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Effect of time on the degradation of *Pasteuria penetrans*-infected females of *Meloidogyne javanica* and the proportion of parasitized nematodes in the roots of tomato planted subsequently

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Abstract

Different parameters were evaluated to confirm that how long *Pasteuria penetrans* infected *Meloidogyne javanica* females took to degrade and for spores to be released from the cadavers into the soil. Regression analysis was carried out to compare the degradation of *P. penetrans* infected females cadavers in different time intervals over two crop cycles. There were highly significant decreases in the total number of egg-masses per plant between the 3 weeks and other treatments. Infected females (%) over both crop cycles were compared by the estimated coefficients of the fitted lines and increased significantly with degradation period and significantly higher in the second crop. The 3 week duration apparently allowed more spores to disperse which was reflected in the observations of more infected female nematodes and reduced numbers of egg-masses and total females per plant compared with the first crop. The results of this experiment suggest that dispersal of spores from the degrading females occurs after 2 weeks and this is reflected in a significant reduction in egg-masses, galling and the female population between the 1 week and 3 weeks treatments. The greatest percentage of infected females and numbers of endospores were recorded in that treatment where *P. penetrans* infected females were left to degrade for 3 weeks.

Key words: Root-knot nematodes, *Pasteuria penetrans*, degradation, infected females, biological control.

Much more effort has been taken since a long to understand the biology of *P. penetrans* isolates which parasitize the economically important root-knot nematode species. *P. penetrans* is a potentially good bio-agent to control root-knot nematodes (Darban *et al*., 2005, Gowen & Ahmed, 1990). It has many attributes required by a successful biological control agent. The spores adhere to with the cuticle of second stage juveniles of *Meloidogyne* spp., (Stirling, 1984; Davies *et al*., 1988) in soil when they search for host roots. These juveniles when invade roots and begin to develop, the parasite is also developing. At maturity they destroy reproductive system of females and infected females were unable to produce eggs and so the build-up of nematode populations prevented (Mankau, 1980; Sayre, 1980). Invasion of roots may be reduced by the spore burden. The number of infected females increases with increasing spore attachment on the juveniles (Stirling, 1984; Davies *et al*., 1988). However, the germination of a single spore was enough to create infection in a *Meloidogyne* sp., female (Stirling, 1984). *Pasteuria penetrans* completed its life cycle inside the female and mature spores are released when the females degrade (Sayre, 1980).

Oostendorp *et al*., (1990) reported that spores of *P. penetrans* have survived for several weeks in dry, moist and wet soils and in soils with fluctuating moisture levels without loss of their ability to attach to their nematode hosts, which is a particularly useful attribute for a biological control agent (Stirling, 1991). In this experiment

an attempt was made to monitor the degradation of infected females to ascertain how soon spores released from female cadavers.

Materials and Methods

The isolate of *P. penetrans* designated (*P. p*3) was cultured on *M. javanica* on tomato plants using the method of Stirling & Wachtel (1980). The plants were harvested after 6 weeks when 750 degree-days had accumulated (base temperature 10 °C) (Stirling, 1981). The roots were washed and kept in a freezer for 5-7 days and then *P. penetrans* infected females were handpicked from thawed roots. Pots (10 cm diam.) were filled with 1 kg of John Innes no. 2 compost. A single *P. penetrans* infected female was put in the middle of each pot at 6 cm depth for degradation. The soil of each pot was kept moist by watering as necessary and then left in a growth room without plants for 1, 2 and 3 weeks. After each interval, 3 ml of water containing 2000 freshly hatched juveniles were added per replicate by pipette in four 2 cm deep holes in the central area of each pot. The pots for the control treatment (without a cadaver) were also inoculated with the same number of juveniles at the same time. After 2 days, four-week old tomato plants, which were grown in multi-cell plant trays transplanted in the pots. All pots were placed individually on saucers to avoid cross contamination and watered by hand very carefully. The experiment was arranged in a completely randomized block design with ten replicates. Five replicates of each treatment of the first crop were harvested after 6 weeks when 750 degree-days had accumulated. The other sets of five replicates for the second crop were harvested after 12 weeks with the completion of 1500 degree-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 roots had been previously stained for 15-20 minutes in an aqueous solution of phloxin B (15 mg/L) (Southey, 1986). 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 µm 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. The same method was followed for the second crop.

Results

Different parameters were evaluated to confirm how long *P. penetrans* infected females took to degrade and for spores to be released from the cadavers into the soil. Regression analysis was carried out to compare the degradation of *P. penetrans* infected females cadavers in different time intervals over two crop cycles. There was no evidence of significant interaction between both crops for egg-masses per plant. Hence a common line model was fitted between 1 week and 3 weeks treatments. There were highly significant ($P < 0.05$) decreased in the total number of egg-masses per plant between the 3 weeks and other treatments (Fig. 1; Table 1). A parallel line model fitted for number of females per plant and it showed that there were lower numbers of females per plant in the treatment where spore-filled females were left to degrade for 3 weeks as compared to the 1 week and control treatments over two crop cycles (Fig. 2; Table 2). The percentage of infected females over both crop cycles was compared by the estimated coefficients of the fitted lines and increased significantly with degradation period and was significantly ($P < 0.05$) higher in the second crop (Fig. 3; Table 3) resulting in significantly high numbers of endospores/mg dried root powder (Fig. 4; Table 4). The number of egg-masses and females did not differ significantly between the 1 week and control treatments. The 3 week duration apparently allowed spores to disperse which was reflected in observations of more infected female nematodes (Fig. 3) and reduced number of egg-masses (Fig. 1) and total females per plant compared with the first crop. The root-galling indices based on the scale of Bridge & Page (1980) provided lower levels of galling in the 3 week treatment (Fig. 5).

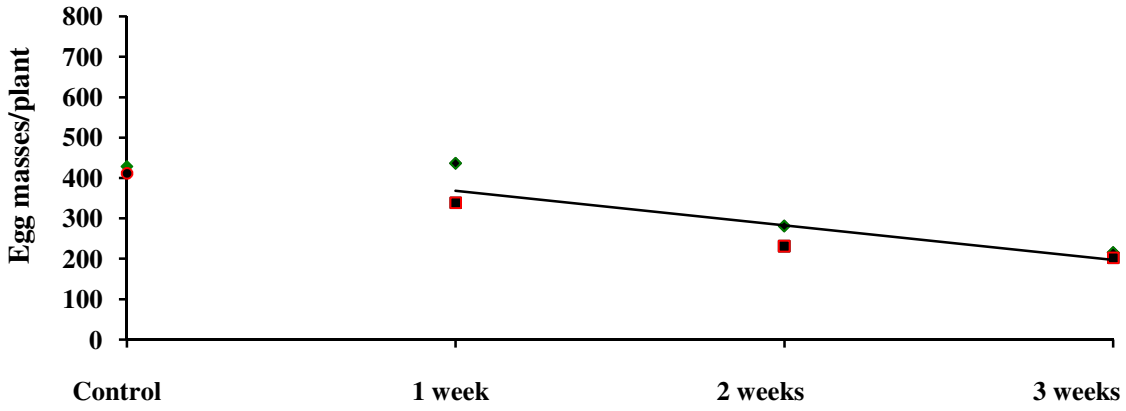

Fig. 1. Effect of degradation of *P. penetrans* infected females over different time intervals on the numbers of egg-masses over two crop cycles, linear regression fitted between 1st (♦) and 2nd (■) crops. Parameter estimates of fitted line is in table 1, LSD = 152.5, $P = 0.05$.

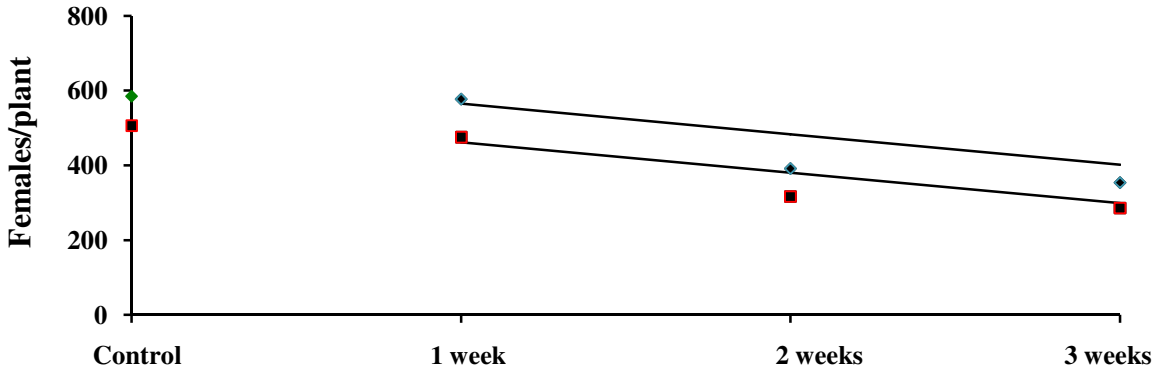

Fig. 2. Effect of degradation of *P. penetrans* infected females over different time intervals on the number of females/plant, linear regression fitted between 1st (♦) and 2nd (■) crops. Parameter estimates of fitted lines are in table 2, LSD = 127.5, $P = 0.05$.

Table 1. Estimated regression coefficients for number of egg-masses/plant over two crop cycles.

Crops	Intercept estimates	S.E.	Slope estimates	S.E.
First crop Second crop	453.3	32.9	-85.8	15

Table 2. Estimated regression coefficients for number of females per plant over two crop cycles.

Crops	Intercept estimates	S.E.	Slope estimates	S.E.
First crop	646.7	37.6	-81.6	26.6
Second crop	543.7	16.3		

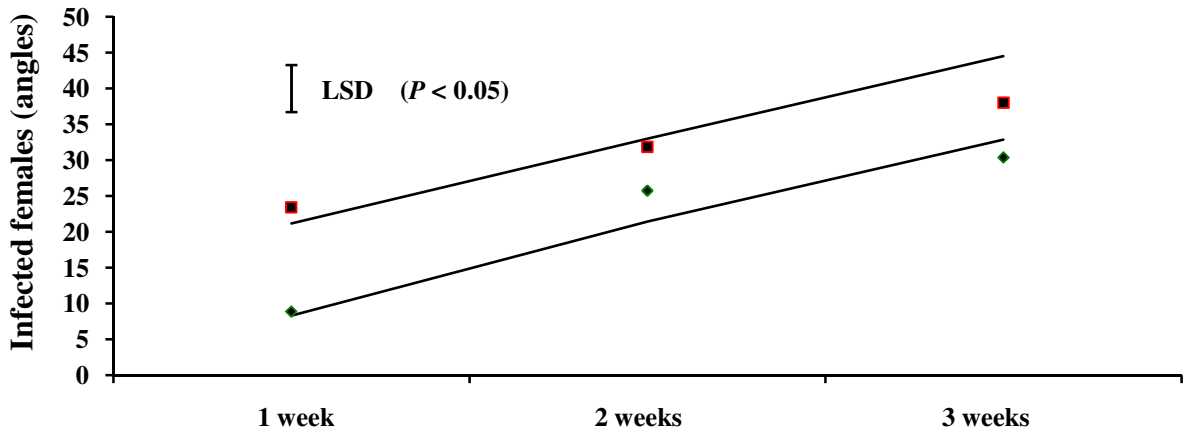

Fig. 3. Effect of degradation of *P. penetrans* infected females over different time intervals on the percentage (shown in angles) of infected females from 20 females/replication over two crop cycles, linear regression fitted to 1st (♦) and 2nd (■) crops.

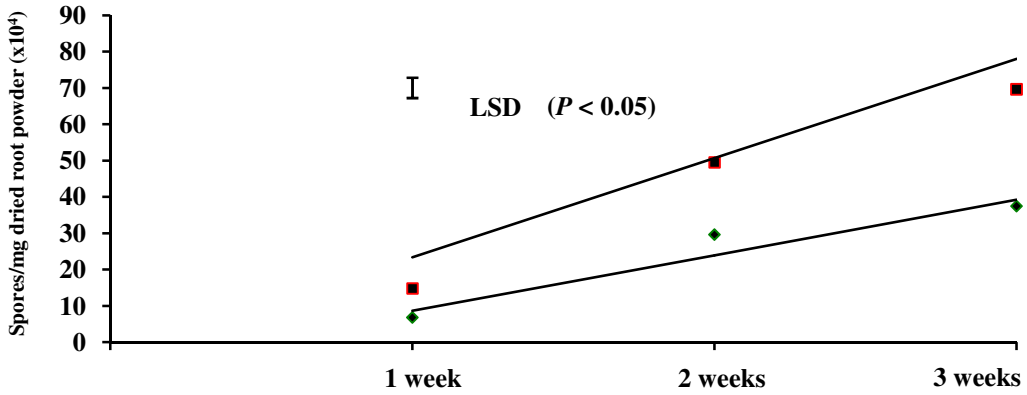

Fig. 4. Effect of degradation of *P. penetrans* infected females over different time intervals on the number of spores produced/mg dried root powder ($x10^4$) over two crop cycles, linear regression fitted between 1st (♦) and 2nd (■) crops.

Table 3. Estimated regression coefficients for percentage of infected females from 20 females/replication over two crop cycles.

Crops	Intercept estimates	S.E.	Slope estimates	S.E.
First crop	-5.67	2.89	11	1.25
Second crop	-15.33	2.04		

Table 4. Estimated regression coefficients for number of spores produced/mg dried root powder (x10^4) per plant over two crop cycles.

Crops	Intercept estimates	S.E.	Slope estimates	S.E.
First crop	-4	5.4	27.3	2.5
Second crop	-6.7	3.8	15.3	1.7

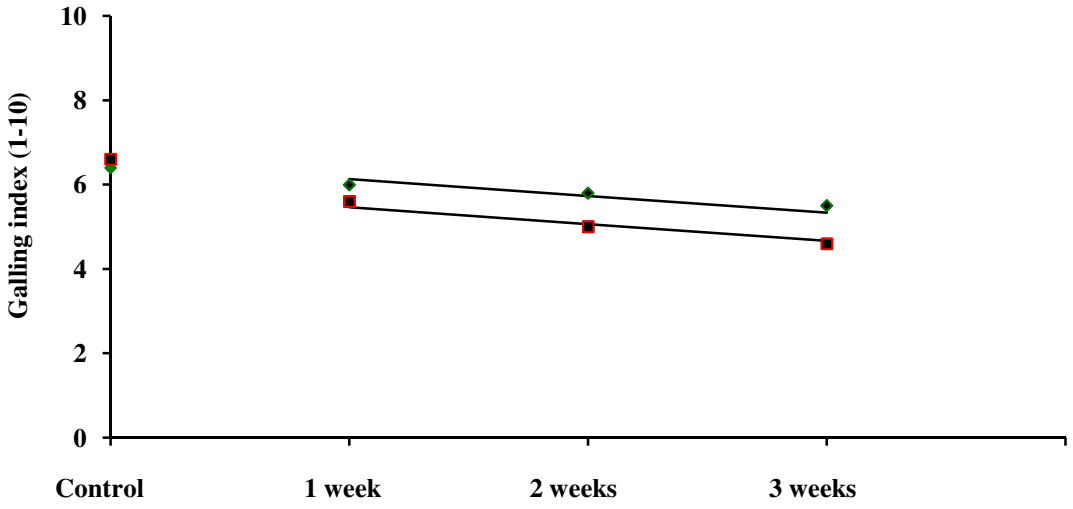

Fig. 5. Effect of degradation of *P. penetrans* infected females over different time intervals on the galling severity over two crop cycles, linear regression fitted between 1st (♦) and 2nd (■) crops. Parameter estimates of fitted lines are shown in table 5, LSD = 0.748, *P* = 0.05.

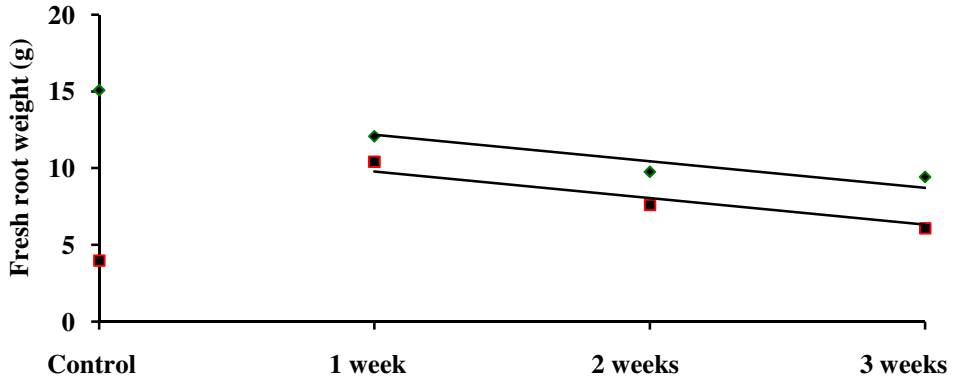

Fig. 6. Effect of degradation of *P. penetrans* infected females over different time intervals on the fresh root weight over two crop cycles, linear regression fitted between 1st (♦) and 2nd (■) crops. Parameter estimates of fitted lines are shown in table 6, LSD = 1.473, *P* = 0.05.

Table 5. Estimated regression coefficients for galling index over two crop cycles.

Crops	Intercept estimates	S.E.	Slope estimates	S.E.
First crop	6.53	0.29	-0.4	0.12
Second crop	5.86	0.20		

Table 6. Estimated regression coefficients for fresh root weight per plant over two crop cycles.

Crops	Intercept estimates	S.E.	Slope estimates	S.E.
First crop	13.91	1.19	-1.73	0.51
Second crop	11.52	0.84		

Discussion

The results of this experiment suggest that dispersal of spores from the degrading females occurs after 2 weeks and this is reflected in a significant reduction in egg-masses, galling and the female population between the 1 week and 3 weeks treatments. The high percentage of infected females and numbers of endospores were recorded in that treatment where *P. penetrans* infected females were left to degrade for 3 weeks (Figure 3 and 4). Presumably the

lower numbers of endospores and higher numbers of egg-masses and females in the 1 week treatment means that the body wall had remained at least partially intact and spores had not been released. After the second harvest there was a significant decrease in numbers of egg-masses and the final population decline in 3 weeks treatment that occurred was probably due to a higher density of *P. penetrans* endospores. The reduced numbers of egg-masses and females in the control treatment could be explained by the over-exploitation of its host and plants were senescing (Gowen *et al*., 1998), which suggested by the fresh root weights.

These results showed that the degradation of the body wall of *P. penetrans* infected females and release of endospores from their cadavers takes places over at least 2-3 weeks. There is published information on what causes the degradation of the females in soil. It can be speculated due to activity of certain soil bacteria and/or fungi, which will be pronounced in moist conditions. Spore-filled females remained intact if the roots in which they contained are allowed to dry (Pembroke, personal communication, 2014). Once the endospores were released from the cadavers their distribution probably assisted by irrigation water. From a practical point of view cultivation of soil after removal of a crop could improve the distribution of *P. penetrans* endospores by enhancing the degradation of the senescing roots.

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