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# Influence of different initial spore concentrations of *Pasteuria penetrans* on the infection of root-knot nematodes over three host crop cycles

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## Abstract

The number of egg-masses, females per plant, over all the crop cycles were compared by accepting a parallel line, logarithmic model. The number of egg-masses per plant over all the crop cycles decreased significantly ($P < 0.05$) more in the ten females treatment as compared with control. A highly significant difference was found in total number of females per plant where ten *Pasteuria penetrans* infected females were originally added as compared to the control treatment. A separate line model was fitted to compare the percentage of infected females by observing twenty females per replicate, but no infected female was recorded in any of the *Pasteuria* treatments after the harvest of the first crop. There was a highly significant difference in endospore production in root systems of the one female and ten females treatments and increased exponentially with the number of females over all the crop cycles. Infected females (%) increased and found significantly higher in the ten females treatment. The soil bioassay after first harvest showed few juveniles encumbered with an average of 1-5 spores and the number of juveniles without spores very high.

**Keywords:** Root-knot nematodes, *Pasteuria penetrans*, hyperparasite, spore concentration.

**P**lant parasitic nematodes are major pests in many countries of the world especially in tropical and subtropical regions where they recognized as the cause of serious yield losses on a wide range of crops (Bridge & Page, 1980). Among the plant parasitic nematodes, root-knot nematodes (*Meloidogyne* spp.) are the most important, limiting world agricultural productivity (Sasser, 1979; Sasser & Carter, 1985). *Pasteuria penetrans* is an obligate, endospore-forming bacterial parasite of *Meloidogyne* spp., and showed potential as a biological control agent against root-knot nematodes (Chen *et al*., 1996; Dickson *et al*., 1994) in pot experiments (Channer & Gowen, 1988) and micro plot experiments (Daudi *et al*., 1990; Trivino & Gowen, 1996). *Pasteuria penetrans* is a highly selective by nature within its host, it does not disturb the nematode feeding ability, only affect the reproduction (Bird, 1986). Thus, the parasitized female nematodes virtually become a bag of spores and each spore is capable of infecting another nematode. Oostendorp *et al*., (1991) observed that *P. penetrans* showed short-term impact on nematode populations at low spore densities because only a few nematodes come in contact with spores. When a spore filled carcass of an infected female degrades and released infective spores into the soil they remain dormant until they attach to the food searching second stage juveniles. With infection of the next generation of host nematodes, the spore densities will increase over time, thereby reaching nematode

suppressive levels. This study was conducted to investigate the relationship of spore density of *P. penetrans* on the infection of nematodes over three host crop cycles.

## Materials and Methods

In this experiment 1.5 litre pots (15 cm diameter) were filled with (John Innes No. 2) a loam based proprietary compost. *Pasteuria penetrans*-infected *M. javanica* females were put in the middle of the pots at 8 cm depth. Crop was grown in three cycles with four replications and twelve treatments. The treatments were one, three and ten infected females per pot. Forty eight hours after adding the *P. penetrans* females, 1000 freshly hatched $J_2$ of *M. javanica* were added per pot with the help of pipette in 5.3 ml of water in four holes in the central area of the pots. The pots were watered sufficiently to keep soil moist. Four days later six-week old tomato plants cv. Tiny Tim was planted. The experiment was arranged in a randomized block design in the growth room. The first set of the four replicates per treatment for the first crop cycle harvested after 60 days and the shoots of the other sets were cut off, the pots were left one week before re-planting with six-week old tomato plants without disturbing the soil. A few days after re-planting due to high residual population plants in the control pots died prematurely so the other plants were cut off and the pots were left without plants to allow the population level decline. Before the next re-planting 50 ml soil samples were taken per pot, prepared for nematode extraction and after 24 h total number of nematodes and attachment of spores/$J_2$ was recorded. After 60 days the pots were planted again with six-week old tomato plants. After the completion of first nematode generation the plants were harvested (24 days). The last four replicate sets of each treatment were left with plants for third crop cycle and harvested after 60 days. The roots of harvested plants were washed gently. The numbers of egg-masses in infected roots were counted by observation with a magnifying lens. The total numbers of females per replicate were counted. The spore density in the tomato roots was determined by grinding 100 mg of the dried root material with a mortar and pestle and the powder suspended in 100 ml of water before sieving through 38 $\mu$m aperture sieve to remove residual root debris and stored in bottles in refrigerator at 4°C. The spore concentration was estimated from counts made by using a haemocytometer at x 400. Some plant roots of the one female, three females and control treatment of the first crop cycle were so severely galled, that egg-masses could not be found on the rotted roots. The number of inoculated females was transformed to ($\log_{10}$+1).

## Results

The number of egg-masses, females per plant, over all the crop cycles were compared by accepting a parallel line, logarithmic model ($P < 0.05$, $F = 11.82$). The number of egg-masses per plant over all the crop cycles decreased significantly ($P < 0.05$) however, more in the ten females treatment as compared with control (Fig. 1 and Table1). A highly significant ($P < 0.05$) difference was found in total number of females per plant where ten *P. penetrans* infected females were originally added as compared with control (Fig. 2 and Table 2). A separate line model was fitted to compare infected females (%) by observing twenty females per replicate, but no infected female recorded in any of the *Pasteuria* treatments after the harvest of the first crop (Fig. 3 and Table 3). There was a highly significant difference ($P < 0.05$) in endospore production in root systems of the one female and ten females treatments and increased exponentially with the number of females over all the crop cycles. The number of spores increased in all *Pasteuria* treatments and there was a significantly ($P<0.05$) greater number of endospores in the ten female treatment than in the one female and three females treatments (Fig. 4 and Table 4). The effect of the parasite was further increased in the third crop cycle. Infected females (%) were two-fold greater after the harvest of the third crop than after the second crop cycle (Fig. 3).

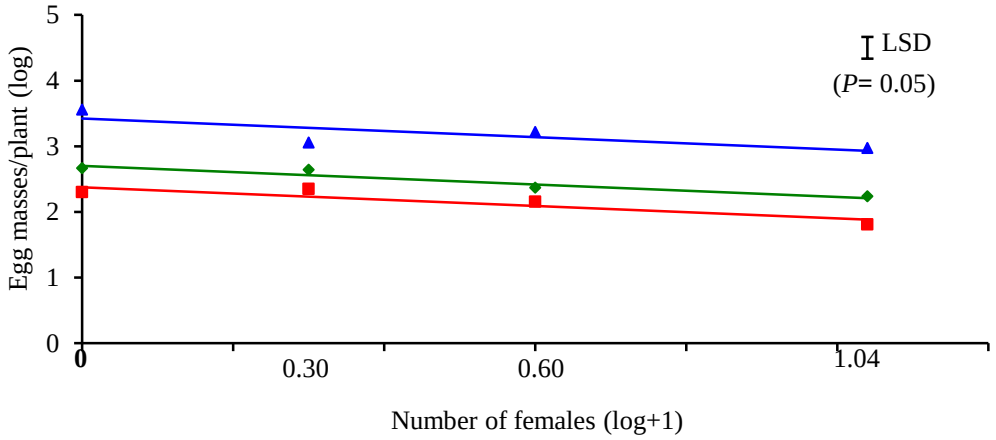


**Fig. 1.** Effect of inoculating the soil with different numbers of *P. penetrans* infected females on the number of egg-masses/plant over three crop cycles, linear regressions were fitted separately for 1st (♦) 2nd (■) and 3rd (▲) crops.

**Table 1. Estimated regression coefficients for the log-log relationship of number of egg-masses per plant over three crop cycles, as a function of the number (+1) of the initial number of infected females.**

| Crops | Intercept estimates | S.E | Slope estimate | S.E |
|---|---|---|---|---|
| First crop | 2.70 | 0.070 | | |
| Second crop | 2.372 | 0.080 | -0.472 | 0.087 |
| Third crop | 3.424 | 0.84 | | |

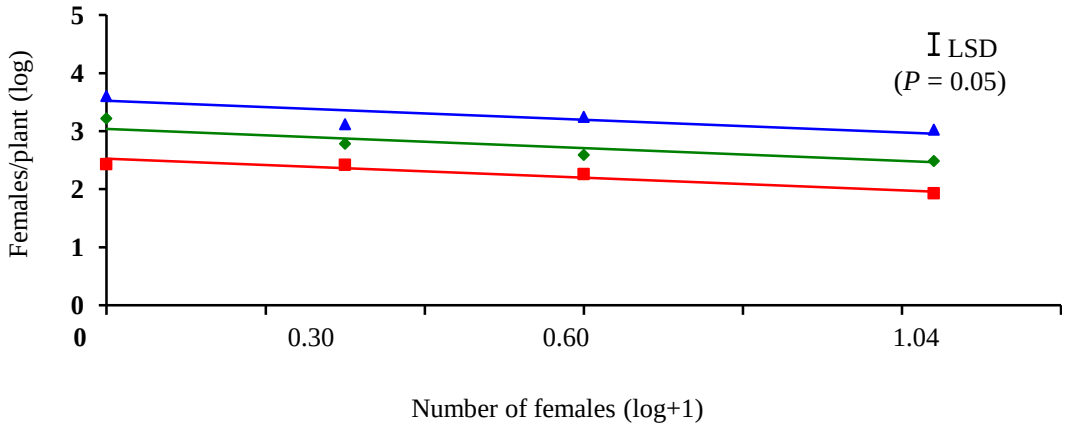


**Fig. 2.** Effect of inoculating the soil with different numbers of *P. penetrans* infected females on the number of females/plant over three crop cycles, linear regressions were fitted separately for 1st (♦) 2nd (■) and 3rd (▲) crops.

**Table 2. Estimated regression coefficients for the log-log relationship of number of females per plant over three crop cycles, as a function of the number (+1) of the initial number of infected females.**

| Crops | Intercept estimates | S.E | Slope estimate | S.E |
|---|---|---|---|---|
| First crop | 3.036 | 0.078 | | |
| Second crop | 2.526 | 0.088 | -0.547 | 0.096 |
| Third crop | 3.013 | 0.093 | | |

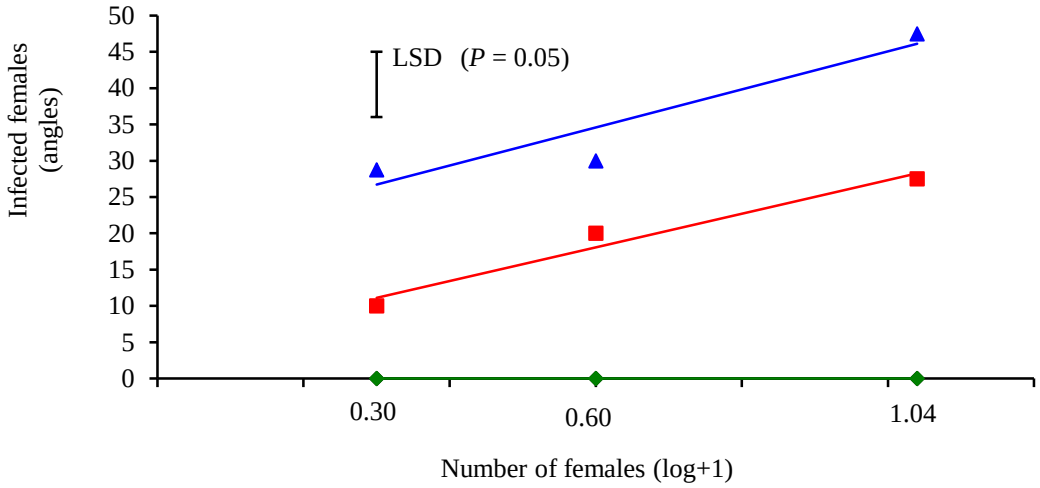


**Fig. 3.** Effect of inoculating the soil with different numbers of *P. penetrans* infected females on the percentage (shown in angles) of infected females out of 20 females/replication over three crop cycles, linear regressions were fitted separately for 1st (♦) 2nd (■) and 3rd (▲) crops.

**Table 3. Estimated regression coefficients for the angular transformation of the percentage of infected females out of 20 females/replicate over three crop cycles, as a function of the logarithm (number + 1) of the initial number of infected females.**

| Crops | Intercept estimates | S.E | Slope estimate | S.E |
|---|---|---|---|---|
| First crop | 0.0 | 3.03 | 0.0 | 4.24 |
| Second crop | 4.19 | 4.29 | 23.16 | 6.00 |
| Third crop | 18.85 | 4.32 | 26.21 | 6.00 |

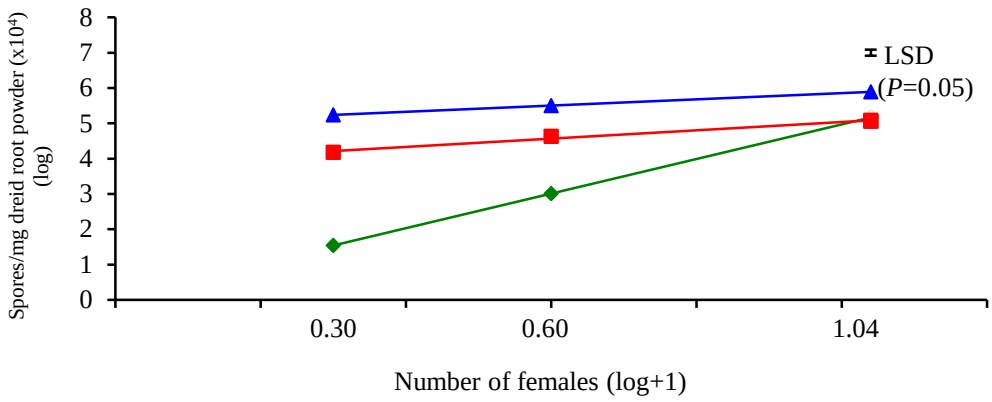


**Fig. 4.** Effect of inoculating the soil with different numbers of *P. penetrans* infected females on the number of spores produced/mg dried root powder (x10$^4$) over three crop cycles, linear regressions were fitted separately for 1$^{st}$ (♦) 2$^{nd}$ (■) and 3$^{rd}$ (▲) crops.

**Table 4. Estimated regression coefficients for the log-log relationship of number of spores produced/mg dried root powder (x10$^4$) over three crop cycles, as a function of the number (+1) of the initial number of infected females.**

| Crops | Intercept estimates | S.E | Slope estimate | S.E |
|---|---|---|---|---|
| First crop | 0.069 | 0.599 | 4.901 | 0.777 |
| Second crop | 3.793 | 0.766 | 3.73 | 1.03 |
| Third crop | 4.902 | 0.805 | 4.02 | 1.06 |

## Discussion

Egg-masses were not significantly reduced and females in the one and three female treatments in the first crop, the number of egg-masses and females per plant significantly reduced with increase in the number of original *P. penetrans*-infected females over all the crop cycles. At the completion of the second crop the numbers of egg-masses and the female population per plant reduced but increase in infection. However, the rate of infection was slow in the first and the second crop than the third crop and could not protect plants from a heavy invasion of nematodes and plants in the one female, three females and control senesced prematurely due to the debilitating effects of the heavy nematode population. In the third crop cycle *P. penetrans* parasitized more nematodes and produced a stronger effect in suppressing the nematode population and there was no sign of plant senescence up to 60 days. Giannakou *et al*., (1999) found that a bigger root system provided better conditions for the development of female nematodes and that in turn provides a better medium for the development of the parasite. Studies by Hatz & Dickson (1992) also showed that there is coincident development by host (nematode) and parasite (*Pasteuria*). There were infected females in the roots in the first crop because spores in the dried root but none were detected in the fresh samples. It seemed that without allowing the 2-3 week time interval for degradation before adding nematodes, even the

relatively high "dose" of ten spore-filled female cadavers could not influence the nematode population. Densities of *P. penetrans* increased during one crop and thus not provided protection against multiple nematode generations in that crop. However, these results indicated that levels of *P. penetrans* on the nematode population can increase over time to an extent that plant growth is improved relative to plants without the biocontrol agent.

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