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RELATIONSHIP BETWEEN *PASTEURIA PENETRANS* INFECTION LEVELS AND DENSITY OF *MELOIDOGYNE JAVANICA*

by
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Summary. The interaction between three different levels of *Meloidogyne javanica* inoculum density and three levels of parasitism by *Pasteuria penetrans* was investigated in a factorial experiment. The highest and the lowest final female densities were observed at 5% initial parasitism, at the highest and lowest inoculum density, respectively. At 15% initial parasitism an increasing effect of *P. penetrans* on nematode multiplication was observed. No dose response effect of female density to increasing juvenile inoculum was observed at 40% initial parasitism. At this parasitism inoculum the highest female parasitism was observed at 2000 initial juveniles per pot. The final juvenile density was positively correlated with the final percentage of juveniles with spores. A *P. penetrans* effect was also observed at the highest density and parasitism levels, on the number of galls and egg masses per g of root. In a second trial with two fertilization levels, plant growth higher than control and lower *M. javanica* density and percentage of parasitized females were observed in the treated plants. Data suggest a density dependent relationship between *P. penetrans* and *M. javanica*. However, no suppression of the nematode population at the inoculum levels used was observed.

The pathogens of the "*Pasteuria penetrans* group" are microbial antagonists of nematodes characterized by obligate parasitism and host specificity (Stirling, 1991). The life-cycle of *P. penetrans* occurs entirely within the infected host tissues. The infective resting spores are free in the soil where they adhere to the nematode cuticle. After spore germination, the host body becomes progressively consumed by spherical microcolonies. After sporulation, resting spores are released into the soil after death of the host and subsequent decomposition of the cadaver (Stirling, 1991).

The development of a *Pasteuria* disease in a nematode population depends on the transmission of infective spores from the soil microcosm to the nematode host due to its movements. Germination occurs after spore adhesion to the cuticle provided that a minimum threshold number of propagules is acquired (Verdejo-Lucas and Jaffee, 1988).

The study of the effect of *P. penetrans* on a nematode population is of importance because of its possible exploitation in biological control. The relationship between parasitism by *P. penetrans* and the corresponding host abundance is density dependent (Kasumimoto *et al.*, 1993; Ciancio, 1995). Little information, however, is available on the interactions between the development of a nematode population in the soil and the level of parasitism achieved. Studies on *P. penetrans* in naturally infested fields showed parasitism persisting at stable but low levels (Verdejo-Lucas, 1992; Ciancio, 1995).

Data on host and parasite growth and mortality, and parasitism efficiency and persistance

are necessary for the application and testing of general density dependent models to *P. penetrans* and its hosts (Hassel, 1978; Anderson and May, 1981). Here we report on the effect of different initial host densities and *P. penetrans* infection levels on abundance and parasitism of the root-knot nematode *Meloidogyne javanica*.

Materials and methods

A *P. penetrans* (Thorne) Sayre *et* Starr isolate (PPM) originating from *M. javanica* females parasitizing banana at Souss-Massa, Morocco, was maintained on the same nematode species on tomato in the glasshouse. Spores were obtained from dried tomato roots containing infected *M. javanica* females as described by Stirling and Wachtel (1981). A spore suspension was prepared by adding 1 g of ground dry root material to 200 ml of tap water in a flask and gently bubbling in air. After 24 hrs the suspension was sieved through a 25 µm sieve to remove roots debris. The spore concentration was determined by counting the number of spores observed in three replicated 5 µl droplets of the suspension on a glass slides at 500 X.

An Italian population of *M. javanica* (Treub) Chitw. collected at Acireale (Catania) and maintained on tomato in glasshouse at Bari was used in this study. Several egg masses were dissected from the tomato roots and hatched on a sieve in a Petri dish containing a thin water film and maintainedat 23 ± 2 °C for five days. The juveniles (J2) collected from the egg masses were stored at 4 °C. Subsequently, approximately 9×10^3 juveniles concentrated in 20 ml of water were added to 200 ml of spore suspension containing 1.9×10^4 spores/ml. The J2 were maintained in the suspension by gently bubbling in air at room temperature for 24 hrs. The nematodes were then extracted from the suspension by sieving with a 25 µm plastic sieve. The percentage of spore adhesion and the number of spores adhering per nematode were measured on three samples of 15 specimens picked up with an eyelash and examined on a temporary mount on a glass slide at 500 X.

In the first experiment, three different initial nematode densities (500, 1000 and 2000 J2 per pot) were combined with three levels of *P. penetrans* parasitism (5, 15 and 40%) to obtain nine treatments. For each treatment the spore encumbered J2 were added to nematodes without spores prior to plant inoculation. Tomato (*Lycopersicon esculentum* Mill.) plants (cv. San Marzano) at the four leaf stage were transplanted into 15 cm diam. pots containing steam sterilized sandy soil. Healthy and spore encumbered nematodes were mixed in 100 ml water and pipetted in two small holes 5 cm from the plant base one week after transplanting. The plants were maintained in a glasshouse at 24 ± 2 °C, randomized every week and irrigated daily. There were four replications of each treatment.

After 9 weeks, the plants were harvested and the number of females/g root and J2/100 cm^3 soil were counted. For each treatment and replication, the *P. penetrans* parasitized females were counted examining 8-12 females dissected from 2-3 g of roots. The females were crushed in a water droplet on a temporary glass slide and examined at 500 X. The presence of spores, vegetative dichotomic thalli or other sporulating forms typical of *P. penetrans* were used to assess parasitism.

Data were analysed using the procedure GLM of the statistical package SAS and Duncan's multiple range test for mean testing (SAS, 1985).

In a second experiment the effect of soil fertility on *P. penetrans* parasitism was studied. *Meloidogyne javanica* J2 with *P. penetrans* spores were inoculated at a density of 1200 nematodes per pot around the roots of tomato plants (cv. San Marzano) as described above. Parasitism by *P. penetrans* was 63% with 2-3 spores per J2. Twelve pots were inoculated and divided into three groups. The first group was

used as control and did not receive any fertilizers. The second and the third were fertilized at ten days intervals using 2.5 and 5 ml of a liquid fertilizing solution (Cifo, Bologna, Italy, 14% N, 7% P, 5% K and 0.2% Fe) dissolved in 1 L of irrigation water. The same amount of water without fertilizer was used as control. The plants were watered daily and maintained at 24 °C ± 2 in a glasshouse. Eight weeks after inoculation the plants were harvested and the level of parasitism examined as described.

Results

In the first esperiment, the level of *in vitro* spore attachment to *M. javanica* J2 used as inoculum was 96% with a mean of 3 (1-8) adhering spores per nematode after 24 hrs. At the end of the experiment different life-stages of *P. penetrans* were observed within *M. javanica* females which showed mainly immature spore stages, sporulating cells, or vegetative thalli. Mature spores were observed adhering to the J2 extracted from the soil at the rate of 1-2 spores per nematode.

Final female density was positively correlated with the initial J2 inoculum level ($r = 0.8053$, $P < 0.01$). When considering each initial parasitism level, a significant higher final density was observed at 2000 J2 and 5% initial parasitism ($P < 0.001$). At this initial parasitism the lowest final female density was observed at 500 initial J2. At higher initial percentages of nematodes with *P. penetrans*, a lesser effect on female density was observed. At 15% initial parasitism, female abundance was significantly lower at 500 J2 inoculum ($P < 0.001$). No difference in final female numbers was observed among the 40% initial parasitism treatments (Fig. 1).

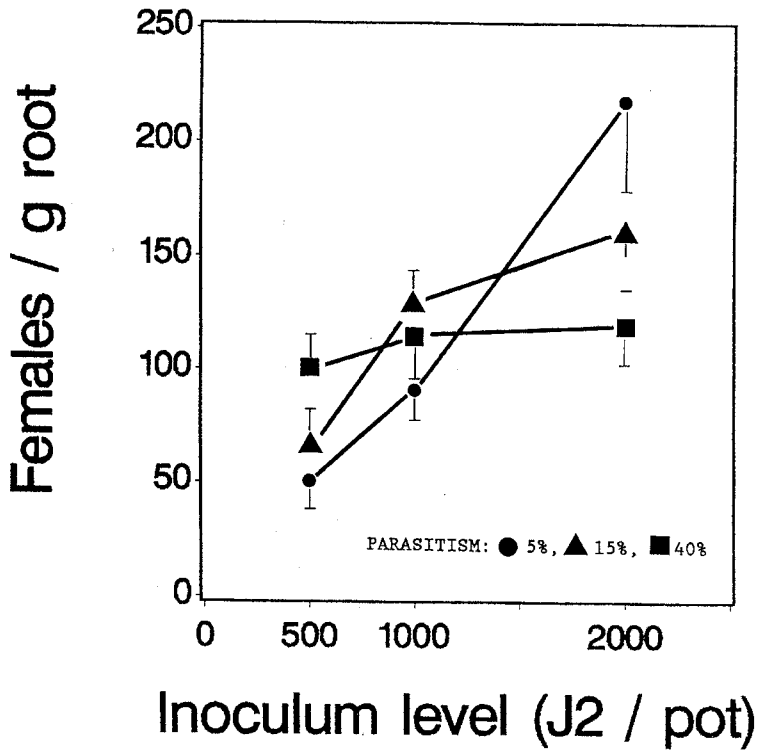

Fig. 1 - Final density of *Melodogyne javanica* females at different initial juvenile densities and *Pasteuria penetrans* parasitism levels. (Vertical bars = 1 standard deviation unit).

Considering each initial density, at the lowest inoculum the highest female density was observed at 40% initial parasitism, significantly higher than the 5% treatment ($P < 0.001$). At an initial 1000 J2 no differences were observed amongst treatments. At an initial 2000 J2 the final density at 5% initial parasitism was significantly higher than that observed at 40% initial parasitism ($P < 0.001$, Fig. 1).

The highest final female parasitism was observed at an initial 2000 J2 and 40% parasitism ($P < 0.001$). When considering each initial inoculum and parasitism level, no other significant differences were observed among treatments (Fig. 2).

Final J2 density was correlated with the initial inoculum ($r = 0.8064$, $P < 0.01$). The final mean percentage of J2 with spores never exceeded 20% and was positively correlated with final J2 density ($r = 0.8706$, $P < 0.01$) and final female parasitism ($r = 0.7411$, $P < 0.05$). A dose response to initial density and parasitism was observed for the final number of J2 in the soil and, to a lesser extent, for the percentage of J2 with spores (Table I). The number of egg masses or galls per g of root showed a similar increase at the lowest initial densities but decreased at higher initial levels of density and parasitism (Table I).

Plant height generally decreased as initial nematode inoculum increased. At an initial 500 J2, plant height was less at 40% parasitism. At a higher initial J2, plant weight was lower at 15% initial parasitism. Root weight increased with parasitism only at an initial 1000 J2 (Table I).

TABLE I - *Effect of initial* Meloidogyne javanica *inoculum and* Pasteuria penetrans *parasitism on final juvenile density and parasitism, and host plant growth.*

	Initial density (J2/pot)					
	500	1000	2000	500	1000	2000
Initial parasitism (%)	J2/100 ^{3} cm soil			Final J_2 with spores (%)		
5	*B* 216 A	*B* 291 A	*A* 2366 a	0.0 a	0.9 A	1.2 A
15	*B* 1000 A	*B* 1483 AB	*A* 5725 b	2.4 ab	4.0 AB	10.9 AB
40	*B* 1149 B	*B* 3233 B	*A* 5433 b	*B* 6.2 b	*B* 10.0 B	*A* 18.3 B
	Egg masses/g root			Galls		
5	*B* 50 A	*B* 90	*A* 202 a	*B* 39 A	*AB* 76	*A* 165 a
15	*B* 66 AB	*AB* 120	*A* 154 ab	*B* 56 AB	*AB* 93	*A* 131 ab
40	100 B	113	116 b	81 B	87	79 b
	Plant height (cm)			Root weight (g)		
5	33 a	28	28 A	8.0	6.1 a	8.6
15	*A* 29 ab	*B* 22	*B* 18 B	7.6	8.2 ab	8.6
40	25 b	31	25 AB	7.8	9.0 b	8.2

* Data flanked in line on the left side by the same letter (italic) are not significantly different according to Duncan's multiple range test (capital letters: $P < 0.01$; lower case: $P < 0.05$). Data flanked in columns on the right side by the same letter are not significantly different according to Duncan's multiple range test (capital letters: $P < 0.01$; lower case: $P < 0.05$).

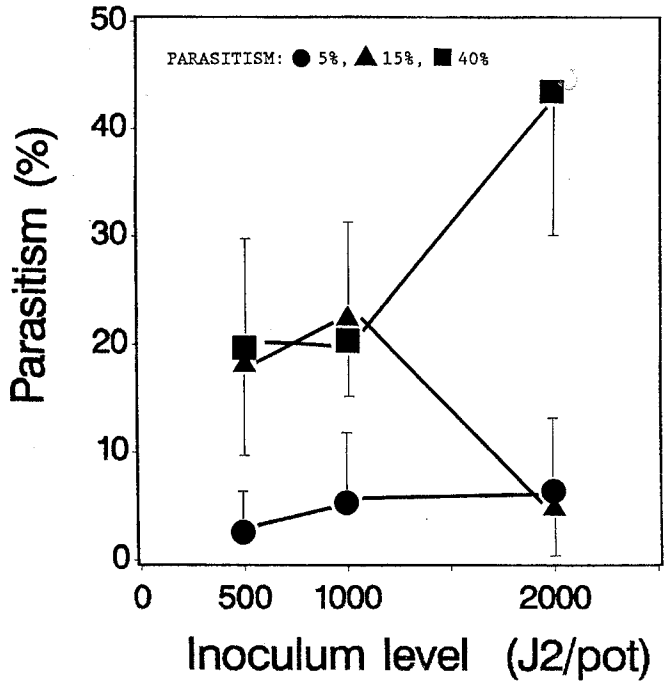

Fig. 2 - Final *M. javanica* female parasitism by *P. penetrans* at different initial juvenile densities and parasitism levels.

The GLM procedure showed a significant effect of initial density and of its interaction with initial parasitism on final female density and the numbers of galls and egg masses ($P < 0.001$). Initial parasitism ($P < 0.001$) and, to a lesser extent, its interaction with initial density ($P < 0.05$) had a significant effect on final female parasitism. Initial parasitism also had a significant effect on plant height ($P < 0.05$).

Fertilization had a positive effect on plant development and the number of females observed per g of root was lower in the treated pots. Female parasitism levels decreased with increasing doses. Fewer galls were observed at higher fertilizer levels, together with higher root and plant weights (Table II).

Discussion

Considering the life-cycle of *P. penetrans* and its thermal requirements (Stirling, 1981) the factorial experiment covered almost two parasite generations, as also confirmed by the presence of immature *P. penetrans* stages in parasitized females. The spores observed on infested J2 thus proceeded mainly from the first parasite generation. Since each female *M. javanica* parasitized by *P. penetrans* produces an average of 2 x 10^6 spores (Stirling, 1991), the total numbers of spores expected after the first generation in the pots ranged from 0.25 to 8 x 10^8, assuming a 50% J2 survival rate. When the concentrations are referred to each unit of soil volume, the observed rates of J2 spore attachment were much lower than those observed for the same nematode species in similar conditions (Stirling *et al.*, 1990). This suggests that a significant fraction of the spores produced in the first parasite generation was removed by the first J2 generation. It also suggests that a lower fraction was still present in the pots at harvest and were adhered to the hatching J2.

The infection of different nematode genera-

TABLE II - *Effect of fertilization doses on density of* M. javanica, *parasitism by* P. penetrans *and plant growth.*

Fertilizer rates (ml)	Females/g root	Galls/g root	Parasitism (%)	Weight (g)	
				Root	Plant
0 (Control)	173 ± 18 A	109 ± 11 A	15 ± 11	6.9 ± 0.5 B	16.3 ± 1.6 B
2.5	104 ± 17 B	80 ± 9 B	12 ± 8	12.2 ± 0.5 A	38.9 ± 5.5 A
5	123 ± 10 B	78 ± 5 B	7 ± 12	9.0 ± 0.5 B	42.9 ± 3.7 A

* Data flanked in columns by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.01$).

tions by spores produced by the same parasite generation may explain the low frequencies of J2 with spores observed and the low numbers of spores per infected nematode counted at the end of the trial. *Meloidogyne javanica* shows a continuous delayed egg hatching lasting several weeks (Huang and Pereira, 1994). Due to the persistance and durability of spores, any *P. penetrans* generation can then infect hosts from several generations as well as the final hatching J2 of the first nematode generation. This particular interaction of host and parasite produces a continuous overlapping of parasitic events in time.

Considering the biological control efficiency of this *P. penetrans* isolate, nematode population growth appeared affected at 40% parasitism and an initial 2000 J2 only. Parasitism around 5% appeared enough to avoid parasite extinction, although no changes were observed in time, irrespective of the initial densities. A marked decline in parasitism was observed at 15% and an initial 2000 J2.

Several factors can affect the efficiency of *P. penetrans* in controlling the host population growth. At high densities of the parasite, the removal of spores by heavily encumbered J2 can act as a limiting factor. In this case, hosts remove spores from the soil microcosm, but are uncapable of parasitizing plants, thus avoiding the subsequent parasite multiplication (Davies *et al.*, 1991). When parasitism occurs, the proportion of propagules produced per spore at the end of the parasite life-cycle (or the "per spore" multiplication efficiency) is also reduced. Finally, the occurrence of time delayed effects between host density and parasitism (Ciancio, 1995) and/or the selection of resistant nematodes (Tzortzakakis and Gowen, 1994) may further explain the low numbers of J2 with spores observed.

Although no linear relationship was observed between final female density and parasitism, the positive correlation between final J2 density and percentages with spores provides an indication of a host-parasite density dependent relationship. Density dependence is a general term describing a situation in which the effect of limiting factors increases at high host density levels, decreasing as the population declines. A density dependent relationship can evolve in stable situations in which both host and parasite co-exist, with regular changes of density and parasitism in time (Hassel, 1978; Jaffee, 1993). Similar situations were observed in some field studies on root-knot nematodes or other nematode species in association with specific *P. penetrans* isolates (Verdejo-Lucas, 1992; Ciancio *et al.*, 1992; Ciancio, 1995). Density dependence can provide a general framework for plant nematode management strategies, although it does not necessarily imply the occurrence of a "natural biological control" or nematode suppression (Jaffee, 1993).

High frequencies of *P. penetrans* spore-encumbered or parasitized nematodes are consid-

ered necessary to reduce the host population at non-damaging density levels (Kasumimoto *et al.*, 1993). In our experiments however, no suppression was observed even at the highest *P. penetrans* treatments and the plants showed significant root damage. Lack of suppressiveness was reported in other experiments carried out in controlled conditions with root-knot nematodes and *P. penetrans* (Stirling *et al.*, 1990; Oostendorp *et al.*, 1991; Kasumimoto *et al.*, 1993). Low parasitism values were also observed in studies on the population dynamics of *P. penetrans* and other host nematodes in naturally infested fields (Verdejo-Lucas, 1992; Ciancio, 1995).

Increased fertilizer levels resulted in reduced numbers of *M. javanica* females per g of root and in improved plant development. This effect appears responsible for the reduction in nematode density. No linear relationship was, however, observed between nematode parasitism and fertilizer doses. Decline in parasitism was not expected and may be the result of a time delayed response to density changes or, indirectly, of a better plant reaction to nematode parasitism.

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