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THE POTENTIAL FOR MANAGING ROOT-KNOT NEMATODES BY USE OF *PASTEURIA PENETRANS* AND OXAMYL

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Summary. The use of *Pasteuria penetrans* and oxamyl singly and in combination for the control of *Meloidogyne javanica* infecting two tomato cultivars was tested under glasshouse conditions. Treatments significantly decreased the number of egg masses produced on cvs. Tiny Tim and Moneymaker compared with the untreated controls. On Moneymaker, egg production was greater in the combined treatments despite there being fewer egg masses. The relationship between the egg masses produced in a root system and the number of eggs per egg mass was negatively correlated.

The widespread use of conventional chemical nematicides to control plant parasitic nematodes has declined in some countries because they have been found to cause environmentally undesirable side effects or toxicity to humans and other non-target organisms. No new efficient, environmentally benign nematicides are available. The problem has introduced a sense of urgency into the search for alternative methods of nematode management.

Pasteuria penetrans (Thorne) Sayre *et* Starr is an obligate, spore-forming parasite of plant parasitic nematodes (Davies *et al.*, 1988). Stirling (1984) found that the addition of the bacterium to soil reduced galling of roots caused by root-knot nematodes and Brown *et al.* (1985) showed that it decreased the pathogenicity of field populations of *Meloidogyne incognita* resulting in yield increases of tobacco, soybean and winter vetch. These authors also suggested that a combination of organisms was a possibility for the sustainable, long term control of root-knot nematodes. Roy (1982) demonstrated synergistic suppression of the galling of tomato roots due to *M. incognita* with the nematophagous endoparasitic fungus *Catenaria anguillulae* Sorokin and the nematicide ethoprophos. Stirling (1984) suggested that a combination of *P. penetrans* and a nematicide might prove effective for nematode population control as *P. penetrans* is apparently not affected by nematicides.

The main objective of this investigation was to test the effectiveness of a combination of the oxime carbamate nematicide oxamyl and *PL. penetrans* for the control of *M. javanica* (Treub) Chitw.

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Materials and methods

Spores of a Malawian isolate of *P. penetrans* (PpMwa) prepared from powdered tomato roots by the technique of Stirling and Wachtel (1980) were mixed thoroughly in commercially produced soil-based compost (John Innes No 2) at a concentration of 40,000 spores per g. The spore-amended compost was put into 15 cm diam pots. In other pots a liquid preparation of oxamyl (Vydate L 240 g a.i./kg) was applied by drenching to unamended compost at 1 mg/kg and to *P. penetrans* spore-amended compost at 0.5 mg/kg. Untreated compost was used as a control. After filling, all pots were lightly watered and left to stand for 24 hours before adding a suspension of 5000 freshly hatched *M. javanica* juveniles from a population originating from Malawi to holes in the compost which were then covered. The pots were left for a further 48 hours before planting 6 week old tomato seedlings cv. Tiny Tim. This was done so that there would be a greater opportunity for the nematodes to become encumbered with spores before invading the host, such as may occur in the field. The pots were arranged in a completely randomised design with three replicates. After eight weeks growth in a glasshouse at 18-43°C (July-August 1990) the plants were washed from their pots and galling assessed on a 0-5 scale (Stirling, 1984). The root systems were examined after staining in phloxine B dye which enabled the counting of egg masses (Hooper, 1986). Nematode eggs were collected from the root systems using 1% sodium hypochlorite (NaOCl), as described by Hussey and Barker (1973).

Compost (John Innes No 2) in 20 cm diam pots was treated with oxamyl at concentrations as in the previous experiment. Because of shortage of *P. penetrans* powder, the *M. javanica* inoculum was pre-encumbered with

spores of the same isolate by leaving the newly hatched nematodes in a spore suspension at 25°C overnight. An assessment was made of spore attachment on the juveniles before they were added to the pots (5000 per pot) as described above. Two days later 6 week old tomato seedlings cv. Moneymaker were planted and the pots were arranged in a glasshouse in a completely randomised design with three replications of each treatment. Eight weeks later the plants were examined as previously described. Fruit weights and plant heights were also recorded. The number of eggs/egg mass was determined by counting the total number of eggs in a root system and dividing by the total number of egg masses from that particular root system. A random sample of 10 mature females with egg masses was examined for presence of *P. penetrans* spores.

Results

Pasteuria penetrans or oxamyl, or the combination of both, significantly reduced the number of egg masses per root system compared with the untreated control ($P<0.001$) (Table I). Root galling was also reduced significantly by the different treatments compared with the untreated control ($P<0.001$) (Table I).

The majority of juveniles used to inoculate the tomato cv. Moneymaker were encumbered with 10-19 spores (Table II).

Egg mass production was significantly reduced with *P. penetrans*, oxamyl or the combination, compared with the untreated control ($P<0.05$). Significant differences were also found between the numbers of eggs produced per egg mass in different nematode control treatments ($P<0.001$), there being a greater number of eggs produced on the root systems of plants that had been treated with oxamyl and/or *P. penetrans*-encumbered nematodes (Table III). Approximately 288750 eggs were produced with the *P. penetrans* + oxamyl treatment compared to the control which had 241500 eggs per root system.

The regression analysis of the relationship between the number of eggs per egg mass and the number of egg masses produced per root system showed a significant negative correlation, – 0.8828 ($P<0.001$) indicating that as the number of egg masses increased the number of eggs per egg mass became fewer (Fig. 1).

No *P. penetrans* spores were found in the females that had produced egg masses.

The weight of tomatoes, root-knot indices and plant heights did not differ significantly between treatments and these data are not presented.

Table I - *Root galling indices and numbers of egg masses of* Meloidogyne javanica *on tomato cv. Tiny Tim as influenced by treatments with* Pasteuria penetrans *and oxamyl.*

Treatment	Root galling index+	Egg masses/root system
P. penetrans	1.50	41
Oxamyl	1.25	20
P. penetrans + oxamyl	1.00	8.5
Control	3.50	162.8
SE Difference ±	0.445	28.27

+ Based on estimates of % galling 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-88% and 5 = 89-100%.

Discussion

Once *P. penetrans* spores are modified by desiccation, a lag period in spore attachment to nematodes is required upon hydrating (Brown and Smart, 1984). Dutky (1978) reported that for maximum spore attachment to occur, spores of *P. penetrans* should be moistened for 48 hours or longer. The *P. penetrans* spores are immobile and the attachment depends on chance contact with the nematodes moving through the soil before entering roots. When deploying under field conditions it would seem wise to delay transplanting a crop for a few days in order to expose nematodes to spores for a period long enough for attachments to occur. The use of oxamyl before planting may also be useful because at low concentrations, carbamate nematicides affect the movement and orientation of nematodes towards host roots rather than killing the nematodes (Wright, 1981). Probably juveniles which are exposed to oxamyl are disoriented and with a spore burden on their cuticle, many juveniles might fail to invade roots and thus fewer egg masses are produced. In the first experiment, the incidence of galling was less in the *P. penetrans* and oxamyl treatments, indicating that root invasion was affected in their

Table II - *Estimation of* Pasteuria penetrans *spore attachment to nematodes before inoculating into tomato plants cv. Moneymaker (from sample of 31 juveniles).*

Spores per nematode	Number of nematodes
0-4	0
5-9	2
10-14	13
15-19	10
20-24	3
Over 25	3

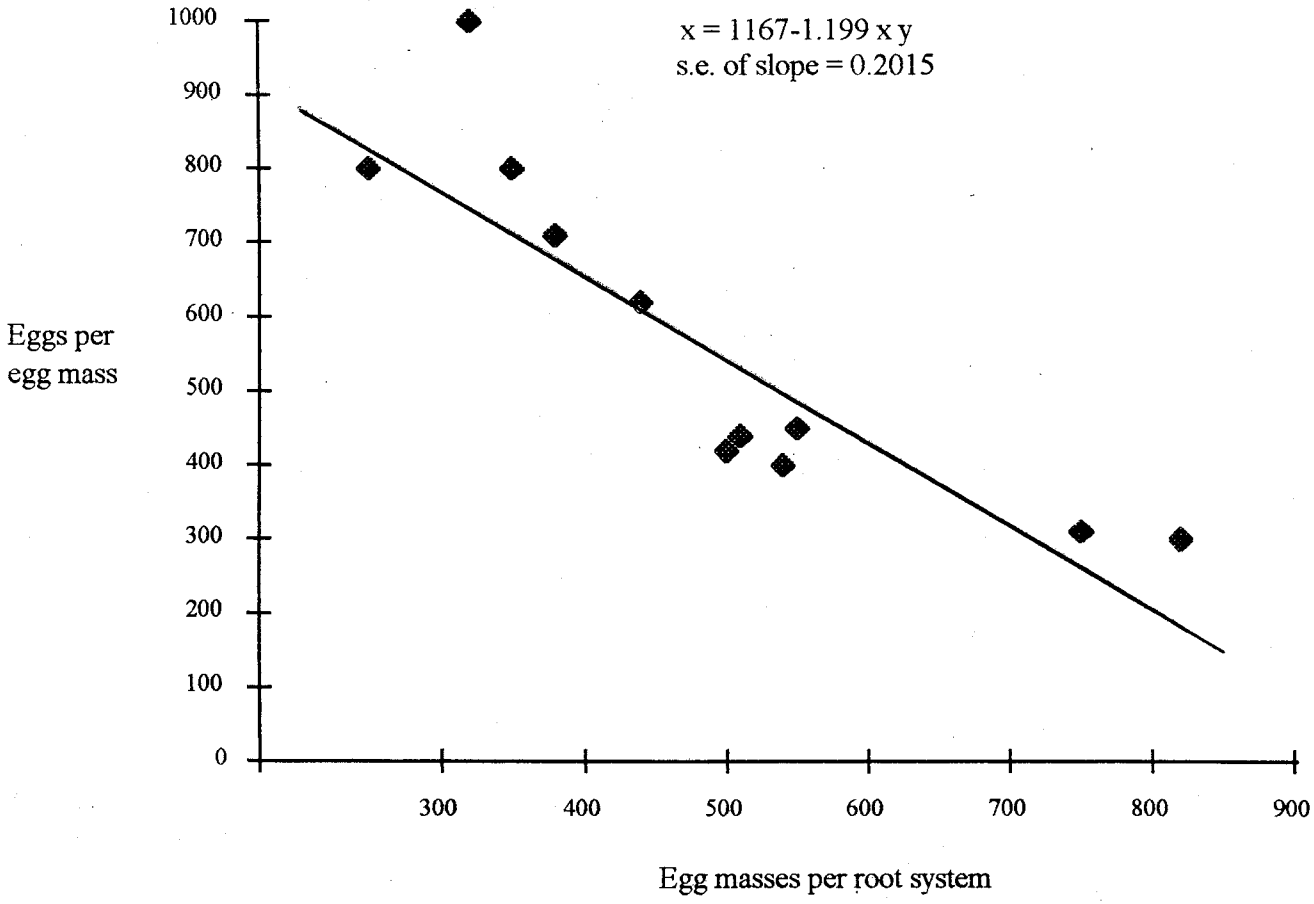

Fig. 1 - Relationship between *Meloidogyne javanica* egg masses on a root system and the number of eggs per egg mass.

Table III - *The relationship between number of egg masses and the number of eggs per egg mass as influenced by different nematode control treatments on tomato cv. Moneymaker.*

Treatment	Egg masses per plant	Eggs per egg mass
P. penetrans	508	551
Oxamyl	432	545
P. penetrans + oxamyl	359	841
Control	693	349
SE Diff	± 93.4	117.7

presence. Although the two experiments are not directly comparable, it would appear that nematode reproduction on the tomato cv. Tiny Tim was less than on Moneymaker. The reasons why the dwarf determinate tomato should be a poorer host than the indeterminate Moneymaker needs further investigation particularly if *P. penetrans* is to be used as a biocontrol agent for container-grown crops where restricted root growth may affect nematode populations and the epidemiology of the parasite.

On Moneymaker, the greater the number of egg masses produced the fewer was the number of eggs per egg mass. It is probable that there is competition for space at high nematode densities which affects the number of eggs produced or slows the rate of egg production. It is possible that *P. penetrans* and/or oxamyl will give short-cycle crops some protection from nematode attack during their early growth although the final population density of root-knot nematodes might be very high. These results show how important it is that a control method should restrict the invasion or developmeent of the first generation of *M. javanica.*

Nematicides may be a useful adjunct to the deployment of *P. penetrans.* However, as *P. penetrans* has resistant,

long-lived spores, treatments will have to be developed which encourage the natural build-up of spore concentrations in soil over repeated crop cycles.

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