atmosphere rather than in

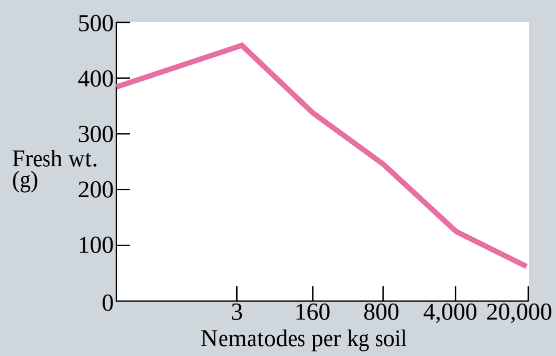


**Figure 7** Effect of different numbers of *Meloidogyne incognita* at planting on the total fresh weights of tomato plants after 135 days.

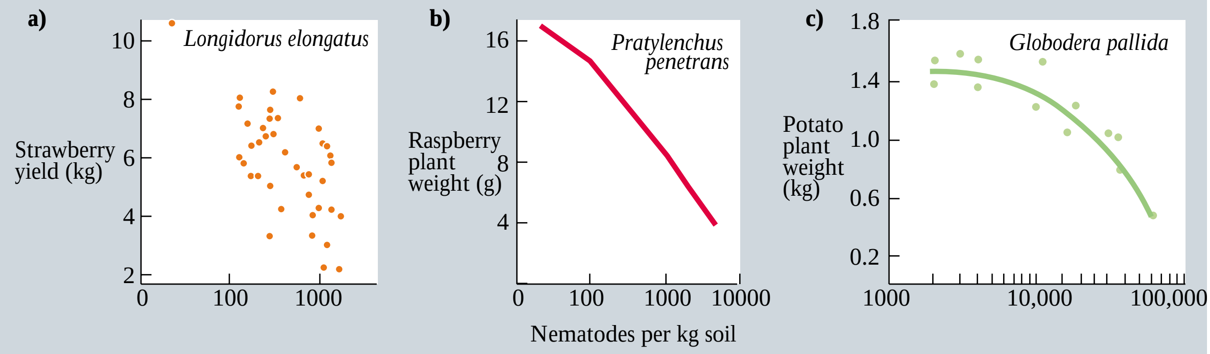


**Figure 8** Relation between numbers of nematodes at planting (log scale) and yield for (a) strawberry infested with *Longidorus elongatus*, (b) raspberry plants in pots with *Pratylenchus penetrans* and, (c) potatoes with white potato cyst nematode *(Globodera pallida)*.

the soil, then a column of air about 4 km high above each treated field would exceed the 17 ppm limit above which a respirator should be worn (Fig. 6).

Environmental concerns Toxicity does not necessarily imply an unacceptable threat to consumers or the environment. In practice, where it is approved for use, aldicarb degrades rapidly to non-toxic products and is used safely. But fumigant nematicides, particularly methyl bromide, which is the third most important ozone-destroying chemical, have recognised environmental costs. The UK is a relatively small user of methyl bromide, but elsewhere much larger amounts are used. In Crete, 600 t are used annually, mainly to control RKN damage and wilt diseases, and the USA uses c. 16,000 t. Under the amended Montreal Protocol, the production and use of methyl bromide is being phased-out in most countries, but is still increasing in others[3]. Consequently, what is done to control nematodes in one part of the world can be of direct relevance to the others. Our contribution to developing a biocontrol agent effective against RKN, which could help replace methyl bromide, will be discussed later.

Nematicides also have a direct financial cost. Consequently, farmers are also interested in the development of strategies to effectively manage soil nematodes whilst minimising nematicide use. To be effective, such strategies often require the integration of several control measures and require a deeper knowledge of nematode ecology and biology than is required for chemical control alone. Four examples of our contribution in developing such knowledge are given below.

Modelling nematode damage and management Effective management of nematodes requires an understanding of the relationship between nematode population density at planting and crop growth. In contrast to aerial pathogens whose epidemiology is typically dynamic, nematodes are relatively immobile and reproduce slowly. Consequently, above a certain threshold, nematode damage is usually directly proportional to the nematode population density at planting (Figs. 7 & 8). Below the damage threshold, nematodes may even stimulate plant growth (Fig. 7). We have shown that the amounts of damage caused vary with the crop and nematode involved. *L. elongatus* (the needle nematode) is a migratory ecto-parasite that is particularly damaging to seedling crops such as carrots and perennial crops such as strawberry. In field trials, the threshold for damage to carrots was as few as 20 *L. elongatus* per kg soil, whereas much larger populations were required to damage strawberry (Fig. 8a). *Pratylenchus penetrans* (the root-lesion nematode) is a migratory endo-parasite that tunnels in plant roots. It has a wide host range and, in the mid-1970s, a series of field trials showed that the growth of newly

Figure 9 Three-year old raspberry plantation growing at a site with *Pratylenchus penetrans* where alternate strips were treated with nematicides prior to planting.

Figure 10 Tomato root system heavily galled by *Meloidogyne incognita*.

Figure 12 Females of white potato cyst nematode (*Globodera pallida*) on a potato root.

planted raspberry was consistently improved where it was controlled (Fig. 9). The growth of raspberry in a pot test was inversely proportional to the numbers of *P. penetrans* at planting, confirming its pathogenicity (Fig. 8b). *M. incognita* (a root-knot nematode) is a sedentary root endo-parasite that causes attacked roots to swell to form galls (Fig. 10). It has a short life cycle and, unlike most soil nematodes, populations can increase rapidly during one growing season. Consequently, it can be extremely damaging with a threshold as low as 60 eggs per kg soil (Fig. 7). *G. pallida* (wPCN) has a similar biology to *M. incognita*, but it is inherently less damaging because it has only one generation per year. Although the threshold for wPCN damage is c. 2000 eggs per kg soil, field trials showed (Fig. 8c) that population densities often become much larger, resulting in severe damage.

Based on knowledge of the relationship between numbers of nematodes at planting and damage, fields can be sampled prior to planting. Sampling errors tend to be large, particularly when nematodes are heterogeneously distributed, and much work has been done to identify appropriate sampling strategies. Currently, most sampling is barely adequate for making decisions with regard to whether to plant a susceptible crop, or apply a nematicide. Long-term management that seeks to keep nematode population densities below the damage threshold, requires both an understanding of nematode population dynamics, and accurate sampling data to determine whether the management is being effective - and if it is not being effective, where it is failing.

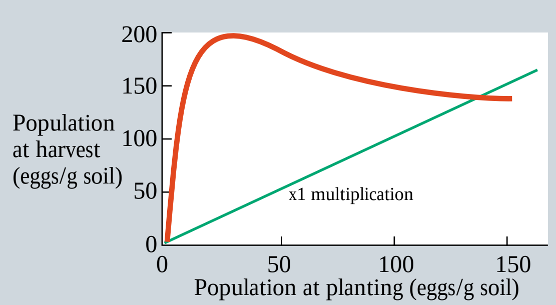


**Figure 11** Relation between the numbers of potato cyst nematode at planting and at harvest for cv. Maris Piper with *Globodera pallida*.

For many nematodes, as the nematode population density at planting increases, so the density at harvest reaches a maximum, and then decreases (Fig. 11) because the size of the root system available for nematode feeding and development is progressively reduced. Our research, often in collaboration with others nematologists, has addressed a range of problems including the wPCN, *Globodera pallida*, tropical RKN, *Meloidogyne* spp., fanging damage in carrots, and replanting problems in raspberry. Progress, or otherwise, in managing each of these problems will be briefly discussed.

### White potato cyst nematode (wPCN)

Our studies on wPCN (Fig. 12) have provided the database required to model the long-term threat that it poses to potato producers in the UK, and options for its management. The threat it poses is not always obvious because the progressive increase of wPCN is almost imperceptible, especially in seed potato land and ware land on long rotations. Modelling has shown that up to five potato crops are required for populations of wPCN to increase to damaging levels. However, wPCN is increasing everywhere, and is proving much more difficult to manage than the yPCN.

| | Main effects of | | | | | |
|---|---|---|---|---|---|---|
| | Scion | | | Stock | | |
| | Cara | P. Dell | P. Javelin | Cara | P. Dell | P. Javelin |
| Total dry wt(g) | 80 | 67 | 47 | 67 | 69 | 61 |
| % decrease due to *G. pallida* | 28 | 32 | - | 21 | 40 | - |
| Tuber dry wt as % of total | 30 | 51 | 70 | 51 | 54 | 50 |

**Table 3** The average stock and scion growth of grafts between potato cv Cara and Pentland Dell or Cara and Pentland Javelin and the effect of *Globodera pallida* damage. Results means of 9 harvests.

**Tolerance of Damage** A basis for modelling the control of wPCN was laid by field trials which enabled us to quantify the effects on crop yield and nematode multiplication of interactions between wPCN population density at planting, soil type and cultivar resistance and tolerance of damage. Soil type was shown to affect rates of juvenile invasion, and, hence, both crop damage and maximum rates of nematode multiplication. Tolerance and resistance were shown to be independent characteristics with some resistant cultivars more tolerant, and some less tolerant of wPCN damage than susceptible cultivars. More detailed studies showed that PCN damage affected potato growth by reduced rates of nutrient uptake. This decreased rates of top growth, the amounts of light interception by the crop canopy, and yield. Vigorous, late maturing cv. Cara, which produces a large canopy, was shown to be more tolerant of damage, but to create long-term problems because it supported much higher populations of wPCN than most other susceptible cultivars. Grafting experiments between early/late maturing, and tolerant/intolerant cultivars showed that both scion and stock genotypes influenced tolerance (Table 3).

**Resistance and virulence** The efforts of the potato breeders at SCRI, and elsewhere, to breed for resistance to wPCN have been supported by screening, each year, up to 10,000 plants. We have also provided the strategic nematological under-pinning to the breeding programme. Achievements include demonstrating that resistance in *Solanum vernei* and *S. andigena* CPC 2802 is quantitatively inherited. We also showed that the virulence of populations of wPCN differed (Fig. 13) but that different wPCN populations still tended to rank resistant clones in the same order. Consequently, it is acceptable to use just one population of wPCN to routinely screen for resistance. Importantly, clones derived from *S. andigena* were shown to have greater and more consistent resistance than those derived from *S. vernei*, emphasising that breeding should concentrate on the former. However, selection for increased virulence in wPCN was demonstrated following the repeated growing of such clones.

**Modelling integrated control of PCN** Our prediction that the repeated growing of potato cultivars resistant only to yPCN (*G. rostochiensis*) would progressively select for the wPCN (*G. pallida*) has been dramatically confirmed. Anticipating this change, our research in the 1980s focused on factors important in the management of wPCN. Achievements include confirming in field trials that wPCN damage and population dynamics can be described by simplified density-dependent equations, and demonstrating that these equations can be given predictive value by incorporating the tolerance associated with potato cultivar

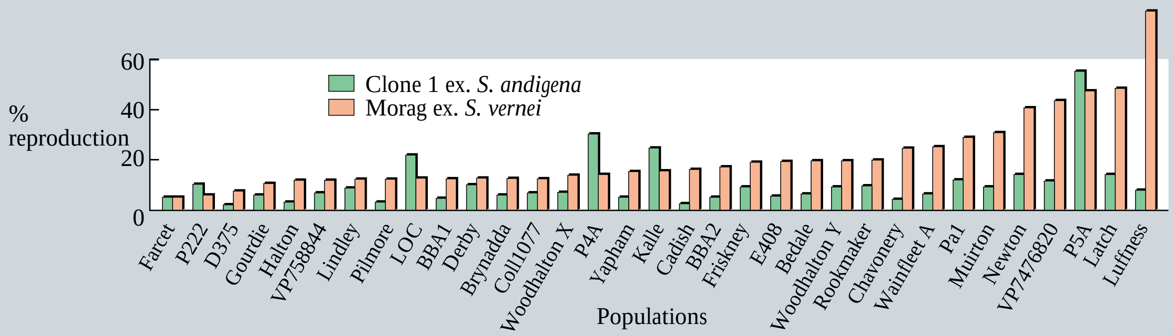


**Figure 13** Percentage virulence on two clones of partially resistant potato, compared with susceptible cv. Desirée, of forty populations of *Globodera pallida* from the UK, Europe and South America.

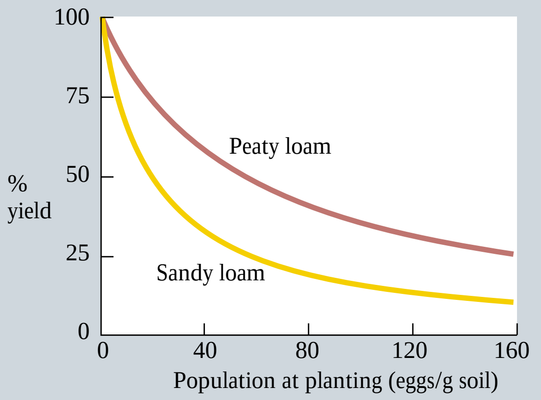


**Figure 14** Percentage yield reduction for cv. Maris Piper due to *Globodera pallida* at a peaty loam and a sandy loam site.

Figure 15 Two strips of nematicides applied across a field infected with potato cyst nematode.

and soil type (Fig. 14). The effects on wPCN multiplication of partial resistance and nematicides were shown to be additive – a crucial observation for their use in the integrated control of wPCN (Fig. 15). With funding from the British Potato Council, these results and equations are being used to develop a computer-based programme able to model the impact of different control measures on the long-term management of wPCN. This model shows that nematicides are most effective in managing wPCN when used against small (when the wPCN multiplication rate is at its maximum) rather than large populations (Table 4). It also shows that in high yielding fields, wPCN is unlikely to be controlled without integrating rotation, nematicides and partial resistant cultivars (Fig. 16). Even so, the effectiveness of this integration depends on rates of wPCN population decline between potato crops, nematicide effectiveness, and the virulence (ability to over-come resistance) of the wPCN, all of which are unknown in most commercial fields. To remedy this information deficit, there need to be fundamental changes in the strategy for monitoring wPCN, including the processing of larger samples, and sampling after, as well as before each potato crop.

| Before Planting (eggs/g) | After Harvest (eggs/g) | |
|---|---|---|
| | No Nematicide | Nematicide |
| 100 | 147 | 190 |
| 20 | 190 | 84 |
| 4 | 84 | 25 |
| 0.8 | 25 | 5 |

**Table 4** Effect on *Globodera pallida* population densities (eggs/g soil) at harvest of applying a nematicide (80% kill).

## Root-knot nematodes

Three species of RKN, *M. arenaria*, *M. incognita* and *M. javanica*, cause extensive root galling in susceptible crops (Fig. 10) and are amongst the world's most important crop pathogens. They have world-wide distributions in tropical and Mediterranean regions, where they attack and seriously damage almost all the major crops. Coffee, cotton, tobacco, fruit-trees, and almost all vegetable and horticultural crops are damaged. Related species damage cereals, including rice. In the UK, *M. hapla* damages carrots and other horticultural crops, and potatoes are threatened by *M. chitwoodi*, recently identified as causing problems in the Netherlands. Without using methyl bromide, RKN are extremely difficult to manage because of their short generation time (1 month at 25°C), high reproductive rate (2000 eggs per female) and very wide host range (most weeds and crops).

**RKN damage in Ecuador** During an EU-funded study, coordinated by SCRI, the damage caused by

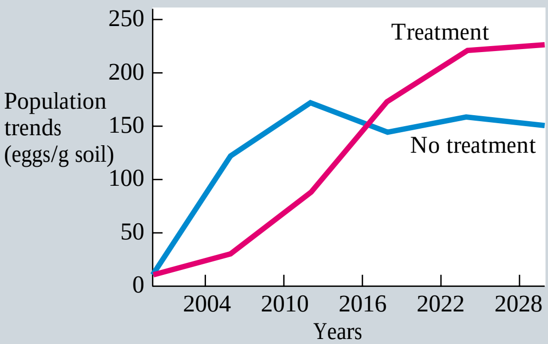


**Figure 16** Increases in population density of *Globodera pallida* on cv. Maris Piper on a peaty loam soil and 6 year rotation, with and without a nematicide (80% kill).

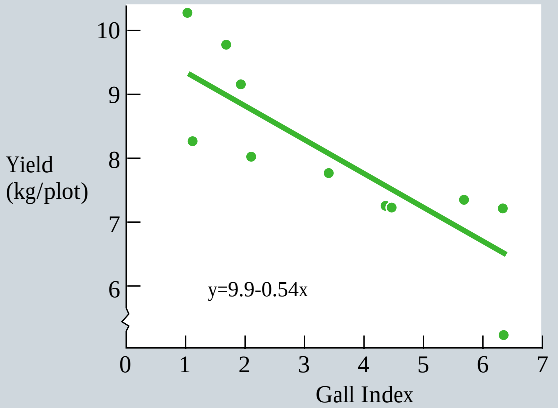


**Figure 17** Relation between root gall index due to damage by *Meloidogyne* spp. and yield of tomato in a micro plot trial in Tanzania.

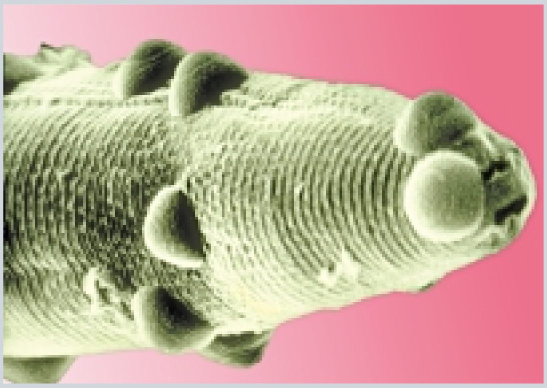

Figure 18 Juvenile *Meloidogyne* spp. carrying spores of *Pasteuria penetrans*. Picture IACR, Rothamsted.

RKN was determined by field trials and surveys in Ecuador, and in several other tropical countries. A total of 207 horticultural crops were sampled in Ecuador (many of them treated with nematicides) and 205 were found to be RKN infected (C. Trivino, pers. comm.). The mean damage (gall) index was 5.5, and the trials in Ecuador and Tanzania showed (Fig. 17) that yield was decreased by between 5% and 6% for every unit increase in the damage index, *i.e.* RKN is decreasing yields in Ecuador by between 25% and 30%.

Biological control of RKN The problems of managing RKN by rotation and with nematicides has encouraged study of alternatives. The project in Ecuador also examined the utility of a bacterium (*Pasteuria penetrans*) as a biological control agent for RKN. This bacterium has several attributes. It has an enormous rate of increase; each infected female RKN releases into the soil c. 2 million spores of *P. penetrans*; the spores persist in the soil for many years; and they are specific, only attaching to the soil migratory, juvenile stage of RKN (Fig. 18). Infected juveniles carry the spores into the plant root when they invade, but the nematodes are not killed until they have developed into large, adult females – each of which is packed with spores.

A survey in Ecuador by Dr Carmen Trivino showed that c. 30% of RKN populations were infected with *P. penetrans*, but that levels of infection were generally low. Laboratory experiments by Dr Mireille Fargette in France showed that the rates of infection probably varied because there are great variations between isolates of *P. penetrans* in their ability to infect RKN, and between populations of RKN in susceptibility to isolates of *P. penetrans*. Attempts to increase suppression of RKN at sites with *P. penetrans* by the repeated and intensive cultivation of crops susceptible to RKN were ineffective. However, the introduction into these trials of small amounts of an exotic isolate of *P. penetrans* resulted in a large increase in the proportion of spore-

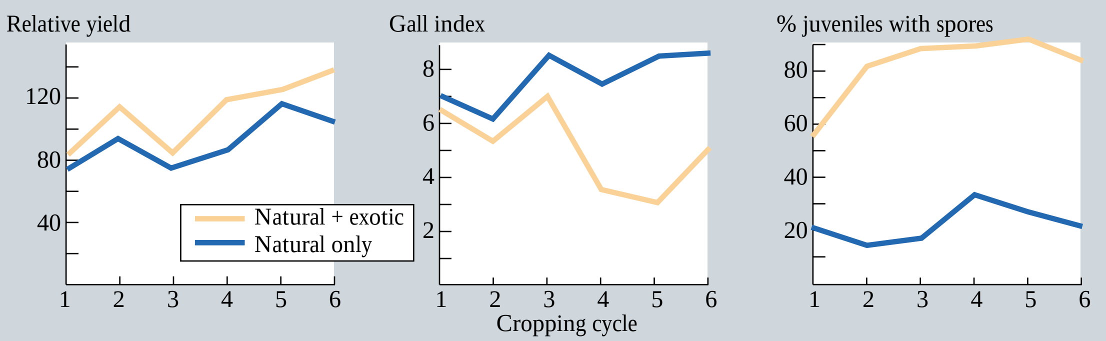


**Figure 19** Relative yields of bean and tomato, gall index and percentage of juveniles of *Meloidogyne incognito* carrying spores of *Pasteuria penetrans* over five cropping cycles. Results are for plots with a natural infestation of *P. penetrans*, without and with the addition of an exotic isolate.

Figure 20 'Fanged' and stunted carrots due to nematode damage.

infected juveniles, decreased root galling, and increased yields (Fig. 19). These results strongly suggest that, in natural infections, a balance develops between the host and parasite such that levels of infection remain relatively low. Introducing an exotic isolate of *P. penetrans* that infected most or all of the RKN juveniles, produced an epidemic and greatly increased suppression.

Figure 21 Aerial view of 3-year old raspberry plantation with 'sick' patches.

## Fanging damage in carrots and parsnips

Damage to the tap roots of seedling carrots and parsnips leads to 'fanging' (Fig. 20). Fanged carrots have to be removed, decreasing yields and increasing costs. The granular nematicide aldicarb (Temik 10G), applied in the drill at planting, is used to decrease fanging, but the specific nematodes involved were unclear. Studies at 20 sites with Dr M. Groom and Ms E. Horring showed that aldicarb decreased fanging from a mean of 21% to 7%, and that the degree of fanging was correlated ($r^2$ = 0.31, $P$ = 0.01) with the numbers of *Longidorus* spp. The presence of RKN (*M. hapla*) and cyst (*Heterodera carrotae*) nematodes accounted for further damage. Despite these results, aldicarb use is not based on an estimate of the potential risk, and many fields are treated unnecessarily.

## Replant problems in raspberry

Raspberries tend to be replanted in the same fields, resulting in raspberry 'sick' land (Fig. 21). Although the causal agents were initially unknown, a pre-planting treatment with the granular soil sterilant dazomet (Basamid) was found to minimise replant problems (Fig. 9). Pot tests demonstrated that the root-lesion nematode (*Pratylenchus penetrans*) was a major cause of the problem (Fig. 22), but an unidentified fungus was also involved at some sites. In pot experiments with soil from problem fields, deep-freezing soil markedly improved subsequent raspberry growth. Treating the soil with the fungicide benomyl or the nematicide aldicarb slightly improved growth but, when applied together, they improved growth as much as deep-freezing (Table 5). These results confirmed that raspberries can suffer replanting problems, and suggest that rotation may be of value. However, once *P. penetrans* has been introduced, it is likely to persist as it has a wide host range. A survey of planting stocks showed that a substantial proportion (20%) carried *P. penetrans* and, based on these results, spawn nurseries were sampled to ensure freedom from *P. penetrans*.

## Fundamental studies

Continued progress in managing nematodes has to be under-pinned by relevant fundamental and basic studies. Such studies usually also have wider scientific relevance. Two examples are given, both involving PCN.

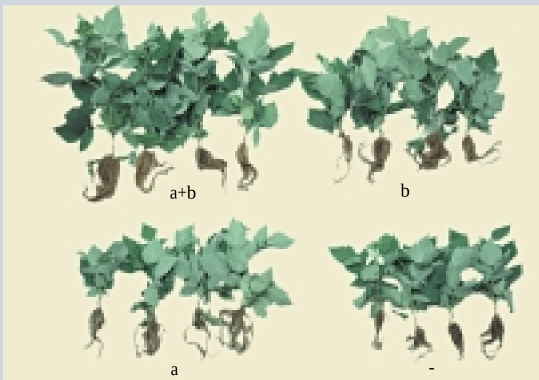


Figure 22 Raspberries grown in soil from a 'sick' plantation treated with a nematicide (aldicarb; a), a fungicide (benomyl; b), or both together.

| Experiment/Site | Untreated | Nematicide | Fungicide | Nematicide + Fungicide | Frozen |
|---|---|---|---|---|---|
| | Mean plant weight (g) | | | | |
| 1. | 18 | 49* | 40* | - | 48* |
| 2. | 16 | 24 | 23 | 49* | 46* |
| 3. | 7 | 6 | 15* | 24* | - |
| 4. | 9 | 13 | 23* | 40* | - |
| 5. | 15 | 13 | 24* | 36* | - |
| 6. | 48 | 39 | 52 | 61* | - |
| 7. | 20 | 45* | 24 | 58* | - |
| 8. (sterilised soil) | 20 | 21 | 23 | 21 | - |

*Significantly different from untreated P>0.01.

**Table 5** Soils from fields with raspberry re-plant problems treated with a nematicide, fungicide or frozen (-22°C). Results are from different experiments and are mean raspberry plant weights.

### Heterogeneity in PCN and RKN

Potato cyst (PCN), and root-knot (RKN) nematodes have been widely spread around the world by agriculture. PCN came from the Andean region of South America, but the origins of the most widespread and damaging RKN species (*M. incognita*, *M. javanica*) are unknown. Each species of PCN and of RKN has been sub-divided into 'pathotypes' and 'races' respectively that differ in their host range. It was suggested that some of the variation in virulence of the wPCN (*G. pallida*) on resistant cultivars of potato (Fig. 13) was due to the introduction of different populations and gene-pools from S. America. A range of molecular techniques was used to explore this hypothesis. At the same time, the hypothesis that variation will be greater with sexually reproducing wPCN than in parthenogenetic RKN was also tested.

RAPDs Short regions of DNA from a range of UK, European and S. American populations of wPCN were amplified at random using the polymerase chain reaction (PCR). Because juvenile wPCN are so small (0.5 mm), many 1000s of nematodes were required to provide the DNA for this analysis (this contrasts with studies on larger organisms, *e.g.* plants, where all of the required DNA, which is guaranteed to be homogeneous, can be obtained e.g. from a single leaf). Whilst there were differences in RAPD profiles between some 'pathotypes', the European populations of *G. pallida* formed a heterogeneous group distinct from two S. American populations and from populations of *G. rostochiensis* (Fig. 23a). Similar studies with populations of six species of RKN from widely distributed origins produced fewer and more reproducible bands, and there was less variation within species, especially for *M. incognita* (Fig. 23b). We concluded that, with wPCN, the variation observed reflected substantial heterogeneity within each population, whereas with RKN, the less complex and consis-

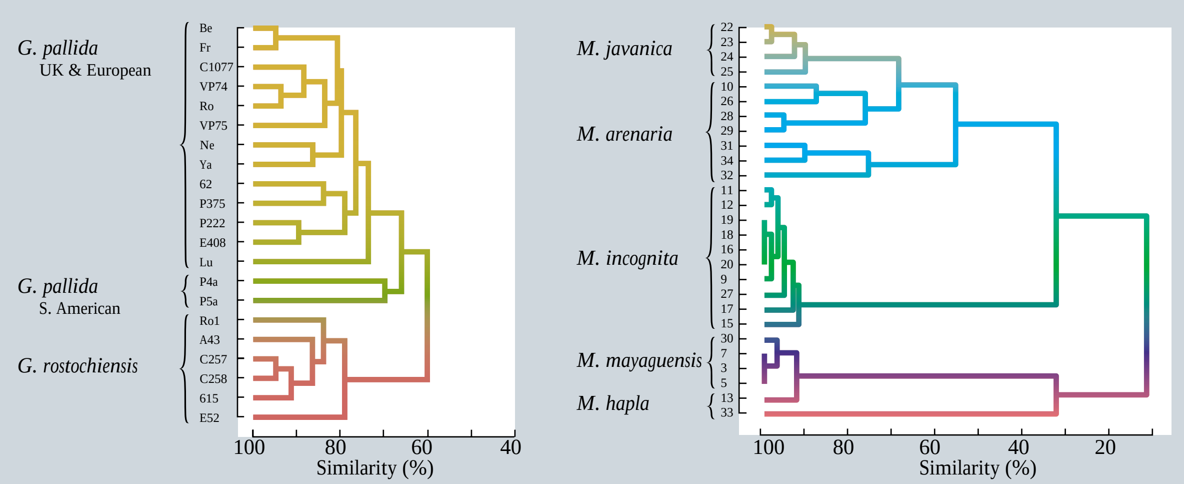


**Figure 23** Dendogram summarising RAPD results for populations of (a) both species of PCN and (b) six species of RKN.

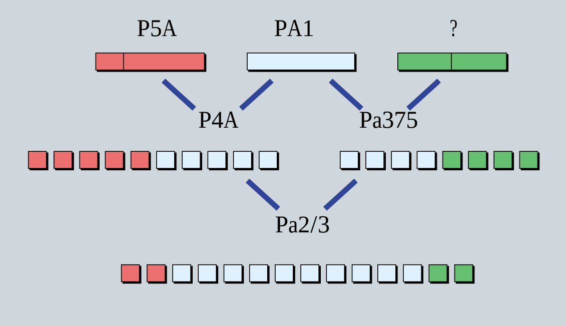


**Figure 24** Differences in ITS ribosomal DNA in populations of *Globodera pallida*. Populations of the common UK pathotype Pa 2/3 contain three types of ribosomal DNA that may have resulted from population hybridization.

tent banding patterns reflected the homogeneity of each population that is a consequence of parthenogenetic reproduction.

Ribosomal DNA (rDNA) For nematodes, the nuclear genome contains multiple copies of tandemly repeated rDNA, which can be amplified by PCR. The internal transcribed region of the rDNA was amplified from various populations of wPCN and the products digested with a range of enzymes (endonucleases) that cut the DNA only where specific sequences occur. This process produces fragments of rDNA which may vary in length. Populations of the wPCN from South America and from Northern Ireland, which represented different pathotypes (P4A, P5A, and Pa1), were distinct, whereas, populations from England were very similar (Fig. 24). However, the English populations appeared to contain the greatest number of restriction sites, apparently resulting from different types of ITS regions occurring within individual nematodes. This suggests that they may be derived from a mixture of distinct gene pools that had interbred. In contrast, for the RKN, *M. incognita*, *M. javanica* and *M. arenaria*, this region has very little sequence variation even when these species are compared. This suggests a recent divergence.

Mitochondrial DNA (mtDNA) The suggestion that there might have been several introductions of *G. pallida* into the UK, was examined by studying the mitochondrial DNA (mtDNA) of a range of populations from the UK and South America. In animals, mtDNA occurs in the mitochondria as a circular molecule coding for 12-13 protein genes and 24 transfer RNAs, which requires a minimum of 13,000 bases. MtDNA is ideally suited for studying colonisation by animals because it is inherited maternally, it evolves more quickly than the nuclear DNA, and each cell contains many identical mtDNA copies. Consequently, distinct introductions of PCN from different parts of South America were likely to have differences in their mtDNA that could be correlated with virulence differences. The results showed that with the exception of the exceptionally virulent 'Luffness' population and the avirulent Pa1 from N. Ireland (Fig. 13), UK populations of *G. pallida* were not distinct, suggesting that they derive from a single introduction.

However, the two populations from South America differed greatly (Fig. 25). The mtDNA from the P4A population was even more diverse than the UK populations, and could have been the progenitor of the UK populations. The P5A population was so different that it suggests that it may be a new species.

Unique organisation of PCN mtDNA. During these studies, the mtDNA of *G. pallida* was found to be organised uniquely, posing fundamental questions regarding the inheritance and functioning of mtDNA. Instead of a single circular molecule of >13,000 bases,

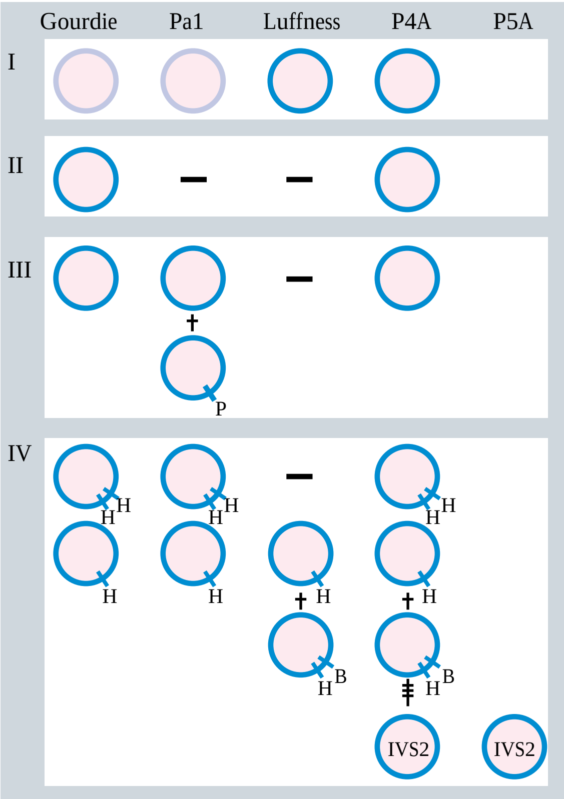


**Figure 25** Occurrences of four mitochondrial mini circles in a typical UK *Globodera pallida* population (Gourdie), in two distinct populations from the UK (Pa1, Luffness), and two populations from S. America (P4A and P5A).

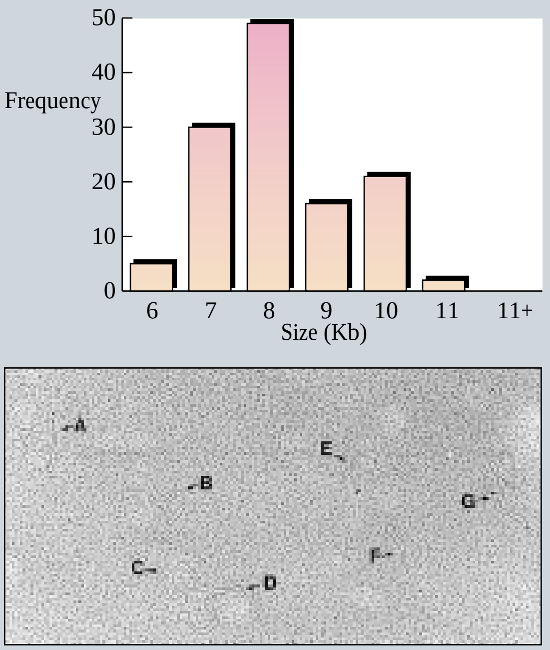


**Figure 26** Electron microscope photograph of mitochondrial mini circles of *Globodera pallida* (A-G), and histogram of their estimated size distribution.

the mtDNA of all the populations studied was comprised of several smaller molecules of 6,000 to 10,000 bases (Fig. 25). Initially, we were concerned that these were artefacts or relatively rare mutants, but their occurrence and lengths were confirmed under the electron microscope (Fig. 26) and by sequence analysis.

### Nematode-plant interactions

Some nematodes induce specialised feeding sites in their hosts. These provide the nematodes with greatly increased amounts of food, enabling them to have high rates of reproduction and, consequently, become serious crop pests. They induce these changes by injecting, through their hollow stylet, secretions from oesophageal gland cells into the root cells around their heads (Fig. 27). These cells become enlarged, mulinucleate and much more active. Understanding the initiation and regulation of these profound changes in plant gene expression is of fundamental interest to both animal and plant scientists, and should also provide novel options for control. Plant resistance to nematodes, and mechanisms of nematode virulence (ability to circumvent plant resistance) are other topics of wide fundamental interest with potential practical relevance.

**Secretions** Studies on nematode secretions are an important area of collaborative research involving colleagues in the UK and Europe. Secretions of nematodes are important for a variety of reasons, including the changes they induce in the plant root cells (see above) which provide endoparasites such as *Globodera* and *Meloidogyne* with an abundant supply of food. Studies using plant promoters coupled to the GUS reporter gene have shown that, in inducing these changes, the nematode manipulates plant gene expression (Fig. 28). The secretions thought to be responsible originate from one or more of the three gland cells associated with their feeding apparatus. These gland cells also produce secretions involved in root penetration, and in the formation of the 'feeding tube' – a complex structure attached to the stylet-tip - that is secreted into the plant cell prior to each bout of ingestion. Nematodes also produce secretions from the two chemosensory amphids on their head, from the 'excretory pore', and from glands associated with the reproductive system. Proteins are also found coating

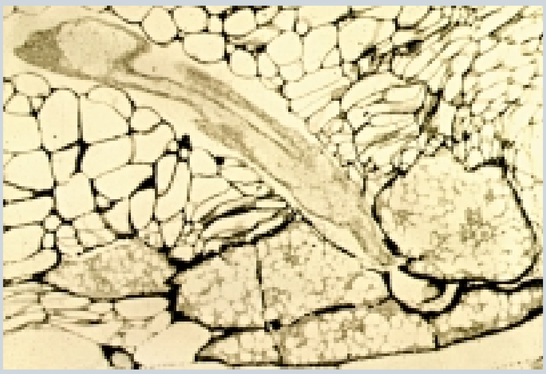

Figure 27 Longitudinal section along a root containing a giant cell system associated with a developing juvenile *Meloidogyne*.

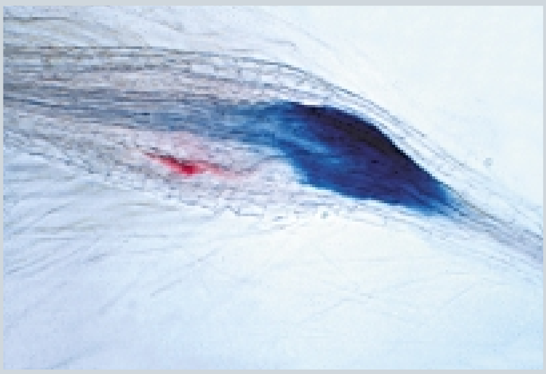

Figure 28 Expression of GUS reporter gene (blue staining) in a feeding site induced by developing *Meloidogyne* juvenile (red).

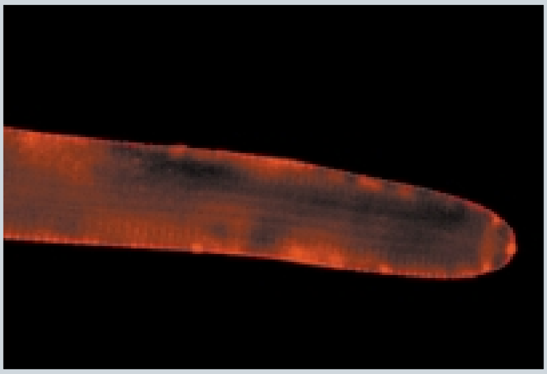

Figure 29 Surface of juvenile *Globodera pallida* stained red by the specific attachment of an antibody raised against a secreted fatty acid-binding protein.

the nematode surface (Fig. 29) that may be secreted directly through the cuticle. We developed various techniques to collect and study these secretions, but the analysis of gene expression using ESTs (expressed sequence tags) has been especially productive. This has led to the identification of a pectate lyase, the first gene of this type to be cloned from any animal. We have also identified genes encoding a thioredoxin peroxidase, glutathione peroxidases and a secreted fatty acid-binding protein. Studies of the sites of expression and properties of these genes suggest that one of the peroxidases and the fatty acid binding protein may be involved in protecting the nematode from host defences. Of particular interest is the indication that nematodes have been recruiting genes from other organisms. The cell wall degrading enzymes (cellulases and pectate lyases) strongly resemble orthologous genes from *Erwinia*. While there is evidence that horizontal gene transfer has occurred in these cases, the mechanisms by which this has happened remain unclear. Researchers at SCRI are now focusing on the development of systems that will allow the function of potentially interesting nematode genes to be tested *in vivo*.

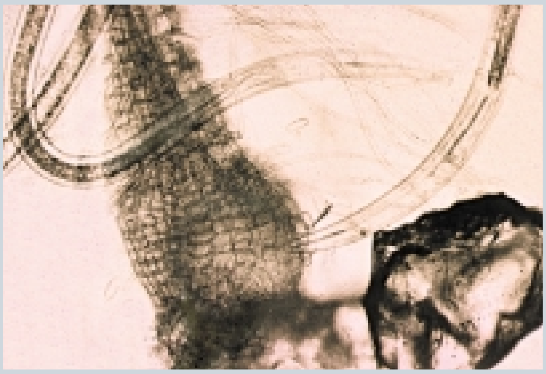

Figure 30 *Longidorus* nematode feeding on a galled root-tip. Note the sand grain (bottom right).

### Virus vectors

The nematologists at SCRI have been at the forefront of research on virus transmission by nematodes since the inception of this relatively young research area. This has partly been achieved by close collaboration with virological colleagues and this unique combination of research skills has resulted in numerous requests both nationally and internationally for assistance and collaboration with nematode and virus problems. The research is highly focused and extends from fundamental core-funded studies to externally funded, pre-commercial PCR-based molecular diagnostics (PCR-MDs).

Some of the achievements include developing methods for conducting transmission tests with *Longidorus*, *Xiphinema* and trichodorid spp., whereby each step in the transmission process (rates of feeding, virus ingestion and retention during the acquisition phase, and rates of feeding and virus infection during the transmission phase) can be quantified (Fig. 30). Using this approach, it became clear that many reports of nematode transmission were of doubtful validity. We established criteria for demonstrating virus transmission and, based on these, almost two thirds of the reports of viruses being transmitted by specific nematodes were rejected. Subsequently, new associations and crop problems have been identified by SCRI staff, including *Paralongidorus maximus* as a vector of an atypical strain of *Raspberry ringspot nepovirus* in vineyards in Germany; *Longidorus arthensis*, a previously undescribed

Figure 31 A cherry tree showing typical bare-branch symptoms caused by *Cherry rosette virus* transmitted by *Longidorus arthensis.*

Figure 32 Yellowing diseased in barley cv. Express caused by *Arabis mosaic virus* transmitted by *Xiphinema diversicaudatum*.

Figure 34 'Spraing' disease in potato caused by *Tobacco rattle virus* transmitted by trichodorid nematodes.

species, as the natural vector of a new nepovirus in cherry orchards in Switzerland (Fig. 31); *X. diversicaudatum* as the vector of a strain of *Arabis mosaic nepoviruses* causing a yellowing diseases in barley in Switzerland(Fig. 32); and *X. intermedium* and *X. tarjanense* as vectors of *Tobacco* and *Tomato ringspot nepoviruses*. We have shown also that *Tobacco rattle tobravirus* transmitted by trichodorid species is widespread not only in northern Europe, but also in northern Greece and Portugal, and that the nematode also causes direct damage (Fig. 33).

By analysing rates of virus transmission, we demonstrated large differences between nematode species in the rates at which they would transmit their associated viruses and, in Europe, a high degree of specificity between viruses and their vectors. This specificity of association sometimes extended even to specific nematode populations and minor serological variants of the viruses. Collaborative studies with colleagues in North America, who are seeking to control virus problems in vines and fruit crops, have demonstrated the converse. The virus-vector species of *Xiphinema* in North America each transmit two or three distinct viruses, and each virus is transmitted by more than one species of nematode. However, within a species, some populations may not transmit virus, or may transmit only one, rather than two or three, viruses.

A recent success has been the development of techniques for studying the specificity of the transmission of the distinct strains of *Tobraviruses* by species of trichodorid nematodes. We are the only centre in the world able to conduct such studies and have demonstrated that each strain of *Tobraviruses* has only one or two species of trichodorid nematode as its vector (Table 6). Consequently, in potato fields with a 'TRV-spraing' problem (Fig. 34 & Fig. 35) in the tubers and where typically two or three trichodorid species will be present, only one species may be transmitting the virus.

These specialised techniques (Fig. 36) also have been

Figure 33 Stubby root system caused by the direct feeding of trichodorid nematodes.

Figure 35 Potato crisps made from: left, healthy potato; right, potato with 'spraing' disease.

| Nematode | Tobravirus | Strain |
|---|---|---|
| *P. allius* | TRV | USA |
| *P. anemones* | PEBV | English |
| | TRV | PaY4 |
| *P. minor* | PRV | Brazil |
| | TRV | USA |
| *P. nanus* | TRV | PRN |
| *P. pachydermus* | PEBV | Dutch |
| | TRV | PRN (PpK20) |
| | | PaY4 |
| *P. teres* | PEBV | Dutch |
| | TRV | Oregon |
| *P. tunisiensis* | TRV | Italian |
| *T. cylindricus* | PEBV | English |
| | TRV | RQ |
| | | TcB28 |
| *T. primitivus* | PEBV | TpA56 |
| | TRV | TpO1 |
| *T. similis* | TRV | Ts-Belgian |
| | | Ts -Dutch |
| | | Ts-Greek |
| *T. viruliferus* | PEBV | English |
| | TRV | RQ |

**Table 6** Associations between *Paratrichodorus* and *Trichodorus* species and serologically distinguishable strains of *Pea early-browning* (PEBV), *Pepper ringspot* (PRV), and *Tobacco rattle* (TRV) tobraviruses.

used successfully in fundamental investigations, in collaboration with the virologists, to study the genetic determinants of nematode transmission of tobraviruses. This has revealed that the virus coat protein and one or two non-structural proteins are involved. Using these same techniques, it was demonstrated that coat protein-mediated transgenic resis-

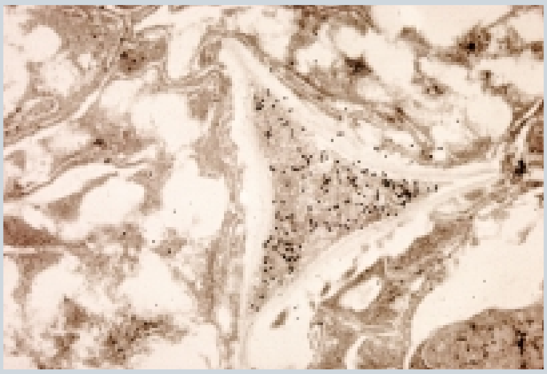

Figure 36 Immunogold (black circular colloidal gold particles) labelling specifically attached tubular *Tobacco rattle virus* particles at the site of virus retention in the feeding apparatus of a trichodorid nematode.

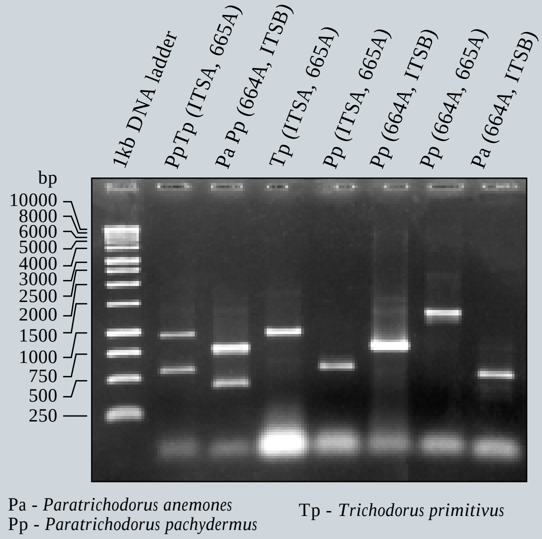


**Figure 37** A PCR based molecular diagnostic using several primer sets distinguishing three trichodorid species that occur commonly in Britain and northern Europe.

tance is ineffective when TRV is naturally transmitted by trichodorid nematodes.

Most recently, PCR-MDs have been developed for pre-plant and post-harvest detection and identification of trichodorid species (Fig. 37) and serologically distinguishable strains of TRV. These techniques can be used to detect and identify individual trichodorid species in nematode populations extracted from soil samples, and TRV has been reliably detected from

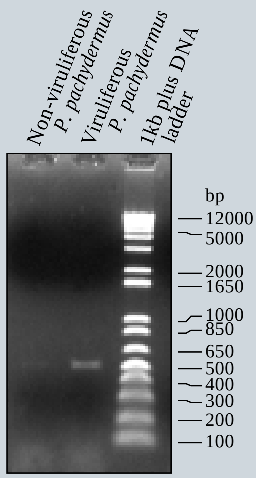


**Figure 38** A reverse transcriptase PCR based molecular diagnostic using a universal primer set for detecting *Tobacco rattle virus* revealing the presence of the virus in an individual trichodorid nematode.

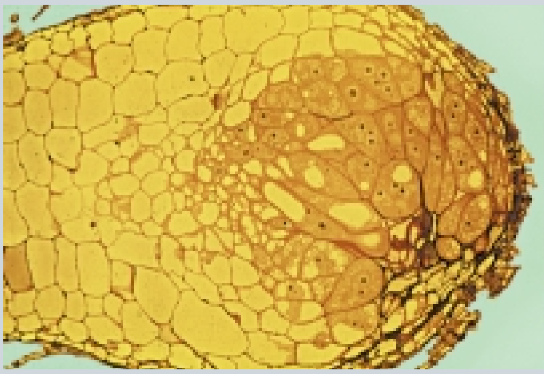

Figure 39 Root tip gall induced by *Xiphinema index* containing multinucleate cells. Photo courtesy of U. Wyss.

individual nematodes (Fig. 38). Current research is focused on obtaining trichodorid species-specific primers for use in PCR-MDs.

### The Future

I have deliberately tried to give a relatively non-technical presentation of nematology, and to demonstrate the difficulties of managing nematodes compared with above-ground pathogens and pests. Nematicides are likely to be required to integrate with other methods to protect many crops for the foreseeable future, and more needs to be done to ensure that they are used effectively and with minimum impact on the wider environment. Nematode-plant interactions are an exciting area of research where molecular studies analysing patterns of gene expression are likely to be especially productive and informative. Some *Xiphinema* spp. induce galls containing modified cells similar to those induced by RKN (Fig. 39), and these large nematodes provide a unique opportunity for studying the expression of their genes during different phases of their feeding. Plant resistance clearly has a central role in improving the management of soil nematodes, but often only as one component in an integrated system. Many are pinning their hopes on transgenic resistance and, for several reasons, we have concluded that seeking to transfer 'natural' resistance genes is the best way forward. We are collaborating with German colleagues to isolate a resistance gene (*Hero*) from tomato with a view to studying its expression in potato. Current EU-funded research is exploring changes in gene expression in plants in both susceptible and resistant interactions. However, there are many uncertainties regarding the long-term durability of all types of resistance, and isolating nematode virulence alleles and understanding their modes of action is a priority. For heterogeneous nematode species, such as wPCN, selection for increased virulence seems probable. But, resistance to more homogeneous species (e.g. the single pathotype of the yPCN introduced into the UK), and particularly for the mitotically parthenogenetic RKN, resistance may be surprisingly durable. However, even when one damaging species is controlled, experience indicates that there is often another, only slightly less dominant species of nematode waiting to take its place. Consequently, nematologists need to understand better the processes involved in the regulation of nematode communities and populations, and the processes involved in spread and colonisation. As c. 40% of soil nematodes reproduce asexually, whereas almost all aquatic and animal parasitic nematodes reproduce sexually, it is clear that the soil environment poses special problems for its inhabitants, including how to find a mate, and how to colonise a distant field. The soil environment also poses particular problems for nematologists, including how to sample effectively, and how to study processes such as gene-flow.

Hopefully, the foregoing provides an insight into the difficulties of studying and controlling soil pathogens, but also examples of quality science and progress. Whilst 'citation indices' are a major criterion for judging scientific quality and impact, specialist nematologists will tend to be at a disadvantage because much of their research is destined to be published in nematological journals with a limited circulation. Even so, papers from the group have been published recently in *Nature*, *Genetics*, *Genome*, *Functional Ecology* and *New Phytologist*, and members of the group have relatively high citation indices. This, and our extensive portfolio of external funded projects, has been achieved by integrating the different aspects of our research, including fundamental and applied, molecular (e.g. sequence) and biological (function), and by ensuring we are attractive partners in collaborative projects because of our expertise, particularly as nematologists.

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