

RESEARCH NOTE/NOTA DE INVESTIGACIÓN

EFFECTIVENESS OF *PASTEURIA PENETRANS* APPLIED TO SEED OR FURROW

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ABSTRACT

Liu, C., and P. Timper. 2020. Effectiveness of *Pasteuria penetrans* applied to seed or furrow. *Nematropica* 50:229-232.

Pasteuria penetrans is a parasite of root-knot nematodes. Endospores of *P. penetrans* attach to the cuticle of second-stage juveniles (J2) and subsequently sterilize the infected female. Previously, we showed that prior exposure of *Meloidogyne arenaria* J2 to root exudates led to reduced attachment of *P. penetrans* spores suggesting that nematodes encountering spores within the root zone may be more resistant to spore attachment than are nematodes encountering spores outside the root zone. This study compared the effect of applying 3×10^5 *P. penetrans* spores in furrow versus with the seed at planting on egg production by *M. arenaria* in greenhouse pots. The results showed that *P. penetrans* spores applied by either method reduced nematode reproduction compared to the untreated control; however, in furrow application reduced egg production more than spores applied along with the seed (57.3% vs 35.6% reduction). Greater exposure of *M. arenaria* J2 to root exudates in the seed compared to the furrow application may explain the difference in application methods. Additionally, furrow application may distribute the spores over a greater area than seed applications, increasing the chances of *M. arenaria* J2 acquiring spores further from the roots which may impede their ability to infect the roots.

Key words: *Arachis hypogaea*, *Meloidogyne arenaria*, *Pasteuria penetrans*, root-knot nematodes, seed treatment

RESUMEN

Liu, C., y P. Timper. 2020. Efectividad de *Pasteuria penetrans* aplicada a semillas o surcos. *Nematropica* 50:229-232.

Pasteuria penetrans es un parásito de nematodos agalladores. Las endosporas de *P. penetrans* se adhieren a la cutícula de juveniles de segundo estado (J2) y posteriormente esterilizan a la hembra infectada. Anteriormente, demostramos que la exposición previa de *Meloidogyne arenaria* J2 a los exudados de la raíz condujo a una menor unión de las esporas de *P. penetrans*, lo que sugiere que los nematodos que encuentran esporas dentro de la zona radical pueden ser más resistentes a la unión de las esporas que los nematodos que se encuentran fuera de la zona radical. Este estudio comparó el efecto de aplicar 3×10^5 esporas de *P. penetrans* en surco contra la semilla a la siembra en la producción de huevos por *M. arenaria* en macetas de invernadero. Los resultados mostraron que las esporas de *P. penetrans* aplicadas por cualquier método redujeron la reproducción de los nematodos en comparación con el control no tratado;

sin embargo, la aplicación en surcos redujo la producción de huevos más que las esporas aplicadas junto con la semilla (57.3% vs 35.6% de reducción). Una mayor exposición del J2 a los exudados de la raíz en la semilla en comparación con la aplicación en surco puede explicar la diferencia en los métodos de aplicación. Además, la aplicación en surcos puede distribuir las esporas en un área mayor que las aplicaciones de semillas, lo que aumenta las posibilidades de que J2 adquiera esporas más lejos de las raíces, lo que puede impedir su capacidad de infectar las raíces.

Palabras clave: *Arachis hypogaea*, *Meloidogyne arenaria*, *Pasteuria penetrans*, nematodos agalladores, tratamiento de semilla

Root-knot nematodes (*Meloidogyne* spp.) are important pests causing significant yield losses to a variety of crops in tropical and subtropical regions of the world. The endospore-forming bacterium *Pasteuria penetrans* is an obligate parasite of these nematodes and has potential as a biological control agent (Chen and Dickson, 1998). The endospores of *P. penetrans* attach to the surface of second-stage juveniles (J2) as they migrate through soil to infect plant roots. When a small number of spores adhere to the J2, the infected female produces few to no eggs; when encumbered by a large number (>7) of spores, J2 are less mobile and their ability to enter roots is reduced (Davies *et al.*, 1991). In a microplot study, galling of peanut roots by *Meloidogyne arenaria* was reduced by 60% and 20% the first year after application of 100,000 and 10,000 *P. penetrans* spores/g of soil, respectively, and in the second year, galling was reduced by 81% and 61%, respectively (Chen *et al.*, 1996).

In a previous study, we showed that when *M. arenaria* J2 were exposed to root exudates from both host plants and nonhost plants, the J2 subsequently acquired 76-80% fewer spores than J2 not exposed to exudates (Liu *et al.*, 2017). These results indicated that *M. arenaria* J2 encountering spores in the root zone may be less susceptible to *P. penetrans* attachment than J2 encountering spores outside the root zone. Therefore, application methods that place spores outside the root zone may be more effective than those that place spores within the root zone. Previous studies comparing application methods for *P. penetrans* have shown mixed results when comparing spores added to the root zone vs. the surrounding soil. Tzortzakakis *et al.* (1997) applied *P. penetrans* to seedlings that were transplanted into soil infested with *Meloidogyne* spp. by either adding spores to the seedling trays or by dipping the seedling roots in a spore suspension. These application methods did not reduce egg production compared to the non-

inoculated control, whereas mixing spores into soil or drenching the soil surface with spores at transplanting significantly reduced egg production by the nematodes. In contrast, Kokalis-Burelle (2015) reported that a seed treatment of *P. penetrans* was as effective as a soil drench in reducing the number of eggs produced by *M. incognita* compared to an untreated control.

To determine whether *P. penetrans* spores applied to the planting furrow is more effective in reducing reproduction of *M. arenaria* than spores applied with the seed, a single spore line SS 16 obtained from University of Florida was used in the experiment. A population of *M. arenaria* was maintained on eggplant (cv. Black Beauty). To obtain nematode inoculum, infected roots were cut into 5 cm pieces, thoroughly mixed, and separated into equal portions (260 g and 210 g in Trial 1 and 2, respectively). Nematode-infected roots were distributed near the top of pots (35-cm diam × 26 cm) containing steam-heated loamy sand and covered with 10 cm of soil. For each trial of the experiment, the same weight of roots added to the pots was placed in a mist chamber to estimate the number of hatched *M. arenaria* J2 after 6 days. The estimated inoculum of *M. arenaria* was 2,000 and 1,600 J2 in Trial 1 and 2, respectively. For the seed treatment, peanut (*Arachis hypogaea*) seeds were placed in 4-cm deep holes every 10 cm along the center line of the pot and 1×10^5 spores of *P. penetrans* were pipetted onto the seed for a total of 3×10^5 spores per pot. For the furrow treatment, a 3-cm wide × 4-cm deep furrow was made in each pot, three seeds were evenly distributed in 10-cm intervals and 3×10^5 spores were evenly distributed along the furrow. The control was similar to the furrow treatment except only water was pipetted into the furrow. There were seven replicates per treatment, and the treatments were arranged randomly on a single greenhouse bench. Thirty days after seed germination, roots from all plants

were cut off and washed well with running water. Roots from the same pot were combined, drained, and left on paper towels to dry for 2 hrs before weighing. Eggs were separated from egg masses using 0.5% NaOCl (Hussey and Barker, 1973) and counted under 40 \times magnification. The experiment was conducted twice.

Data were analyzed using the Standard Least Squares Option (analysis of variance, JMP Pro v. 11). Eggs per gram root and root weight per pot in each replicate were used as data points. Treatment, trial, as well as their interactions were used to construct the model. LS Means Student's t-test was used to test pairwise comparison of model effects.

The data for the two trials were combined because there was no treatment \times trial interaction. The number of eggs per gram root was highest in the control, intermediate in the seed treatment, and lowest in the furrow treatment ($P < 0.0001$, Fig. 1). There was no difference among treatments in root weight ($P = 0.91$). *Pasteuria penetrans* applied both in furrow and as a seed treatment decreased egg production of *M. arenaria* compared to the control; however, the bacterium was more effective in reducing nematode reproduction when applied to the furrow (57.3% reduction) compared to the seed treatment (35.6% reduction). These results support the hypothesis that spores applied outside the root zone will be more effective in suppressing nematode reproduction than spores applied within the root zone. Exposure to root exudates within the root zone may result in J2 that are more resistant to *P. penetrans* spore attachment than J2 outside the root zone (Liu *et al.*, 2017). Irrespective of the

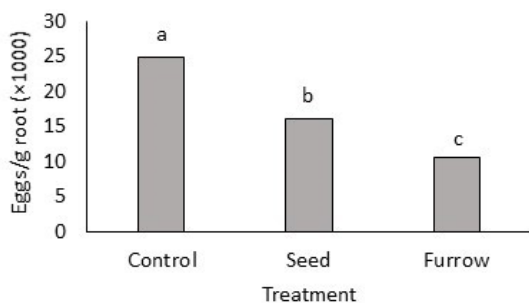


Fig. 1. Comparison of mean egg production by *Meloidogyne arenaria* in different application methods of *Pasteuria penetrans* spores. Control was not treated with spores. Bars with different letters indicate significant difference ($P \leq 0.05$). Each bar represents the mean of seven replications and two trials ($N = 14$).

influence of root exudates, furrow application may distribute the spores over a greater area than seed applications, increasing the chances of J2 acquiring spores further from the roots, which may impede their ability to complete their journey and infect the plant (Stirling, 1984; Davies *et al.*, 1991). For both application methods, movement of spores with irrigation water will distribute the spores downward, and even laterally to a lesser extent (Cetintas and Dickson, 2005).

Seed treatments are becoming the preferred means of applying chemicals and biologicals for crop protection because they reduce the amount of product delivered per hectare, thus decreasing costs, increasing concentrations of the product around the plant, and reducing impacts on non-target organisms (Sharma *et al.*, 2015). However, our results showed that applying *P. penetrans* spores as a seed treatment was less effective than applying the same number of spores in furrow. Perhaps higher spore concentrations can be used around the seed to compensate for reduced efficacy. Greenhouse pots provide only a limited test of the hypothesis that furrow application of *P. penetrans* will be more effective than seed treatments with spores; therefore, additional studies should be done in the field.

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