NEW APPROACHES FOR POTATO CYST NEMATODE MANAGEMENT

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ABSTRACT

Evans, K. 1993. New approaches for potato cyst nematode management. Nematropica 23:221–231.

The yield losses caused by potato cyst nematodes, the variation in tolerance between cultivars, and the principles which control nematode population dynamics are reviewed. The white potato cyst nematode, *Globodera pallida*, is becoming increasingly important in many countries, both because it is becoming more prevalent and because it is more difficult to control than the golden nematode, *Globodera rostochiensis*. A number of previously unexploited control strategies may help in the control of *G. pallida*; these include the use of artificial hatching agents, trap crops, potato cultivars that act as poor hosts, and biocontrol agents. In addition, molecular technology may give rise to exploitable novel resistance mechanisms and will almost certainly provide rapid and accurate diagnostic aids.

Key words: biological control, DNA, *Globodera pallida, Globodera rostochiensis*, immunodiagnosis, integrated control, potato, potato cyst nematodes.

RESUMEN

Evans, K. 1993. Nuevas estrategias para el manejo de los nematodos quiste de la papa. Nematrópica 23:221–231.

Las pérdidas causadas por el nematodo quiste de la papa en la producción de papa, así como la variabilidad entre cultivares y los principios que controlan la dinámica de poblaciones de nematodos son analizados en este trabajo. El nematodo blanco quiste de la papa *Globodera pallida* está adquiriendo una mayor importancia debido a su creciente expansión y dificultad en su control, en comparación con el nematodo dorado *Globodera rostochiensis*. Diversas estrategias de control, hasta el momento no explotadas, tales como el uso de agentes artificiales estimuladores de la emergencia del nematodo, cultivos atrapadores, agentes biológicos y la utilización de cultivares huésped-deficiente, podrían contribuir al control de *G. pallida*. Asimismo, técnicas moleculares podrían contribuir al desarrollo de nuevos mecanismos de resistencia, y con seguridad contribuirían al desarrollo de métodos de diagnóstico más rápidos y precisos que los existentes.

Palabras clave: ADN, control biológico, control integrado, *Globodera pallida, Globodera rostochiensis*, inmunodiagnóstico, nematodos quiste de la papa, papa.

LOSSES CAUSED BY POTATO CYST NEMATODES

White and golden potato cyst nematodes (PCN), *Globodera pallida* Stone and *Globodera rostochiensis* (Woll.) Skarbilovich, are serious pests of potato in the U.K., where the direct and indirect crop losses they cause have been estimated to reach a value of 9% of crop production annually (9). These nematodes occur in more than 50 other countries (1), in many of which they undoubtedly cause large losses.

Potato yields are related to the number of PCN eggs in the soil at planting time and the general form of this relationship is shown in Fig. 1. When PCN are few, yield is little affected because plants compensate for the trivial injury to their root systems but, as their numbers increase beyond the so-called tolerance threshold (T), yield begins to decline and eventually reaches a minimum. The value of the minimum yield and the nematode population density at which it is reached depend

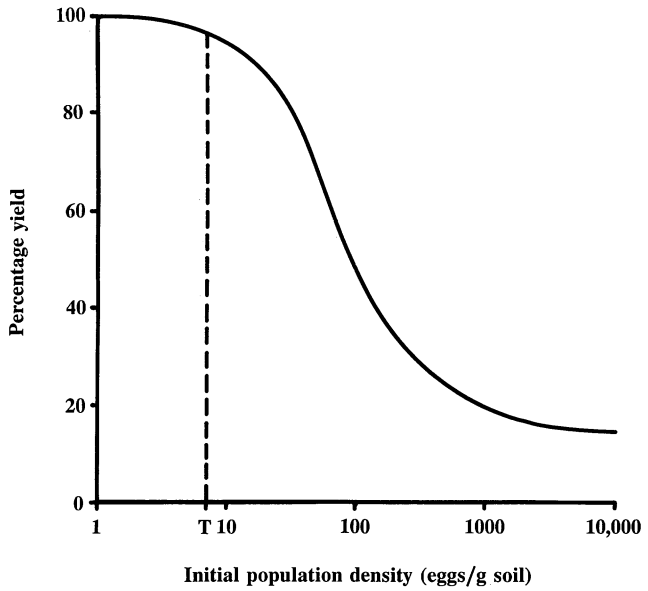

Fig. 1. The relationship between yield and the number of potato cyst nematodes per gram of soil. T is the tolerance threshold, *i.e.*, the nematode population density beyond which the plant cannot compensate for injury.

largely on an interaction between the potato cultivar grown and the soil type. The nematode population density in Fig. 1 is plotted on a logarithmic scale and this gives a sigmoid shape to its relationship with yield. If the central part only of the curve is considered, a linear scale can be used and the relationship between yield and nematode population density can be estimated by linear regression analysis.

Figure 2A shows the yield loss relationship for 24 different cultivars grown at nematode population densities ranging from 0 to 100 eggs per gram of soil and plotted on a linear scale (8). There is a great diversity of potential yields and relative yield losses over this range of nematode densities. Tolerance is an individual characteristic of a cultivar, best measured by the relative yield loss per unit increase in initial nematode population density (Pi) and, therefore, best visualised by scaling yields to the same starting value. This has been done in Fig. 2B. Some common cultivars are identified in the two halves of Fig. 2 and this highlights the fact that, in this experiment, 'Pentland Dell' showed greater tolerance than 'Désirée'; its yield loss per unit increase in nematode density was smaller, despite a yield similar to Désirée at 100 eggs per gram of soil, because it had a lower yield potential. 'Cara' was clearly the most tolerant cultivar with both a high yield potential and a very small loss per unit increase in numbers of nematodes.

POPULATION DYNAMICS OF PCN

In the same way that yield can be related to Pi, the final population density after harvest (Pf) can also be related to Pi. Both Pf and Pi are plotted on a logarithmic scale in Fig. 3 and their relationships are shown in four different situations (A–D). The line (A) at 45° is the 1× or

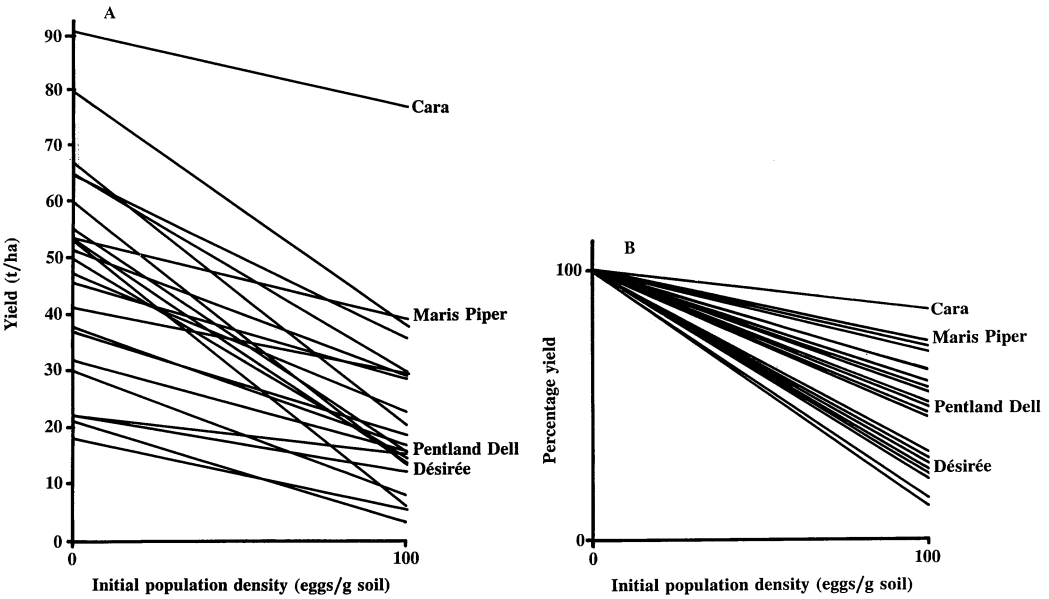

Fig. 2. Relationships between yield and numbers of potato cyst nematodes for 24 cultivars. A) Lines fitted to original data showing variation in potential yield and yield loss. B) Yields of the same cultivars, expressed as a percentage of yield obtained in the absence of nematodes, to show variation in tolerance. (Data from Evans and Russell, 1990).

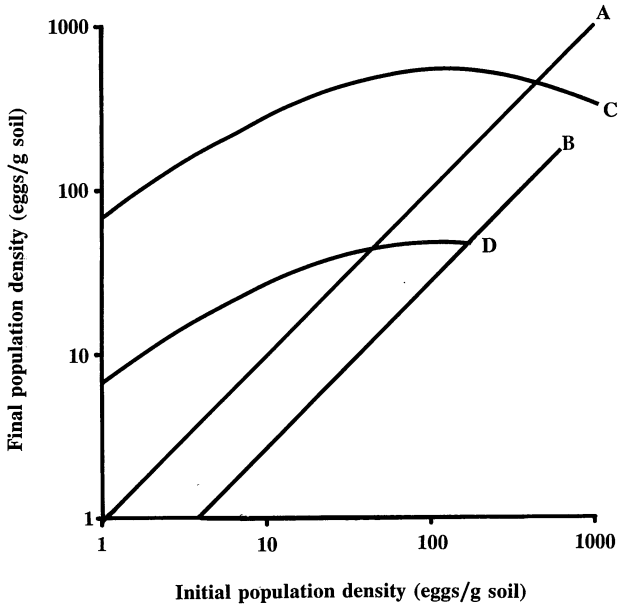

Fig. 3. Theoretical relationships between initial (Pi) and final (Pf) population densities of potato cyst nematodes. A) If Pf = Pi. B) If a resistant host crop is grown (Pf = 0.33Pi). C) If a non-resistant potato cultivar is grown. D) If a partially resistant potato cultivar is grown.

maintenance line, where Pf = Pi. If no host crop is grown, approximately one third of a *G. rostochiensis* population will die per year (11) but, if a fully resistant crop is grown (as at B) approximately two thirds of the nematodes will die. These effects are independent of population density whereas multiplication on a susceptible host (C) is at first great but, at higher values of Pi, decreases as competition between individual nematodes and plant damage both increase. Eventually this curve crosses the maintenance line (at the so-called equilibrium population density) and, at higher values of Pi, Pf is smaller than Pi. When a partially resistant host is grown (D), multiplication is less and again is density-dependent. Both C and D will, of course, turn upwards and run along line B at still higher values of Pi.

IMPORTANCE OF *GLOBODERA PALLIDA*

Distribution maps prepared in the 1960's (2) showed that about half of the PCN populations in England were essentially pure *G. rostochiensis* and susceptible to control by the H_1 resistance gene. Repeated growing of cultivars possessing this gene has, however, allowed selective reproduction of *G. pallida* and it is estimated that no areas of pure *G. rostochiensis* now remain. Single major gene resistance to the common types of *G. pallida* has not been found, so attempts to breed for resistance to this species have used polygenic sources. No fully resistant cultivars have yet been produced. Further concern regarding options for the long-term control of *G. pallida* were expressed when it was discovered that crop rotation and nematicides were also less effective against *G. pallida* than against *G. rostochiensis* (A. G. Whitehead, personal communication).

An important difference in hatching behaviour exists between the two species of PCN (Table 1). Over an initial period of 6 weeks, more juveniles hatched from populations of *G. rostochiensis* than from *G. pallida* (19). Hatch from *G. pallida* was little more than half that from *G. rostochiensis* and this slowness of hatch has important implications for the effectiveness of nematicides that act as nematostats on the second-stage juveniles in the soil. The hatching patterns of the two species of PCN differ in the field and in respect to the decay curves for an oximecarbamate nematicide, such as oxamyl, with half-lives of 2 or 3 weeks (Fig. 4). At the slower decay rate, peak of hatching is reached in *G. rostochiensis* whilst 50% of the nematicide still remains, compared with only 30% at the peak of *G. pallida* hatching. At the faster decay rate, barely 10% remains by the time peak hatch of *G. pallida* is reached. Clearly, a nematicide of this type will work less well with *G. pallida*, whatever the decay rate.

Rotation is an effective control measure for PCN because potatoes are the only widely grown field crop to act as a host

Table 1. Mean cumulative percentages of juveniles emerged from cysts of *G. rostochiensis* and *G. pallida* over a period of 6 weeks in potato root diffusate at temperatures alternating between 15 and 20 °C (12 hr/12 hr).[z]

Species	Number of populations	Cumulative percentage of juveniles emerged
G. rostochiensis	12	78.6
G. pallida	27	45.8
Standard error of the difference		1.02

[z]Data from Whitehead, 1992.

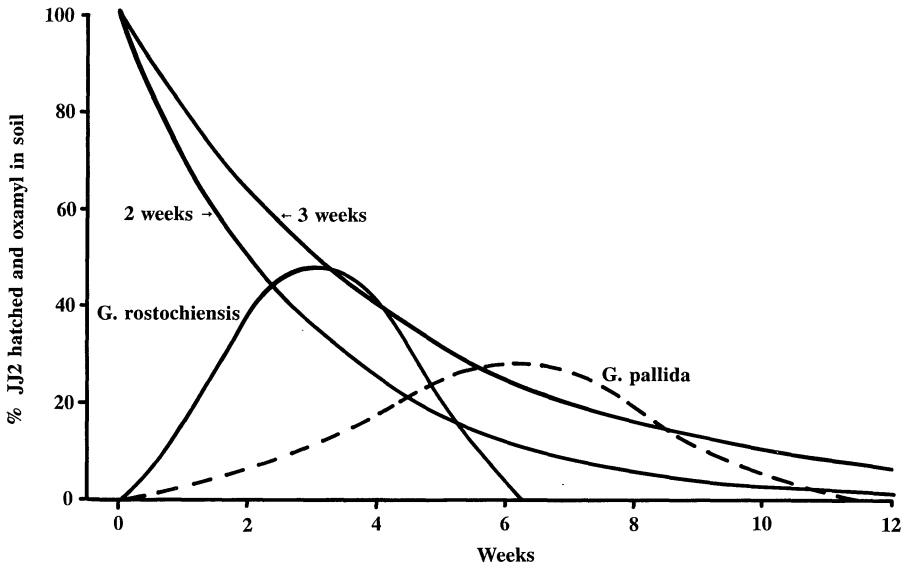

Fig. 4. Hatching patterns for *G. rostochiensis* and *G. pallida* under a potato crop and decay curves for oxamyl with 2 or 3 week half-lives.

and because PCN populations decrease when no host crop is grown. Surveys in the U.K. have shown the decline rate to be density independent and to be about 33% per annum for *G. rostochiensis* (11). However, careful measurement of the decline rate for *G. pallida* has shown that it may be as low as 15% (A. G. Whitehead, personal communication). Even if this figure were as high as 20%, *G. pallida* would take 18 years to decline to a non-damaging level compared with 10 years for *G. rostochiensis* (Fig. 5).

The greater persistence of *G. pallida*, its lesser susceptibility to oximecarbamate nematicides, and the absence of potato cultivars with full resistance combine to make the design of control packages more difficult than for *G. rostochiensis*. Some assistance may be gained, however, from the careful choice of any non-resistant cultivar to be grown. Under field conditions, net multiplication rates of *G. pallida* populations may differ markedly on different potato cultivars with no resistance to nematode reproduction *per se* (Table 2) (from almost 25× on Désirée to 3.0× on Record). The reasons for these differences are unknown but may depend on the varying effectiveness of hatching factors in the different cultivars (7). Other novel treatments that may help in the control of *G. pallida* include growing a trap crop of potatoes that is harvested before females mature and the use of artificial hatching agents. Sodium metavanadate solution (10^{-3} M), for example, applied to summer fallow reduced nematode population densities by 80% (19).

INTEGRATED CONTROL

The aim of integrated control of PCN is to keep population densities within acceptable limits through the use of several control measures. Based on experimental

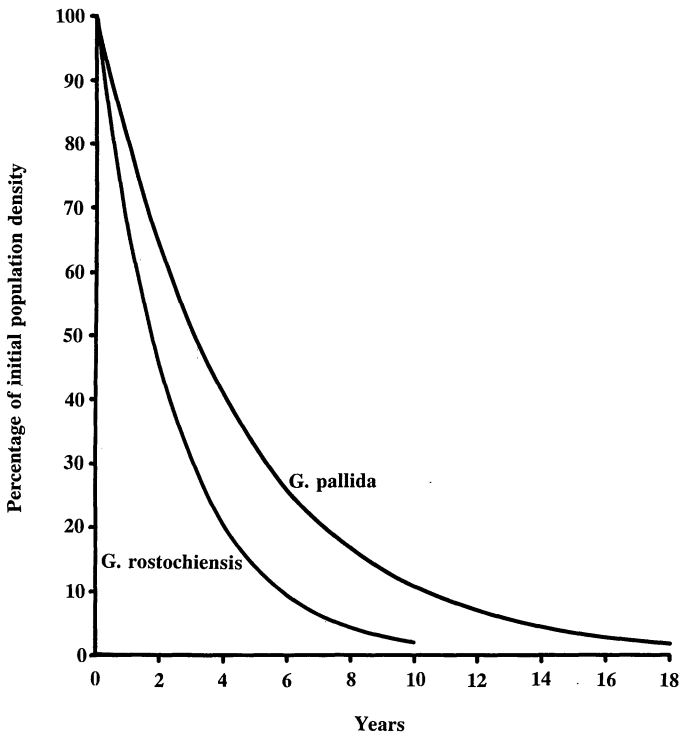

Fig. 5. Decline of *G. rostochiensis* (33% per annum) and *G. pallida* (20% per annum) in the absence of host crops.

data, the control measures that might be used in consecutive years or in combination, are assigned multiplication factors (F) which can be multiplied together to project the population density in the final year of the cropping cycle. These factors may be greater or less than one, depending on how the control measure affects the population in one year. Algebraically expressed,

$$Pf = Pi(F_1)^a(F_2)^b(F_3)^c \ldots\ldots (F_n)^x,$$

where $F_1 \ldots\ldots F_n$ are various control measures and the exponents a, b, *etc.*, denote the number of years for which each is practised.

Adequate control (Pf of 38 from a Pi of 100) of *G. rostochiensis* (Table 3) is obtained by growing three non-host crops in rotation with one resistant potato crop and one non-resistant potato crop treated with nematicide (*i.e.*, potatoes 2 years in 5). For a similar degree of control of *G. pallida* (Pf of 42 from a Pi of 100), a non-

Table 2. Multiplication (Pf/Pi) of two *G. pallida* populations on some susceptible cultivars grown at two different sites.[z]

Cultivar	Site 1	Site 2
Désirée	24.5	19.0
Estima	8.9	19.7
Wilja	17.3	8.9
Pentland Squire	11.3	12.2
Record	3.0	9.5
Romano	3.7	4.3

[z]Data from A. G. Whitehead, personal communication.

Table 3. Examples of the use of population multiplication (or reduction) factors associated with potato cyst nematode control measures to project the final population (Pf) after a number of years of integrated control, assuming an initial population (Pi) of 100.

Control measure	*Globodera rostochiensis*		*Globodera pallida*		
	Multiplication factor	Years measure employed	Multiplication factor	Years measure employed: Control program I	Years measure employed: Control program II
Non-host crop	0.67	3	0.8	6	6
Resistant cultivar	0.25	1	2	1	1
Non-resistant cultivar + nematicide (N)	5	1	20	1	
Non-resistant cultivar + N + hatching stimulant			4		1
Soil fumigation			0.2	1	
Trap crop			0.2	1	1
Calculations					
G. rostochiensis:	Pf = $(100(0.67)^3(0.25)(5)$				= 38
G. pallida (control program I):	Pf = $(100)(0.8)^6(2)(20)(0.2)(0.2)$				= 42
G. pallida (control program II):	Pf = $(100)(0.8)^6(2)(4)(0.2)$				= 42

host crop has to be grown for 6 years, a partially resistant cultivar grown, a non-resistant cultivar treated with nematicide, the soil fumigated, and a trap crop grown. If the nematicide could be made more effective, perhaps by stimulating hatch with an artificial hatching agent, such that multiplication were only 4× (rather than the more likely 20×), either fumigation or trap-cropping could be omitted and the same degree of control still obtained. Either way, this would only permit two potato crops to be grown every 9 years, a situation not readily accepted by an intensive potato farmer but more acceptable than a rotation in which fumigation or trap-cropping were not used (*i.e.*, non-host crops, one partially resistant crop and one non-resistant crop treated with nematicide as the control measures). In such a situation, non-host crops would have to be grown for a total of 20 years during a 22 year cycle (reducing to 13 during a 15 year cycle if the nematicide efficacy were increased as discussed above).

ELECTROPHORETIC DIAGNOSIS

A prerequisite for sound management of PCN is to know which species has to be dealt with. The identity of field populations of PCN, and also the composition of populations which are mixtures of the two species, can be routinely checked by biochemical techniques. The best of these uses two species-specific proteins, which occur in equal concentrations in the two species and which can be separated, visualised, and quantified very easily by isoelectric focusing (10).

IMMUNOLOGICAL DIAGNOSIS

Any protein differences between species that can be detected electrophoretically can also be detected immunologically. From monoclonal antibodies raised against whole body homogenates of second-stage juveniles of PCN, it has been possible to select a number of antibodies that show species specificity (14).

In ELISA (enzyme-linked immunosorbent assay), the reactions of two of these antibodies differ to increasing concentrations of protein in extracts from the two species of PCN (Fig. 6). AFRC MAC 356 recognises *G. pallida* but not *G. rostochiensis* and AFRC MAC 357 does the reverse. In addition, each antibody shows a linear relationship between optical density and antigen concentration of the species that it recognises, which means that they can be used for the quantification as well as qualitative recognition of PCN species. Use of monoclonal antibodies for such a purpose has been proposed previously (16).

DNA DIAGNOSIS

Within each species of PCN, pathotypes or races can be distinguished by their ability to multiply on *Solanum* clones with different major genes for resistance. The H_1 gene from *Solanum tuberosum andigena* distinguishes two pathotypes of *G. rostochiensis* and the H_2 gene from *Solanum multidissectum* distinguishes two pathotypes of *G. pallida*. Breeding for resistance to the pathotypes virulent towards these resistance genes has used polygenically inherited resistance (*e.g.*, from *Solanum vernei*) and this has produced clones with differing amounts of resistance. Nevertheless, these clones were used in schemes to classify PCN population variants (4, 12). These schemes have been subject to considerable criticism (17) because some of the pathotypes are artefacts of the classification system or are not distinct, consisting of continuously varying populations of individuals with different virulence genes. They should be replaced by the concept of virulence groups distinguishable by their behaviour on standard clones or cultivars.

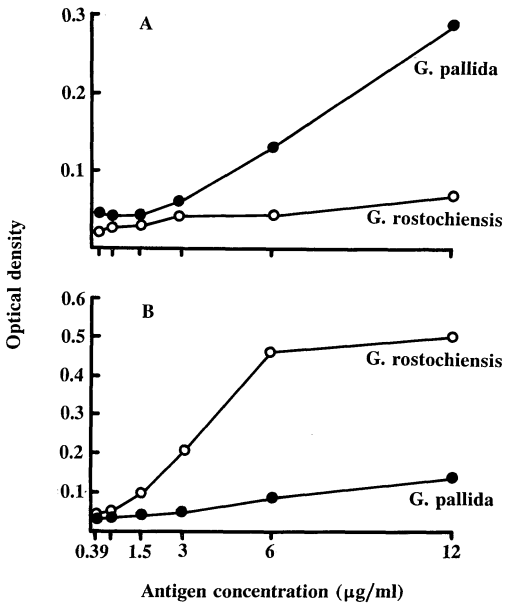

Fig. 6. Reactivity of monoclonal antibodies with different coating concentrations of homogenates of *G. rostochiensis* (open circles) and *G. pallida* (closed circles) in indirect ELISA. A) Antibody AFRC MAC 356. B) Antibody AFRC MAC 357. (Re-drawn from Robinson *et al.*, 1993.)

The absence of major gene resistance to many populations of *G. pallida* has led to the release of commercial potato cultivars with partial resistance based on polygenes. The repeated growing of such cultivars in *G. pallida* infested soil will select increased levels of virulence in the nematode population (18). When six *G. pallida* populations were maintained for 11 successive generations on the ex *S. vernei* clone 62.33.3, multiplication rates on the resistant clone increased from only 10% of that on the susceptible control in year 1 to almost 90% in year 11 (Table 4). Clearly, it will be important to identify the level of virulence in a field population of PCN in order to predict the performance of a partially resistant potato cultivar.

It is most unlikely that protein analyses or immunoassays will be developed that can distinguish differences in virulence levels. However, this may be possible with

Table 4. Mean Pf/Pi ratios for six *G. pallida* populations maintained for 11 successive generations on ex *Solanum vernei* potato clone 62.33.3.[y]

Generation	Pf/Pi	Relative Pf/Pi[z]
1	0.59	10.4
3	1.69	10.7
5	15.4	42.3
7	13.7	68.3
9	47.2	62.6
11	58.0	88.8

[y]Data from Turner, 1990.
[z]Expressed as the percentage of the Pf/Pi obtained for the non-resistant cultivar Arran Banner.

DNA technology. DNA probes have been used to differentiate the two species of PCN (3) and RFLP (restriction fragment length polymorphism) analysis has shown potential for detecting sub-specific differences in *G. pallida* populations (13,15). Further advances in technology, perhaps using PCR (polymerase chain reaction) techniques, may lead to proper understanding of virulence and resistance and to methods of detecting and quantifying virulence routinely.

BIOLOGICAL CONTROL

There have been reports of natural decline of populations of PCN and this is thought to be due largely to parasitism by fungi (5). Soils which have been intensively cropped with potatoes are likely to be good sources of potential biological control agents and 10 candidate species of fungi have been isolated from such soils (6). *Cylindrocarpon destructans* was the most promising of these and has been used as a model organism for the study of methods of culture and incorporation in soil. When an inoculum of straw colonised by *C. destructans* was placed around potato seed tubers planted in PCN infested soil in pots kept in a glasshouse, the numbers of juvenile stages of *G. rostochiensis* and *G. pallida* in roots were decreased by 62% and 84%, respectively (Table 5). These results are particularly promising as they were from a method of inoculation of the fungus which should be easier to utilise in the field than one which requires inoculum to be mixed throughout the bulk of soil.

CONCLUSIONS

The management of *G. pallida* is a much less tractable problem than that of managing *G. rostochiensis*, because of its greater persistence, its lesser susceptibility to nematicides, and the absence of good resistant cultivars. However, there are as yet unused additional measures, and it may be possible to incorporate them in integrated control packages. These include selective use of non-resistant cultivars that permit relatively little net multiplication (for as yet unknown reasons), the use of trap crops, the use of artificial hatching agents (perhaps in combination with nematicides in order to improve nematicide efficacy), and the use of biological control agents. The informed application of appropriate control measures will be assisted by improved methods

Table 5. Effects of *Cylindrocarpon destructans* on the numbers of juvenile stages of potato cyst nematodes in potato roots.[z]

Nematode species	*C. destructans*	Juveniles per gram of root	Percentage of control achieved
G. rostochiensis	–	510	
G. rostochiensis	+	193	62
G. pallida	–	445	
G. pallida	+	73	84

[z]Data from Crump and Flynn, 1992.

of diagnosis and quantification of nematode populations (using both DNA and protein based assays) and, in the slightly longer term, studies of the interactions of nematodes and their hosts at the molecular level should lead to the deployment of novel mechanisms of resistance.

ACKNOWLEDGEMENTS

I would like to thank all colleagues at Rothamsted, especially A. G. Whitehead, who so freely gave of their time and results in order to allow this review to be compiled.

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Received: *3.VII.1993*
Recibido:

Accepted for publication: *26.IX.1993*
Aceptado para publicación: