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The effect of different initial densities of nematode (*Meloidogyne javanica*) on the build-up of *Pasteuria penetrans* population

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Abstract: *Pasteuria penetrans* will build-up faster where there is a high initial nematode density and can suppress root-knot nematode populations in the roots of tomato plants. The effect of different initial densities of nematode (*Meloidogyne javanica*) (150, 750, 1500, 3000) and *P. penetrans* infected females (F1, F3) densities (F0=control and AC=absolute control without nematode or *P. penetrans* inoculum) on the build-up of *Pasteuria* population was investigated over four crop cycles. Two major points of interest were highlighted. First, that within a confined soil volume, densities of *P. penetrans* can increase >100 times within 2 or 3 crop cycles. Second, from a relatively small amount of spore inoculum, infection of the host is very high. There were more infected females in the higher *P. penetrans* doses. The root growth data confirms the greater number of females in the controls particularly at the higher inoculum densities in the third and fourth crops. *P. penetrans* generally caused the fresh root weights to be higher than those in the control. *P. penetrans* has shown greater reduction of egg masses per plant at most densities. The effects of different initial densities of *M. javanica* and *P. penetrans* on the development of the pest and parasite populations were monitored. And no attempt was made to return the *P. penetrans* spores to the pots after each crop so the build-up in actual numbers of infected females and spores under natural conditions may be underestimated.

Key words: *Pasteuria penetrans*, *Meloidogyne javanica*, Densities, Population build-up
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INTRODUCTION

Pasteuria penetrans is an obligate hyper parasite of root-knot nematodes. *Pasteuria* species are gram positive, dichotomously branched endospore-forming bacteria (Mankau and Imbriani, 1975). The life cycle of *P. penetrans* starts when the endospores attach to the cuticle of free living second stage juveniles of *Meloidogyne* spp. (Stirling, 1981). The endospores that attach to the nematode cuticle germinate within 4 to 10 d after the endospore-encumbered juveniles enter a plant root and begin to feed (Sayre and Wergin, 1977; Serracin *et al*., 1997). *Meloidogyne* is the most important nematode genus causing heavy losses to growers and is found in all tropical and subtropical areas as well as in green houses all over the world. More than 2000 different plant species comprising monocotyledons and dicotyledons, herbaceous and woody plants, shrubs and trees can all be parasitized by species of *Meloidogyne*, particularly damaging to all vegetable crops grown in warmer areas and in infested areas (Hussey, 1985). Root-knot nematodes increase rapidly on highly susceptible host plants over a growing season. Reliable control could be improved by a better understanding of host-parasite population dynamics (Pinnock and Brand, 1981). An increase in numbers of nematodes may result in increasing numbers of spores that in turn will increase the probability that nematodes will contact a spore. When the parasites are plentiful, hosts are suppressed and parasites then decline. The spores of *P. penetrans* adhere to passing second stage juveniles (Davies *et al*., 1991; Stirling, 1991). Contact is by chance and therefore effective control does depend upon the density and distribution of the spores in the soil. The spores of *P. penetrans* persist and remain viable in

soil. However, information is lacking on the fate of spores: if they are rapidly carried to lower soil horizons and whether spores are ingested by soil fauna. To decline the nematode population from infested soil the parasitism and spore density must remain high.

Davies *et al.*(1988) reported that root-space and food competition between nematodes may enhance mortality induced by *P. penetrans*. Also, the invasion of spore-encumbered juveniles on roots is negatively correlated with the density of nematodes. The nematodes invading roots from an initially applied inoculum or from residual populations in soil will come in contact with spores more frequently than juveniles hatching from an egg mass on a root-system and perhaps having little distance to migrate to locate a root (Stirling, 1984).

The following experiment was carried out to monitor the effects of different initial densities of *M. javanica* and *P. penetrans* on the development of the pest and parasite populations (and plant growth) over four tomato crop cycles.

MATERIALS AND METHODS

Estimation of the spore concentration per female

One hundred endospore-filled females of *M. javanica* were picked by hand with fine forecep from tomato roots. Then ten females were selected at random and squashed separately in 2.5 cm diameter Petri dishes in 0.5 ml water. Suspensions made by adding 9.5 ml water were poured separately into glass universal bottles. The suspensions were homogenised for 30 s with an electric homogeniser. The number of spores/female was estimated by counting 3 sub samples from each sample. There were an average of 1679000 (±4702) spores/female.

Experimental procedure

Pots (15 cm diameter) were filled with 1.5 L of John Innes No. 2 compost. One or three (F1, F3) *P. penetrans* infected *M. javanica* females were placed in the middle of each pot at 8 cm depth and pots were then left in a growth room at 25~28 °C (without plants), watering as necessary to keep soil moist. After 4 weeks, water suspensions of freshly prepared eggs of *M. javanica* were applied by pipette on the soil surface at densities of 150, 750, 1500 and 3000 eggs per pot. The same numbers of eggs were also added to control treatments (F0) (without *P. penetrans*-infected females). After 4 d, six-week old tomato plants (*Lycopersicon esculentum* Mill, cv Tiny Tim, a dwarf determinate variety) grown in multi-cell plant trays were transplanted in the pots. As an absolute control (AC) (without *P. penetrans*-infected females and Nematodes), one set of plants was grown in the same size of pots without eggs or *P. penetrans*-infected females. All pots were placed individually on saucers to avoid cross contamination and watered by hand very carefully. The experiment was arranged in a completely randomised block design with four replicates. The roots of first crop plants were harvested after eight weeks and washed gently. The numbers of egg masses in infected roots were counted by observation with a magnifying lens. The roots had been previously stained for 15–20 min in an aqueous solution of phloxin B (15 mg/L) (Southey, 1986). The total numbers of females per root system were counted. *P. penetrans* infection was estimated by assessing 20 individual females selected randomly. The second crop was harvested after 12 weeks; the subsequent third and fourth crops were harvested after 15 weeks. The same procedures were followed and similar parameters were measured after each crop cycle. The data were analysed by using the analysis of variance statistical technique of GENSTAT-6 (Laws Agricultural Trust, Rothamsted Experimental Station, UK).

RESULTS

The increase in the numbers of *P. penetrans*-infected females in relation to initial *P. penetrans* and nematode density and crop cycles is demonstrated in Fig.1. In general, there were more infected females in the higher *P. penetrans* doses. The number of females/plant was lower in the 150 nematodes treatment as compared to the other densities so that the actual number of infected females was low. Often, but not always there were significantly fewer females per root system in the *P. penetrans* treatments particularly at the higher nematode inoculum densities (Fig.2). To take into consideration the root growth, data are also presented (Fig.4) which

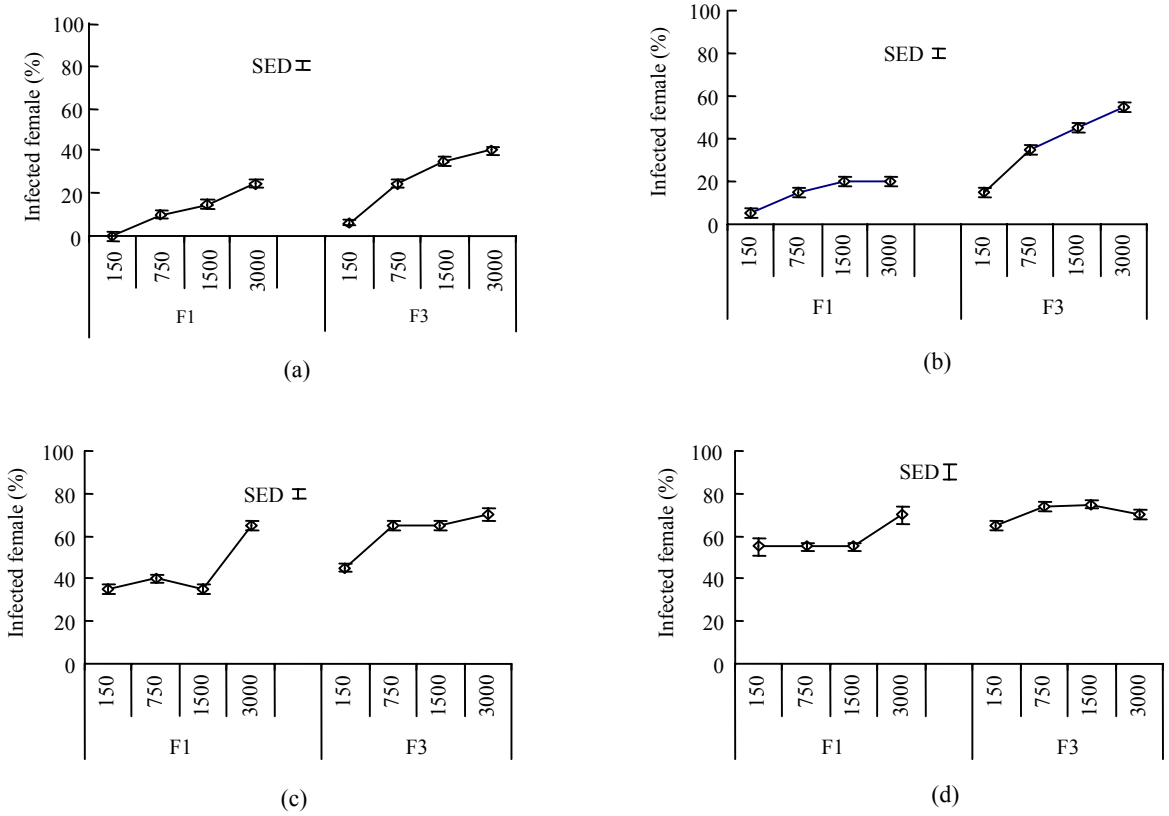

Fig.1 Effect of initial *P. penetrans* density (F1 and F3=*P. penetrans* infected females) and initial nematode density (150, 750, 1500 and 3000) on percentage of infected females produced on tomato plants over 4 successive crops. Vertical bars (where larger than the points on lines) represent the standard error (s.e.) of variability, whereas the separate ones represent the standard error of differences (SED) within means. (a) First crop; (b) 2nd crop; (c) 3rd crop; (d) 4th crop

confirms the significantly greater number of females in the controls particularly at the higher inoculum densities in the third and fourth crops. The effects of *P. penetrans* on the reduction of egg masses per plant were highly significant (P<0.001) at most densities (Fig.3). After the completion of the 3rd and 4th crops the greatest reduction in the number of egg masses was generally found in the higher initial inoculation densities. *P. penetrans* treatments generally caused the fresh root weights to be higher than those in the control treatment (Fig.4). However, in the first crop, root weights were significantly (P<0.001) higher than those in the absolute control in the nematode treated plants at the two lower inoculum densities but significantly lower at the highest density. In the second crop all treatments except the lowest (150) showed heavier roots than in the absolute control. And in the final two crops the nematode control (without *Pasteuria*) had the lowest root mass (Fig.4).

DISCUSSION

This experiment highlighted two major points of interest. First, that within a confined soil volume densities of *P. penetrans* can increase >100 times within 2 or 3 crop cycles. Second, from a relatively small amount of spore inoculum, infection of the host is very high. Because of the reproductive potential of *Meloidogyne* species, only a few individuals escaping infection are sufficient to produce eggs that could result in crop damage. A major "failing" of biological control is that absolute control is unlikely to be achieved and certainly not at the required level of >99% as suggested by Whitehead (1998). At the completion of the 4th crop, up to 70% of females in the root systems were infected with *P. penetrans* (Fig.1). There was a dose effect of *P. penetrans* in that the numbers of infected females were greater in the higher does *Pasteuria* treatment.

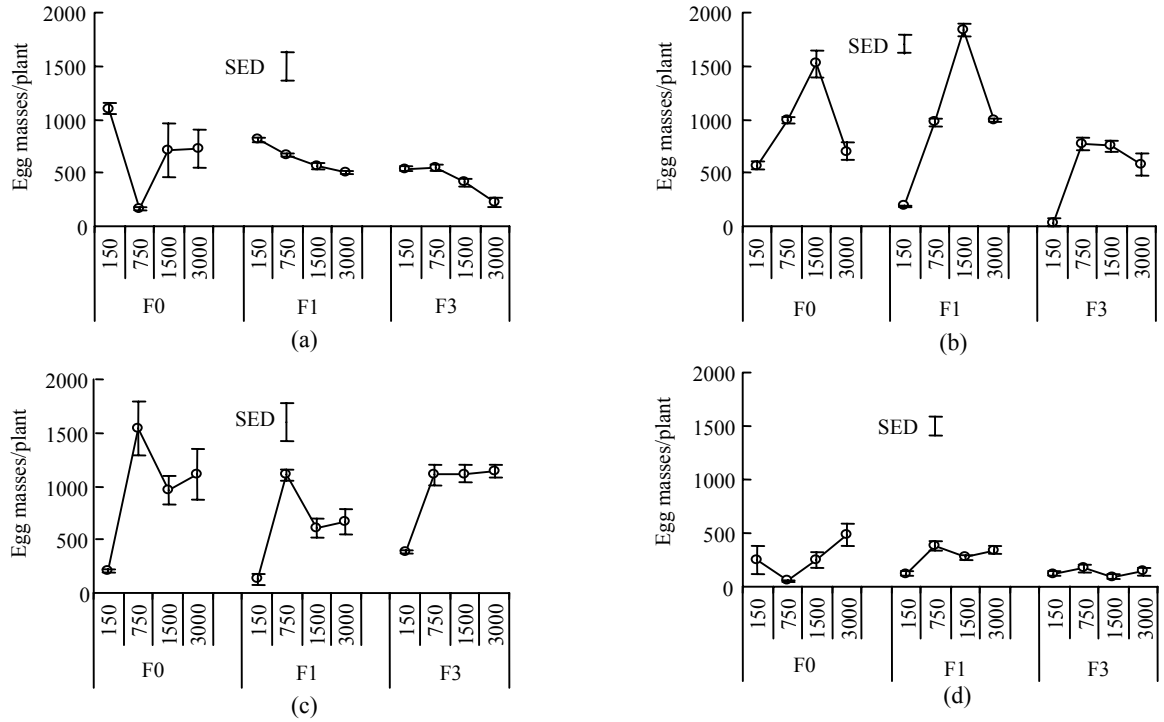

Fig.2 Effect of initial *P. penetrans* density (F0=Control, F1 and F3=*P. penetrans* infected females) and initial nematode density (150, 750, 1500 and 3000) on number of egg masses produced on tomato plants over 4 successive crops. Vertical bars (where larger than the points on lines) represent the standard error (s.e.) of variability, whereas the separate ones represent the standard error of differences (SED) within means. (a) First crop; (b) 2nd crop; (c) 3rd crop; (d) 4th crop

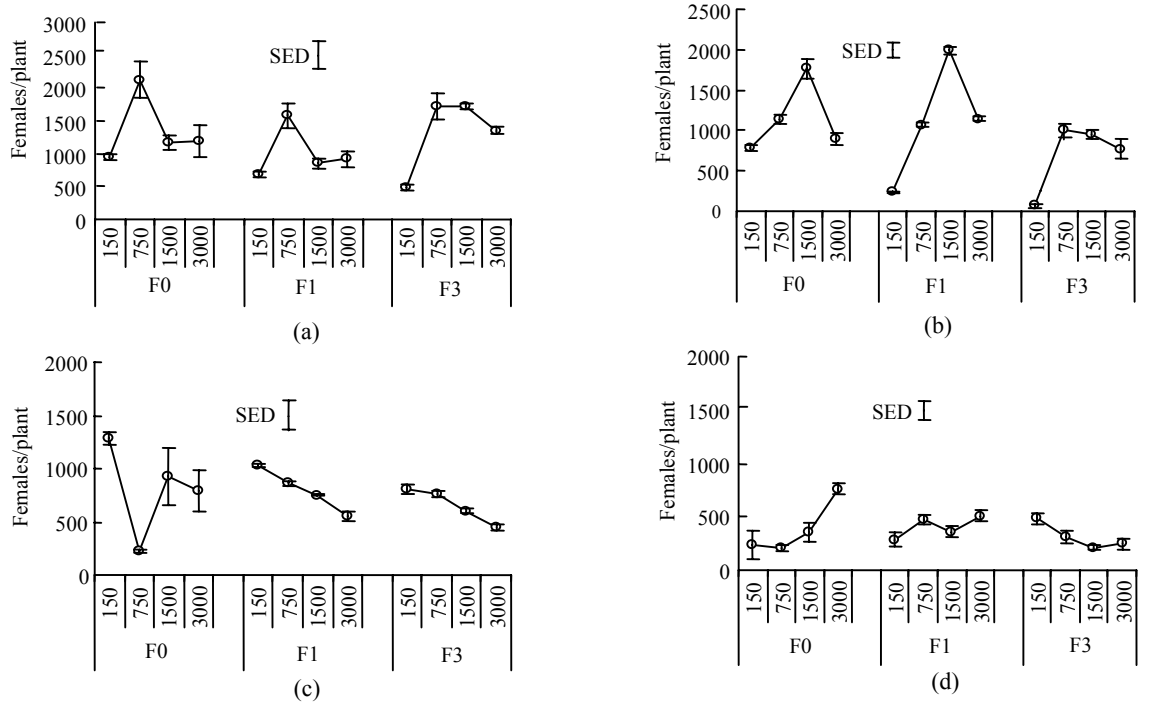

Fig.3 Effect of initial *P. penetrans* density (F0=Control, F1 and F3=*P. penetrans* infected females) and initial nematode density (150, 750, 1500 and 3000) on number of females produced on tomato plants over 4 successive crops. Vertical bars (where larger than the points on lines) represent the standard error (s.e.) of variability, whereas the separate ones represent the standard error of differences (SED) within means. (a) First crop; (b) 2nd crop; (c) 3rd crop; (d) 4th crop

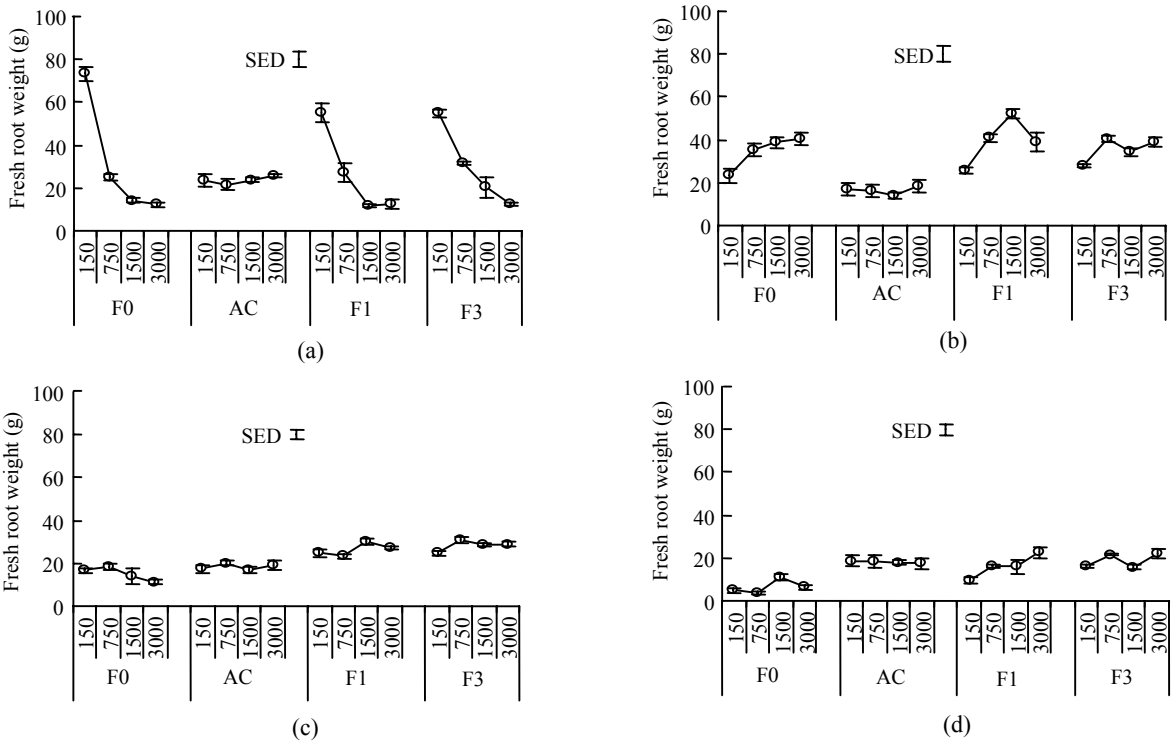

Fig.4 Effect of initial *P. penetrans* density (F0=Control, AC=absolute control, F1 and F3=*P. penetrans* infected females) and initial nematode density (150, 750, 1500 and 3000) on fresh root weight of tomato plants over 4 successive crops. Vertical bars (where larger than the points on lines) represent the standard error (s.e.) of variability, whereas the separate ones represent the standard error of differences (SED) within means. (a) First crop; (b) 2nd crop; (c) 3rd crop; (d) 4th crop

In this experiment no attempt was made to return the *P. penetrans* spores to the pots after each crop so the build-up in actual numbers of infected females and spores under natural conditions may be underestimated. Also the numbers of uninfected nematodes would have been higher if the root systems with egg masses had been returned to the pots. But, if they had, there would have been severe effects on the host plant growth.

Both higher initial densities of *P. penetrans* showed a suppressive effect on root galling and egg mass production over the successive crop cycles. However, with the lower initial nematode density (150) both of the *Pasteuria* treatments suppressed the nematode populations less, presumably because there were fewer nematodes in the system to become infected and increase the density of the parasite.

Generally the number of egg masses was greater in the one-female treatments of all densities as compared to the three-female treatments. The findings of Tzortzakakis *et al*.(1997) support these results that the number of egg masses were higher where there had been a lower initial nematode density. In the first crop untreated control plants of the higher nematode inoculum densities senesced prematurely due to the debilitating effects of the heavy nematode burden and were harvested after 8 weeks. However, the duration of the 3rd and 4th crop cycles was prolonged until 15 weeks instead of 12 weeks (in the second crop) which favoured the build-up of *P. penetrans*. There was no sign of senescence on the *Pasteuria* treated plants, however, control plants were dying, which helps to explain the lower fresh root weights in the controls. (Fig.4). This is an indication of the benefit of *Pasteuria* by prolonging the duration of the crop. The duration of 15 weeks supported the build-up of *P. penetrans* and on the other hand it also provided enough root space to support the low numbers of uninfected nematodes which were then able to produce egg masses with high numbers of eggs which was reflected in the production of egg masses in lower density (150) treatments at the 1st and the 4th crop harvests.

It can be concluded that *P. penetrans* success-

fully suppressed egg mass production, but could not achieve total control of nematodes. These results have shown that *P. penetrans* may effectively help to manage the nematode population but as Tzortzakakis and Gowen (1994) had also reported, *P. penetrans* will only have a useful role in nematode management if deployed with other control measures.

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