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Soil bioassay with time interval and spore density factors affecting the infection of *Meloidogyne javanica* by *Pasteuria penetrans*

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Abstract

A bioassay study was conducted to confirm the effect of different time intervals on the degradation of *M. javanica* females cadavers filled with of *P. penetrans* endospores, release and dispersal in the soil. Three time intervals, of one week, two weeks and three weeks were allowed to observe the degradation of infected female cadavers and the dispersal of released endospores. The hypothesis of this experiment was that, the spores of *P. penetrans* contained in *M. javanica* females that have been placed in soil would be released as the cadavers decompose and would attach to fresh J_2s added to the soil. A very highly significant difference was observed between the treatments as compared to control treatments of both crop cycles. The bioassay revealed a significant decrease in the total number of second stage juveniles from 50 g of the soil samples from second crop than the first crop soil samples. Three weeks duration allowed more endospores as compared to one week and two weeks treatment. These results showed that dispersal of endospores form the degrading female cadavers occurred after two weeks.

Keywords: Root-knot nematodes (*Meloidogyne javanica*), *Pasteuria penetrans*, spore density, degradation, dispersal, tomato plants.

A great deal of research work has been done to understand the biology of a hyper-parasite *P. penetrans* isolates which parasitize the economically important root-knot nematode species. The *P. penetrans* is a potentially good prospect for the control of root-knot nematodes (Darban *et al*., 2005; Gowen & Ahmed, 1990). *Pasteuria penetrans* has many of the attributes required by a successful biological control agent. The spores adhere to the second stage juveniles of *Meloidogyne* spp. (Darban *et al*., 2004, Stirling, 1984) in the soil when they search for host roots. The second stage juveniles of nematodes invade roots and begin to develop to maturity and the parasite also develops with the juveniles. When infected females reach maturity, the parasite destroys their reproductive system and the female become unable to produce eggs; and consequently the build-up of nematode population stops (Mankau, 1980; Sayre, 1980). The invasion of the roots may also be reduced by the spore burden. The infection rate of female nematodes increases with increasing spore attachment on the juveniles (Davies *et al*., 1988; Stirling, 1984).

However, the germination of a single spore is enough to create infection in *Meloidogyne* sp. females (Stirling, 1984). The hyper-parasite *Pasteuria penetrans* completes its life cycle inside the female and mature spores are released into the soil when the females start to 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 may be 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 diameter) were filled with 1 litre of John Innes no.2 compost. A single *P. penetrans* infected female was put in the middle of each pot at 6 cm depth to degrade. The soil of each pot was kept moist by watering as necessary and then was left in a growth room without plants for 1, 2 and 3 weeks. After each interval, 3 ml of water containing 2000 freshly hatched J_2s were added per replicate by pipette in four, 2cm 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 J_2s at the same time. After 2 days, four-week old tomato plants, which were grown in multi-cell plant trays, were 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. For bioassay after both crop harvests the soil of each pot was mixed thoroughly and 50g soil samples were taken from each replicate/pot. All samples were labelled and dated. Small saucers were filled with 100ml tap water and 38µm aperture sieves were placed separately on the top of each saucer and whatman tissue papers double folded were put on sieves and then 50 g soil samples were placed into the sieves and kept at room temperature for 24 h to let spores and second stage juveniles reach down in the water. After 24 h all 100ml water samples containing spores and second stage juveniles were collected and examined under microscope to determine the total number of juveniles and number of spores attached with juveniles.

Results and Discussion

A very highly significant difference ($p< 0.001$) was observed between the treatments as compared to control treatments of both crop cycles (Fig. 3). There was an average of 3-5 spores attached to each juvenile extracted from the soil. However, the number of encumbered juveniles and the level of attachment varied between the treatments (Fig. 1 and 2). The greater numbers of encumbered juveniles were found in the 3 weeks treatment. After the second crop the bioassay showed that numbers of juveniles without spores decreased significantly as compared to the first crop. The number of J_2s with higher spore attachment increased and total number of encumbered juveniles also increased. Further the bioassay revealed that there was a significant decrease in the total number of juveniles extracted from 50 g soil samples of the second crop soil as compared with the first crop soil samples (Fig. 3). The 3 weeks duration apparently allowed more spores as compared to 1 week and 2 weeks treatments to disperse more which was reflected in observation of more encumbered juveniles after the harvest of both crops.

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 the total number of juveniles collected as compared to control treatment. The recovery of J_2s from the soil samples suggests that more spores of *P. penetrans* had been released from the cadavers of infected females in the 3 weeks treatment.

More spores were attached to juveniles in the 3 weeks treatment than the others (Fig. 1 and 2). However, after the second crop, there were more juveniles encumbered with >10 spores/J_2 in the 2 weeks treatment than the 3 weeks treatment; which indicated that the originally added *P. penetrans* infected female was not yet fully degraded and the spores were still not fully distributed. The attachment of >10 spores/J_2 might be due to the close contact of juveniles with the degrading female cadaver. These results show that the degradation of the body wall of *P. penetrans* infected nematode females and release of endospores from their cadavers takes place over at least 2-3 weeks. There is published information on what causes the degradation of the females in soil. It has shown that spore-filled females will remain intact if the roots in which they are contained are allowed to dry. Once the endospores are released from the cadavers, their distribution is probably assisted by irrigation water. From a practical viewpoint, cultivation of soil after removal of a crop could improve the distribution of *P. penetrans* endospores by enhancing the degradation of the senescing roots.

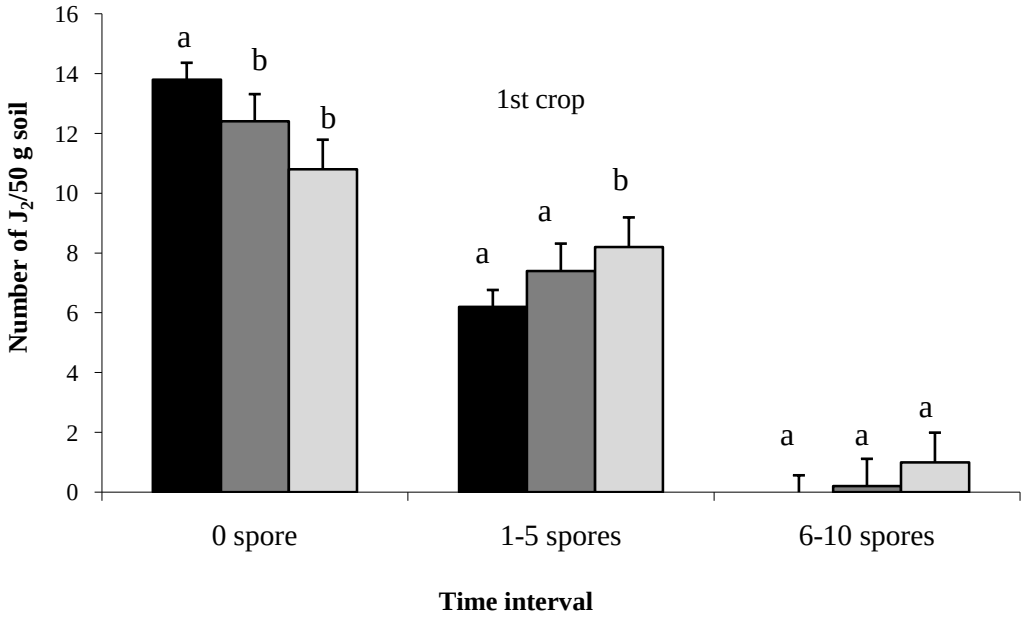

Fig. 1. The level of spore attachment per juvenile after the first crop harvest. Degradation periods of *P. penetrans*-infected females of *M. javanica* were 1 week (■) 2 weeks (■) and 3 weeks (■). Analysis was based on five replicates, with the observation of 20 J_2/replicate to determine the number of spores/J_2. Error bars represent 2x standard errors of means (Data are means of 5 replicates).

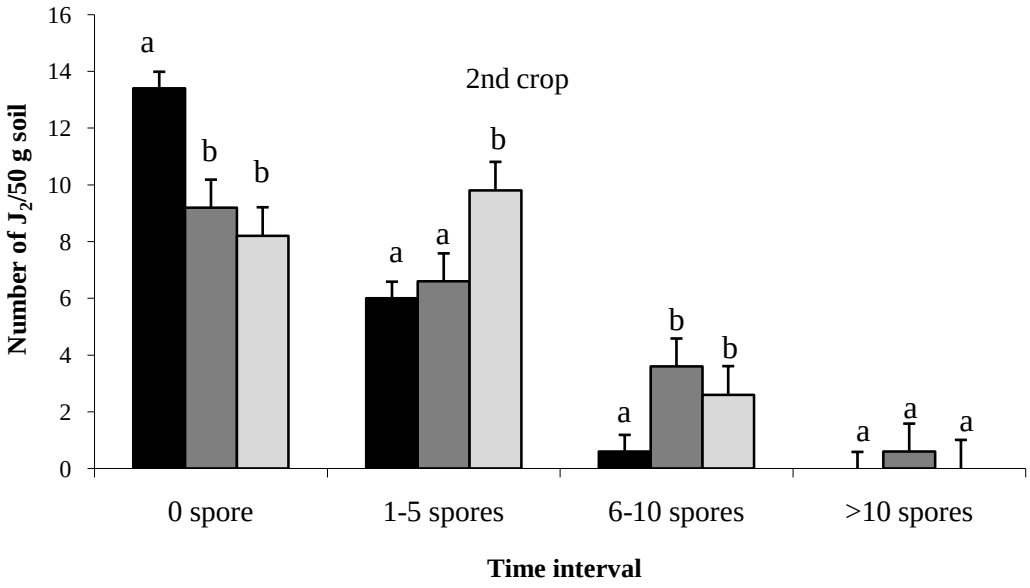

Fig. 2. The level of spore attachment per juvenile extracted from 50ml soil samples after the second crop harvest. Degradation periods of *P. penetrans*-infected females of *M. javanica* were 1 week (■) 2 weeks (■) and 3 weeks (■). Analysis was based on five replicates, with the observation of 20 J_2/replicate to determine the number of spores/J_2. Error bars represent 2x standard errors of means (Data are means of 5 replicates).

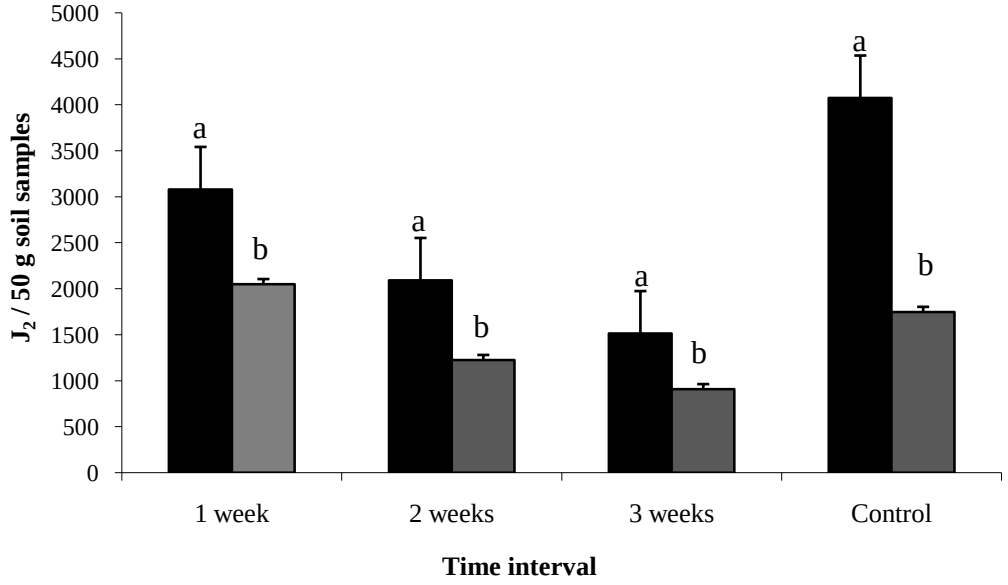

Fig. 3. Number of second stage juveniles (J_2) in 1st (■) and 2nd (■) crops of tomato. Analysis based on means of five replicates to determine the total number of J_2/treatment. Error bars represent 2x standard errors of means (Data are means of 5 replicates).

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