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ROOT INVASION OF DIFFERENT PLANT HOSTS BY JUVENILES OF *MELOIDOGYNE* SPECIES ENCUMBERED WITH *PASTEURIA PENETRANS* SPORES

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Abstract

The effects of *Pasteuria penetrans* encumbered juveniles of *Meloidogyne incognita*, *M graminicola* and *M. javanica* with two levels of attachment (5-10 and > 11 spores / J_2) were studied on the invasion of roots of tomato, barn yard grass, sorghum and egg plant. Invasion by spore encumbered juveniles was significantly less as compared to juveniles without spores. There was no statistically significant difference in root invasions between the two attachment levels in case of tomato, barn yard grass and sorghum. However, a statistically significant difference was observed between the two attachments levels in case of *M. javanica* invading roots of egg plant. The encumbered juveniles (5-10 spores / J_2) of *M. incognita*, *M. graminicola* and *M. javanica* caused 41.76 % reduction in invasion of tomato roots, 24.58 and 33.13 % reductions of barn yard grass and sorghum roots and 11.20 and 19.55 % reductions of tomato and egg plant roots, respectively. The reductions in invasions caused by the juveniles encumbered with > 11 spores / J_2 were: 46.47 % in tomato by *M. incognita*; 25.54 and 38.40 % in barn yard grass and sorghum by *M. graminicola* and 23.87 and 43.73 % in tomato and egg plant by *M. javanica*, respectively.

Pasteuria penetrans is a very promising biological control agent against root-knot nematodes. The role of *P. penetrans* in suppressing plant parasitic nematodes has been tested on many crops, mostly in green house pot tests (Eddaoudi & Bourijate, 1998; Gowen *et al.*, 1998) and field micro plots (Chen *et al.*, 1996; Melki *et al.*, 1998; Mukhtar *et al.*, 2002, 2003, 2005). The ability of *P. penetrans* in the suppression of root knot nematode populations is attributable to (i) reduced root penetration by the spore encumbered juveniles and / or (ii) failure to form egg masses by the infected females. There is a relationship between the numbers of spores attached to the cuticle and their invasion capacity. Mankau & Prasad (1977) observed that juveniles encumbered by spores are less mobile. Moreover, they are less able to invade (Brown *et al.*, 1985). Stirling (1984) found that juveniles encumbered with 40 spores were unable to invade. Channer & Gowen (1988) found that higher doses of *P. penetrans* significantly reduced invasion and led to almost 100 % infection of the nematodes, thus preventing reproduction. Davies *et al.*, (1988) reported that 15 spores per nematode can reduce invasion by more than 70 %. Minton & Sayre (1989) found that more than 25 spores per juvenile of *M. arenaria* were sufficient to prevent them from penetrating plant roots or to cause infection on the developing stages of individuals that succeeded in invading the roots. The objective of these studies was to explore this relationship between spores attachment and invasion and to find a better plant host to withstand maximum invasion.

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Materials and Methods

Seedlings of tomato (*Lycopersicon esculentum*), barn yard grass (*Echinochloa colonum*), sorghum (*Sorghum bicolor*) and egg plant (*Solanum melongena*) were grown in 5 cm pots containing sandy loam soil.

The populations of *Meloidogyne* spp. used in the studies were *M. incognita*, *M. javanica* and *M. graminicola*. *M. incognita* and *M. javanica* were maintained in the glasshouse on tomato var. Money maker and *M. graminicola* on barn yard grass and sorghum var. Cv Tek 1055 R.

An isolate of *P. penetrans* designated as Pp^3 derived from *M. javanica* and originating from South Africa was used in the studies. The isolate of *P. penetrans* was mass produced on *M. javanica* on tomato var. Money maker in the glass house by the method described by Stirling & Wachtel (1980). A spore suspension of *P. penetrans*, Pp3 was prepared from dry root powder and adjusted to a concentration of 30000 spores / ml. Freshly hatched juveniles of *M. incognita*, *M. javanica* and *M. graminicola* were separately encumbered in spore suspension of *P. penetrans*, Pp3 and two levels of attachment (5-10 and > 11 spores / J_2) were obtained by leaving the juveniles in the suspension for different periods of time.

Seedlings of tomato were separately inoculated with 1000 encumbered juveniles of *M. incognita* and *M. javanica* with two attachment levels and those of barn yard grass and sorghum with 1000 encumbered juveniles of *M. graminicola*. The seedlings of eggplant were inoculated with 1000 encumbered juveniles of *M. javanica*. The experiment was run in a glass house at $25 \pm 5^{\circ}$ C. After 7 days, the roots were washed, stained (Bridge *et al.*, 1982) and macerated with a Silverson homogenizer for quantitative estimation of juveniles in the roots.

Results

The results on the invasion of roots of different hosts by different *Meloidogyne* spp. are given in Tables 1 to 5. It is evident that the encumbered juveniles significantly reduced root invasion as compared to the juveniles having no spores. The juveniles of *M. incognita* having different levels of spore attachment significantly reduced the invasion of tomato roots and those of *M. graminicola* caused significant reductions in root invasion of barn yard grass and sorghum as compared to juveniles without spores. However, there was no statistically significant difference between the juveniles of *M. incognita* and *M. graminicola* encumbered with two attachment levels in % invasion of tomato and barn yard grass and sorghum roots respectively (Tables 1, 2 and 3).

Table 1. Effect of different levels of spore attachment of *P. penetrans* on the ability of *M. incognita* juveniles to invade tomato roots.

Level of attachment	Nematodes per root system	% invasion	% reduction over control
0	318.50	31.85	-
5-10 spores / J_2	185.50	18.55	41.76
> 11 spores / J_2	170.50	17.05	46.47
SED	32.07	-	-

Data are means of four replicates.

Table 2. Effect of different levels of spore attachment of *P. penetrans* on the ability of *M. graminicola* juveniles to invade barn yard grass roots.

Level of attachment	Nematodes per root system	% Invasion	% reduction over control
0	207.50	20.75	-
5-10 spores / J_2	156.50	15.65	24.58
> 11 spores / J_2	154.50	15.45	25.54
SED	16.14	-	-

Data are means of four replicates

Table 3. Effect of different levels of spore attachment of *P. penetrans* on the ability of *M. graminicola* juveniles to invade sorghum roots.

Level of attachment	Nematodes per root system	% Invasion	% reduction over control
0	407.50	40.75	-
5-10 spores / J_2	272.50	27.25	33.13
> 11 spores / J_2	251.00	25.10	38.40
SED	94.50	-	-

Data are means of four replicates

There was no significant difference in % invasion of tomato roots by *M. javanica* between the juveniles without spores and the juveniles having 5-10 spores / J_2. The invasion was significantly reduced by the juveniles of *M. javanica* encumbered with > 11 spores / J_2 (Table 4). On the other hand in case of eggplant the spore-encumbered juveniles of *M. javanica* with both attachment levels the invasion was significantly less as compared to juveniles without spores. There was also a statistically significant difference in the invasion between the juveniles having two attachment levels. The juveniles with > 11 spores / J_2 significantly reduced invasion as compared to the juveniles with 5-10 spores / J_2 (Table 5). The different plant hosts showed variation for invasion by *Meloidogyne* species and maximum invasion was by *M. javanica* on eggplant and tomato.

Table 4. Effect of different levels of spore attachment of *P. penetrans* on the ability of *M. javanica* juveniles to invade tomato roots.

Level of attachment	Nematodes per root system	% Invasion	% reduction over control
0	375.00	37.50	-
5-10 spores / J_2	333.00	33.30	11.20
> 11 spores / J_2	285.50	28.55	23.87
SED	53.17	-	-

Data are means of four replicates

Table 5. Effect of different levels of spore attachment of *P. penetrans* on the ability of *M. javanica* juveniles to invade eggplant roots.

Level of attachment	Nematodes per root system	% Invasion	% reduction over control
0	524.25	52.43	-
5-10 spores / J_2	421.75	42.18	19.55
> 11 spores / J_2	295.00	29.50	43.73
SED	52.30	-	-

Data are means of four replicates.

Discussion

The juveniles of different *Meloidogyne* spp., with two levels of spore attachment (5-10 & > 11 spores / J_2) significantly reduced the invasion of roots of different hosts as compared to the juveniles without spores. These results are in keeping with those of Mankau & Prasad (1977) and Brown & Smart (1985). Reductions in root penetration have been observed when nematodes had 15-20 spores attached (Brown & Smart, 1985; Davies *et al.*, 1988), but as many as 50 spores per nematode may be needed before there are marked reductions in the root infection (Stirling, 1984). Nematode movement was significantly reduced when juveniles were encumbered with an average of 7 spores per J_2 (Davies *et al.*, 1991). Presumbly, heavy encumbrance of bacterial spores on the nematode cuticle across the striae tends to obstruct the bending of nematode body, rendering it less mobile, thus leading to reduce root invasion. Stirling (1984) found that 40 spores attached to J_2 prevented invasion, whereas Davies *et al.*, (1988) reported that 15 spores per nematode could reduce invasion by more than 77 %. Moreover, according to Davies *et al.*, (1988) the invasion is density dependent and it was reduced when more nematodes were added and competition for root surface invasion sites increased. Lower infectivity despite higher attachment has been reported by some workers (Tzortzakakis & Gowen, 1994; Espanol *et al.*, 1997) suggesting the incompatibility between the spore isolate and the nematode population or adhesion may be independent of infection. The failure of *P. penetrans* to infect nematodes has been reported to be due only to poor recognition of the spore surface but also to some degree of resistance to germination, penetration or even mycelial development in the developing nematode (De Silva & Gowen, 1990; De Silva, 1992).

It is interesting that a high level of spore attachment may still leave many nematodes uninfected even if root galling and egg production are significantly reduced. Furthermore, it is clear that a *Pasteuria* isolate that attaches at higher rates does not always guarantee greater reduction of egg-laying. The reasons could be high variance in spore attachment or spore detachment during movement of juveniles in soil (Ratnasoma *et al.*, 1991; De Silva *et al.*, 1996). It is not clear what is the minimum numbers of spores, which have to be attached in order to ensure good infectivity. The contradictory results reported by the previous workers (Stirling, 1981; Davies *et al.*, 1988; Rao *et al.*, 1997; Giannakou, 1998) might be due to the nematode population or isolate of *Pasteuria* used. Also, individuals within a single nematode species or a mixture of species may resist spore attachment and infection (Tzortzakakis & Gowen, 1994; Tzortzakakis *et al.*, 1996). Some of these spores may not be viable or able to germinate at that time. Also some of them might not be compatible with the population of root knot used while others are. Davies *et al.*, (1988) also found significant difference between the numbers of females that developed from juveniles encumbered with different *Pasteuria* isolates at a similar level of attachment. If we compare the mean numbers of juveniles that invaded the roots in both attachment levels, the higher attachment level affected the invasion rate. From a practical point of view, attachment, even without subsequent infection, is useful in that juveniles encumbered with many spores are less capable of invading roots (Stirling, 1981; Brown & Smart, 1985; Davies *et al.*, 1988).

It is evident that different plants showed variation for invasion by *Meloidogyne* species and maximum invasion was by *M. javanica* on eggplant and tomato. Weekes (1988) compared three common hosts of *M. incognita* and found that tomato was the most productive for maximum invasion and the eventual number of *P. penetrans* spores produced per root system. Giannakou & Gowen (1996) compared different plant hosts to determine whether the plant host could affect the development of the parasite (*P. penetrans*) or whether the number of endospores produced within nematode (*M. javanica*) females differed between plant hosts. The development of the parasite as well as the number of endospores that developed in individual females was found to differ significantly between some of the hosts, with the highest number of endospores being produced in females reared in okra roots. Shahid (1999) reported that plant hosts exhibited differential host susceptibility to root-knot nematodes and okra was found to be the best plant host for nematode development.

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