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STUDIES ON THE INFECTION OF *MELOIDOGYNE* SPP WITH ISOLATES OF *PASTEURIA PENETRANS*

by
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Summary. Juveniles of *Meloidogyne incognita* and *M. graminicola* were encumbered with spores of three isolates of *Pasteuria penetrans* and the subsequent infection of these nematodes at 20, 25, 30 and 35°C was recorded on tomato and *Echinochloa colonum*. An isolate from South Africa was the most pathogenic. Temperature affected pathogenicity and influenced the parasitic life cycle which is shorter at 30 and 35°C than at 20 and 25°C. Spore burdens of »11 per juvenile decreased the invasion potential of *M. incognita*, *M. javanica* and *M. graminicola*. When tomato plants were inoculated with *M. incognita* in soil containing different concentration of *P. penetrans* spores the numbers of female that produced egg masses were decreased. There were 66% fewer egg masses in pots treated with 9000 spores per g soil than in the controls.

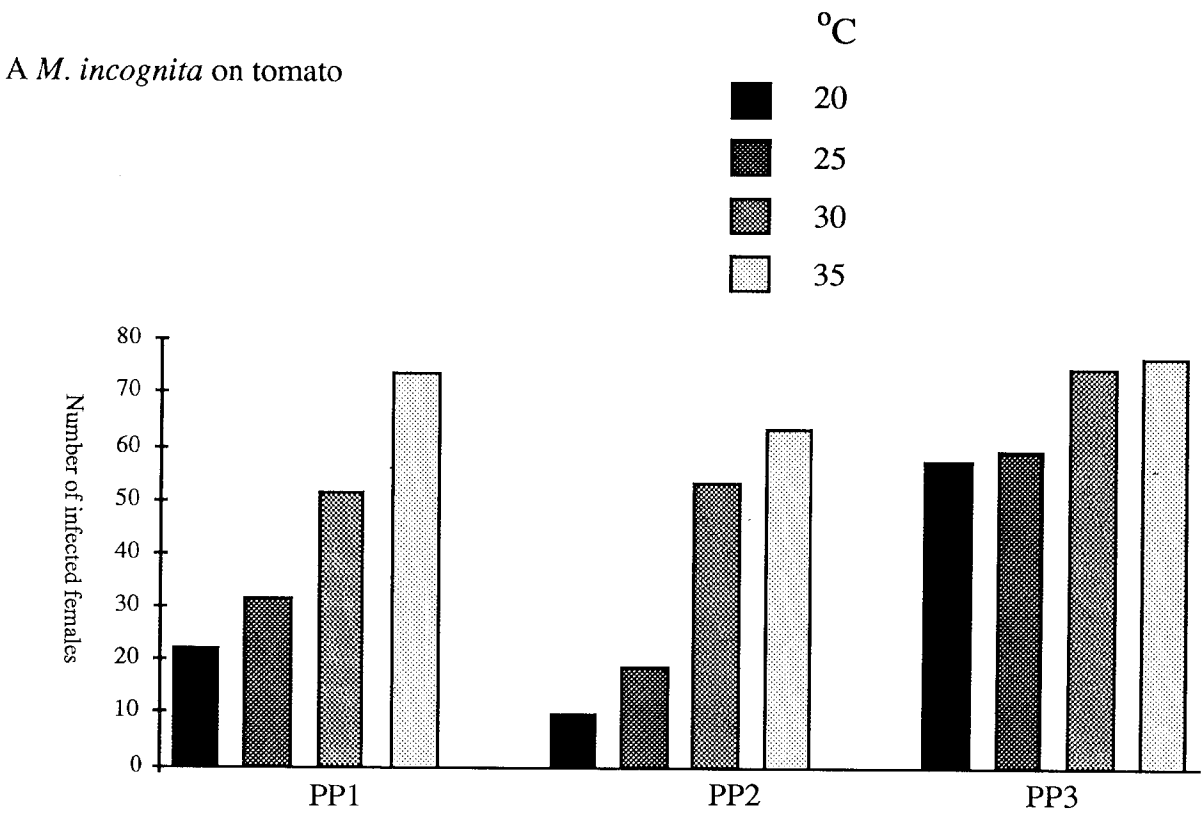
Pasteuria penetrans (Thorne) Sayre *et* Starr is an obligate spore-forming bacterial parasite which has been found infecting mature female root-knot nematodes in many tropical and subtropical countries (Mankau, 1980; Spaull, 1981; Channer and Gowen, 1988). *P. penetrans* is widely distributed but its presence may be overlooked because the infective and mycelial stage of the bacterium can only be seen under high power (x200) magnification. *In vivo* production systems (Stirling and Wachtel, 1980; Gowen and Channer, 1988) offer the prospect of using *P. penetrans* for control in container-grown crops and for small holders and market gardeners. However, isolates of *P. penetrans* maintained on root-knot nematodes in the glasshouse at Reading differ in their specificity for attachment to root- knot nematode species and to populations within species (Channer and Gowen, 1988). The present study is an assessment of the infectivity of three isolates on different nematodes and under different conditions.

Materials and methods

The infection of *Meloidogyne incognita* (Kofoid *et* White) Chitw. and *M. graminicola* Golden *et* Birchfield juveniles was tested with different isolates at 20, 25, 30 and 35°C and with different spore burdens. Spore suspensions of isolates of *P. penetrans* from Australia (PP1), USA (PP2) and South Africa (PP3) were prepared from infected tomato roots following the technique of Stirling and Wachtel (1980). Juveniles of *M. incognita* and *M. graminicola* originating from Bangladesh were collected from infested roots over a 24 hour incubation period using a modified Baermann-funnel technique (Hooper, 1986). The juveniles were added to spore suspensions (30,000 spores/ml) at room temperatures (18-23°C) for up to 48 hours to obtain two levels of spore attachment, 3-10 (Mean = 6) and »11 (Mean = 15) spores per J_2.

Tomato (*Lycopersicon esculentum* Mill.) cv. Moneymaker and barnyard grass (*Echinochloa colonum* L.) plants growing in 7.5 cm pots in a sterile loam soil were inoculated with 2,000 spore- encumbered juveniles of *M. incognita* and *M. graminicola* respectively. Four replicate pots were placed on heating boards which maintained soil temperatures at 20, 25, 30 and 35°C. Small portions of galled roots were taken at 3-5 days intervals to check for the development stages of *P. penetrans* in female nematodes. When the mature endospore stage of *P. penetrans* was known to have developed, the roots were washed and 10 mature females were selected randomly from each root system and the presence of *P. penetrans* infection determined by squashing the females and observing the body contents under x200 magnification.

To study the invasion of host roots by *Meloidogyne* spp. encumbered with spores of *P. penetrans* juveniles of *M. incognita*, *M. javanica* (Treub) Chitw. and *M. graminicola* were placed in spore suspension of an unknown isolate for periods up to 48 hours to obtain groups with 5-10 and »11 spores attached per nematode. Host plant seedlings of tomato, barnyard grass, egg plant (*Solanum melongena* L.) and sorghum (*Sorghum bicolor* L.) growing in 5 cm pots were inoculated with 1,000 J_2 with and without spores attached. The batches of spore-encumbered juveniles with different burdens were not available at the same time so



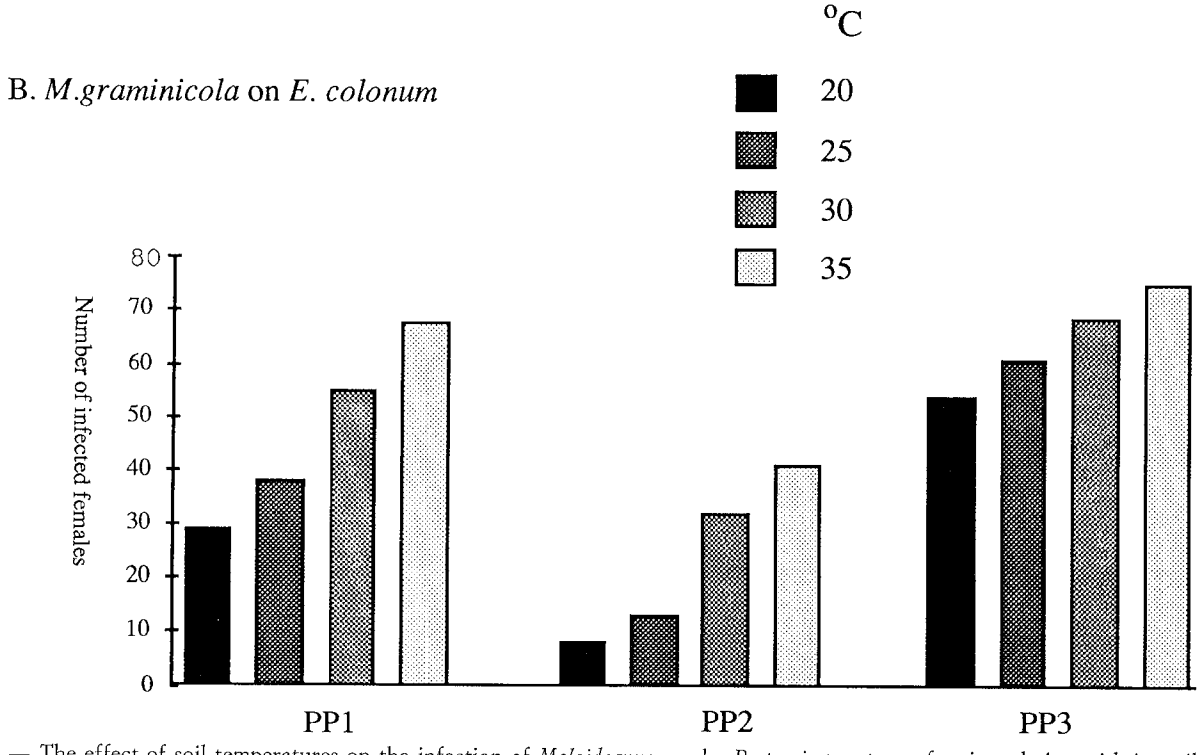


Fig. 1 — The effect of soil temperatures on the infection of *Meloidogyne* spp by *Pasteuria penetrans* after inoculation with juveniles encumbered with spores of different isolates: PP1 Australia, PP2 USA, PP3 South Africa. (Totals of 80 females sampled randomly after determining that the life cycle of *P. penetrans* was completed). Main effects of isolate and temperature significant for P = «0.001. Interactions not significant (from analysis of arcsin data).

each batch was applied with its unencumbered controls and analysed separately. Four replicate plants of each treatment were grown in a growth cabinet on a 16 hr 30°C day 8 hr 25°C night regime. After 7 days the roots were washed, stained in lactoglycerol with acid fuschin (Hooper, 1986), homogenised and quantitative estimates of J_2 in the roots were made.

To determine the invasion and development of *M. incognita* in soil treated with different spore concentrations of isolate PP3 from South Africa in powdered tomato roots were mixed in a sandy loam soil at dosage af 75, 150 and 300 mg/kg to give concentration of 2,250, 4,500 and 9,000 spores per gram. Two week old tomato plants (cv. Ailsa Craig) were planted in pots of 1 litre capacity containing 1 kg of the treated soil (when dry) and were inoculated with 3,000 J_2 of *M. incognita* 4 days later. Four replicate treatments were grown for 6 weeks in a glasshouse at 25-30°C. They were then harvested, weighed and the numbers of females with egg masses and eggs per egg mass recorded.

Results

All isolates of *P. penetrans* infected *M. incognita* and *M. graminicola*. With *M. incognita*, endospores were first found in adult females 20, 25, 35 and 80 days after inoculation of tomatoes when grown at 35, 30, 25 and 20°C respectively. At the same temperatures, *M. graminicola* females in roots of *E. colonum* contained endospores 18, 24, 40 and 80 days after inoculation. Overall, *P. penetrans* from South Africa infected a greater number of nematodes then those from Australia and USA (Figures 1A and B). Infection was influenced by soil temperature and by the time endospores had formed, significantly fewer females of both species were found to be infected at 20°C than at 35 and 30°C. *P. penetrans* from South Africa appeared to be more infective than the other isolates at the lower temperature. There was no significant interaction between temperature and spore burden (P = «0.05) and these data were bulked for presentation (Figs 1A and 1B).

The infection of both species was greater if the juveniles were encumbered with »11 spores than with 10 spores or less (Figure 2). The invasion potential decreased when juveniles were encumbered with spores. Fewer of the encumbered nematodes invaded roots than unencumbered controls but with burdens of 3-10 spores this difference was not significant. However, spore burdens of > 11 per J_2 significantly decreased invasion of *M. incognita*, *M. graminicola* and *M. javanica* on tomato, *Echinochloa* and egg plant respectively but not *M. javanica* on tomato (Table

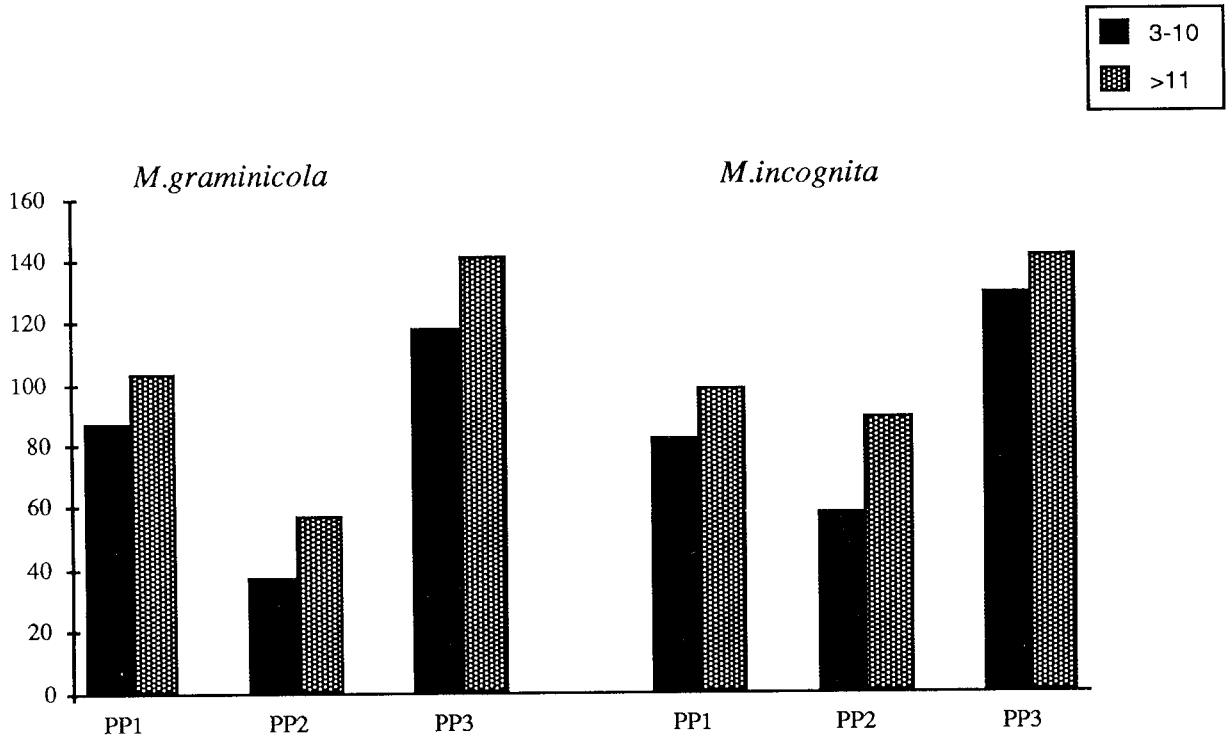


Fig. 2 — Numbers of infected females of *Meloidogyne graminicola* and *M. incognita* in host roots after inoculation with juveniles encumbered with 3-10 or »11 spores of *Pasteuria penetrans* isolates from Australia (PP1), USA (PP2) and South Africa (PP3). Totals of 160 females randomly sampled within each treatment combination. Differences between spore attachment groups only significant (P = «0.05) for PP2 (USA). Overall main effects of isolates and spore attachment levels highly significant (P = «0.001) (from analysis of arcsin transformation).

TABLE I - *The invasion of different host plants by root-knot nematodes after inoculation with 1,000 juveniles encumbered with different numbers of* Pasteuria penetrans *spores.*

Spores attachbed per juvenile	Number of nematodes per root system								
	Tomato				*Echinochloa colonum*		Sorghum	Egg plant	
	M. incognita		*M. javanica*		*M. graminicola*		*M. graminicola*	*M. javanica*	
0	312	318	375	358	193	207	407	524	527
5 – 10	185	—	333	—	156	—	272	421	—
> 11	—	171	—	285	—	154	—	—	295
SED	62	32	53	56	16	21	94	52	64
	NS	**	NS	NS	NS	*	NS	NS	**

* ** Differences significant at P = < 0.05 and P = < 0.01 respectively (Means of 4 replicates).

I). Of the nematodes that invaded and developed to maturity significantly fewer produced egg masses in soil treated with different concentrations of *P. penetrans* spores (Table II). Females that were apparently uninfected by *P. penetrans* developed egg masses with fewer eggs than those in the control. The presence of nematodes significantly decreased the shoot weight of tomatoes in the treatments with lower concentrations of *P. penetrans* and the control without it.

TABLE II - *Fecundity of* Meloidogyne incognita *in tomato plants grown in soil treated with different concentrations of* P. penetrans.

Spores/g	Shoot weight (g)	Females with egg masses	Eggs par egg mass
0	3.25	83	433
2,250	4.39	49	294
4,500	4.97	38	311
9,000	5.84	28	243
0 (No nematodes)	6.72	—	—
2,250 (No nematodes)	6.21	—	—
SED	0.855	5.70	58

Means of 4 replicates.

Discussion

Under optimal conditions the parasitic growth of *P. penetrans* is in synchrony with the growth and development of the host nematode. Stirling (1981) showed that at 20°C the relative rate of development of *P. penetrans* in *M. javanica* was slower than at 25 and 30°C. Fully developed ovaries were observed in infected females at 20°C whereas at 25 and 30°C the proliferation was much faster and females with fully developed ovaries were uncommon. Although it takes substantially longer for the endospore stage to develop at 20°C the results presented suggest that the *P. penetrans* isolates are less pathogenic at the lower temperature. As the nematodes were encumbered with spores before inoculation this effect was on the infection that occurred after the nematodes invaded the host root.

Successful field deployment of *P penetrans* isolates may be dependent on their temperature optima. The indications are that *P. penetrans* from South Africa is more adaptable to ranges of temperature that might be experienced in many subtropical locations than are populations from Australia and USA. The former came from Natal where it had been recently isolated from *M. javanica*. The precise origins of the others are unknown but if as is likely these had been in glasshouse culture for many generations they might have adapted to a narrower temperature range. Future work should address the possibility of variation in temperature optima and of adaptation, particularly if these could influence the efficacy of isolates when *P. penetrans* is taken for field evaluation in other locations.

When juveniles are encumbered with spores they are less able to invade host roots and this effect increases with increasing spore burden (Stirling, 1984; Davies *et al.*, 1988). When juveniles are encumbered with less than 10 spores there is a greater chance of nematodes escaping infection. The effect of spore burden on invasion would appear to be influenced by the plant host, an observation that requires further investigation. It seems that only 20-30% of attached spores germinate (Sayre and Wergin, 1977; Stirling, 1984) which is confirmed by De Silva and Gowen (1991) who found that only 27% of single spore attachments resulted in infection. Spores may become detached during invasion (Ratnasoma *et al.*, 1991) and infection will depend on the number of spores germinating and penetrating the body of the nematode.

Under certain conditions nematodes may withstand infection (De Silva and Gowen, 1991) or infection may be at levels which are difficult to detect. Low levels of infection may explain the decreased fecundity of females in roots of plants grown in *P. penetrans* infested soil.

P. penetrans spores are non-motile and their attachment to root-knot nematode juveniles depends on chance contact in the soil. The numbers of spores that come into contact with the nematode will be related to the duration of the pre-infective migratory phase. Freshly hatched nematodes that are attracted to nearby roots may not come in contact with spores if the migratory distance and duration are short.

P. penetrans is likely to be more effective when large numbers of spores are mixed thoroughly in the soil. Although the application of 9,000 spores per g of soil was beneficial, a considerable proportion of nematodes failed to become infected and the progeny of these would have left a large infestation in the soil at the completion of the crop cycle. It would seem that higher concentrations of spores will need to be applied to soil to obtain a better management of the nematode population.

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