

## Effect of *Hirsutella rhossiliensis* on Infection of Potato by *Pratylenchus penetrans*

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**Abstract:** We evaluated the ability of the nematode-pathogenic fungus *Hirsutella rhossiliensis* (Deuteromycotina: Hyphomycetes) to reduce root penetration and population increase of *Pratylenchus penetrans* on potato. Experiments were conducted at 24 C in a growth chamber. When nematodes were placed on the soil surface 8 cm from a 14-day-old potato cutting, the fungus decreased the number entering roots by 25%. To determine the effect of the fungus on population increase after the nematodes entered roots, we transplanted potato cuttings infected with *P. penetrans* into *Hirsutella*-infested and uninfested soil. After 60 days, the total number of nematodes (roots and soil) was  $20 \pm 4\%$  lower in *Hirsutella*-infested than in uninfested soil.

**Key words:** biological control, *Hirsutella rhossiliensis*, migratory endoparasite, nematode, nematophagous fungus, potato, *Pratylenchus penetrans*, root lesion nematode, *Solanum tuberosum*.

Migratory endoparasites, such as *Pratylenchus* spp., are considered difficult targets for biological control (16). These nematodes spend most, and sometimes all, of their life cycle within plant roots, where they are believed to be protected from infection by antagonists that inhabit soil. Consequently, the effectiveness of an antagonist as a biological control agent will depend, in part, on the amount of time the nematodes are outside roots.

In annual crops, many *Pratylenchus* spp. leave the roots when the plants begin to senesce or are harvested. At the end of the growing season, from 50–96% of the population has been found in the soil (12,14). When a new susceptible crop is planted, the nematodes migrate to and penetrate the roots. Once within the roots, most individuals remain there during the growing season. MacGuidwin et al. found that ca. 80% of *P. scribneri* were within the roots of corn and potato during plant growth (12).

We have been examining the nematode-pathogenic fungus *Hirsutella rhossiliensis* as a possible biological control agent of *Pratylenchus penetrans* on potato. In a previous study, the fungus killed 40% of *P. penetrans* in soil without a host plant (17). The ob-

jective of this study was to determine the effectiveness of *H. rhossiliensis* when a plant is present. We determined the effect of the fungus on nematode penetration of potato roots and population increase after initial root penetration.

### MATERIALS AND METHODS

**General methods:** We obtained juveniles and adults of *P. penetrans* from alfalfa callus culture (15) using the Baermann piepan method (9). The nematodes were stored at 10 C for 1–3 days before an experiment. We reared the entomopathogenic nematodes *Steinernema glaseri* and *Heterorhabditis bacteriophora* in *Galleria mellonella* (5) and stored them after harvesting in distilled water at  $23 \pm 5$  C.

A loamy sand (88.7% sand, 4.6% clay, and 6.6% silt; pH 5.4 in water) was moistened, heated to 60 C for 30 minutes to eliminate most organisms, and air dried. In one experiment, the loamy sand was mixed (1:1) with a medium-fine sand (98% of grains between 100–500  $\mu$ m). Dauer juveniles of *S. glaseri* were infected with *H. rhossiliensis* by adding healthy individuals to a 3- to 5-week-old sand culture of the fungus (6) and extracting them 4 days later. A portion of the heat-treated soil was infested with *H. rhossiliensis* by mixing the infected nematodes into moistened (8% w/w) soil (18).

The relative amount of conidia in the soil was determined at least once per ex-

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periment. We assayed conidia in the soil by adding either  $3,246 \pm 109$  (root penetration experiment) or  $8,074 \pm 1,098$  (population increase experiment) *H. bacteriophora* to pots containing *Hirsutella*-infested soil. The nematodes were extracted 24 hours later, and 40/pot were examined at random for adhering conidia. Acquisition of *H. rhossiliensis* conidia by this nematode is correlated with the density in soil (13). Conidial-assay pots were identical to experimental pots. The percentage of *H. bacteriophora* with conidia was used to compare fungal concentrations among trials (repetitions of the experiment).

We used *Solanum tuberosum* clone NY85, a known host for *P. penetrans*, in all experiments (3). Potato cuttings were dipped in RootTone and placed in moist vermiculite for 4–10 days before being transplanted to the experimental pots. Plants were kept in a growth chamber at 24 C under 15 hours light/day ( $318 \mu\text{E}/\text{second}/\text{m}^2$ ). We applied deionized water when the soil surface was dry and liquid fertilizer (23-19-17) once per week. Deionized water was used because we were concerned that the heavily chlorinated tap water would suppress sporulation of *H. rhossiliensis* conidia. At the end of an experiment, roots were rinsed free of soil, weighed, stained with acid fuchsin-lactoglycerol (2), and homogenized in a blender at high speed for 30 seconds (1). Root debris was separated from the nematodes using a 250- $\mu\text{m}$  sieve. We adjusted the nematode suspension to a volume of 50 ml and averaged the number of stained *P. penetrans* counted in three 1-ml aliquots. Extraction efficiency of nematodes from roots was 80%.

*Effect of H. rhossiliensis on root penetration:* We added 520  $\text{cm}^3$  of a sand:soil mixture (1:1) to 1,055- $\text{cm}^3$  rectangular pots ( $L \times W \times H = 15 \times 10.5 \times 6.7$  cm). The soil was either infested with *Hirsutella* ( $4.3 \pm 0.2$  infected *S. glaseri*/ $\text{cm}^3$ ) or uninfested. We transplanted a 5-day-old potato cutting 4 cm from one end of the pot and, after a 9-day incubation, added  $2,944 \pm 113$  *P. penetrans* to the pots. The nematodes were applied on the soil surface 8 cm from the

plant. The plants were watered from below to avoid dislodging conidia from the conidiophores (13). We removed the plants after 6 days and counted the number of nematodes in the roots. *Hirsutella* conidia were assayed 10 days after potato was transplanted into the fungus-infested soil. The experiment was repeated three times with 12–13 pots per treatment (infested and uninfested soil). We compared the number of *P. penetrans* entering roots in *Hirsutella*-infested and uninfested soil using analysis of covariance (11). Root weight was considered a covariate because of its potential influence on nematode penetration. Within a cultivar, root weight is directly related to root size. More nematodes may penetrate larger root systems than may penetrate smaller root systems.

*Effect of H. rhossiliensis on population increase:* Potato cuttings were grown in 150- $\text{cm}^3$  pots filled with medium-fine sand for 10–15 days before  $1,925 \pm 282$  *P. penetrans* were pipetted onto the sand surface. After 4 days, we removed the plants, rinsed the roots, and transplanted them into 13-cm-d pots containing 925  $\text{cm}^3$  of *Hirsutella*-infested ( $5.0 \pm 0.1$  infected *S. glaseri*/ $\text{cm}^3$ ) or uninfested loamy sand. The number of *P. penetrans* in the roots at the time of transplanting was  $800 \pm 142$  ( $N = 6$  plants/trial). At 30 days, we added  $3,050 \pm 180$  healthy *S. glaseri* to *Hirsutella*-infested pots to maintain the fungus at a high level. Healthy *S. glaseri* were also added to uninfested pots to serve as a control for any influence they might have on the population of *P. penetrans*. At 60 days, we extracted *P. penetrans* from 100  $\text{cm}^3$  of bulk soil, and from the total rhizosphere soil (trials 2 and 3) and roots. The rhizosphere soil was collected by first gently shaking excess soil from the roots and then sonicating the roots with the adhering soil in a plastic bag containing 250 ml water for 1 minute. Nematodes were extracted from bulk and rhizosphere soil by wet sieving through 250- and 38- $\mu\text{m}$  sieves followed by centrifugal flotation (8). Extraction efficiency of *P. penetrans* from soil was 42%. The soil was assayed for *H. rhossiliensis*

conidia at 10, 30, and 60 days. There were five pots per assay day, except for the final assay, when we added the *H. bacteriophora* to all *Hirsutella*-infested pots 1 day before harvest. The experiment was repeated three times with 10–13 replicate pots per treatment (infested and uninfested soil). We performed a two-way analysis of variance to determine the effects of *H. rhossiliensis* and trial on the population of *P. penetrans*.

## RESULTS

**Effect of *H. rhossiliensis* on root penetration:** The results from the three trials were combined into a single analysis because the difference between *Hirsutella*-infested and uninfested soil did not vary among trials (treatment  $\times$  trial interaction,  $P = 0.42$ ). There were 25% fewer nematodes ( $P = 0.01$ ) in roots from *Hirsutella*-infested ( $403 \pm 33$ ,  $\bar{x} \pm SE$ ) compared with uninfested pots ( $539 \pm 38$ ). At the conclusion of the experiment, the potato roots had reached the end of the pot opposite the plant. Root weight influenced penetration ( $P = 0.003$ ), with more nematodes entering larger than entering smaller root systems. Only a few *Hirsutella*-infested nematodes ( $2 \pm 1$ ) were observed within the roots. Infested nematodes were identified by the presence of hyphae protruding from their cuticle. The percentage of assay nematodes with at least one conidium adhering to their cuticle was  $75 \pm 5$ .

**Effect of *H. rhossiliensis* on population increase:** Because the difference between the *Hirsutella*-infested and uninfested pots was similar among the trials (treatment  $\times$  trial interaction,  $P = 0.6$ ), we combined the trials into a single analysis. The fungus reduced ( $P = 0.04$ ) the total number of nematodes per pot by  $20 \pm 4\%$  after 60 days. Most *P. penetrans* were in the roots (Fig. 1); only  $23 \pm 2\%$  were in the soil (bulk + rhizosphere soil). The number of nematodes in soil was similar in pots with and without the fungus. However, fewer nematodes were found in roots from pots containing *H. rhossiliensis* ( $P = 0.05$ ) than

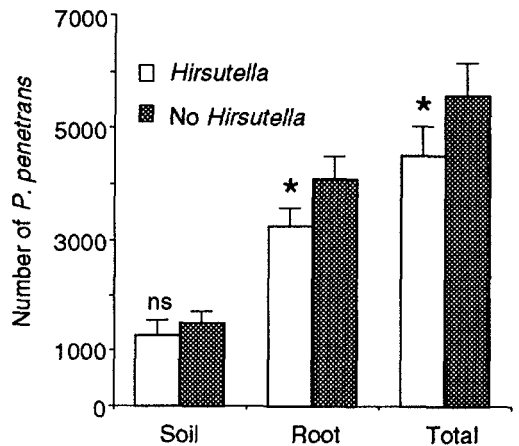


FIG. 1. *Pratylenchus penetrans* in soil and roots of potato after 60 days in the presence and absence of *Hirsutella rhossiliensis*. Columns are the means  $\pm$  SE per pot ( $925 \text{ cm}^3$  soil) of 35 replicates per treatment (pooled from three experimental trials). Columns with an asterisk are different from the control ( $P \leq 0.05$ ). The number of nematodes within roots at the start of the experiment was  $800 \pm 142$ . The percentage of *H. bacteriophora* with at least one *H. rhossiliensis* conidium adhering to their cuticle was  $59 \pm 12$  (day 10),  $56 \pm 13$  (day 30), and  $30 \pm 8$  (day 60).

from pots without the fungus. We found no *Hirsutella*-infested nematodes within the roots. The percentage of assay nematodes with at least one conidium adhering to their cuticle was  $59 \pm 12$  (day 10),  $56 \pm 13$  (day 30), and  $30 \pm 8$  (day 60).

## DISCUSSION

Older plants are generally more tolerant to nematode damage than are younger plants (4); therefore, the critical time to protect plants from nematode infection is when they are young. We found that *H. rhossiliensis* reduced the number of *P. penetrans* entering roots of young potato plants by 25%. The effect of the fungus on root penetration is dependent on the distance the nematode has to move through *Hirsutella*-infested soil to reach a root and on the density of infective conidia. In our study, the distance the nematodes moved to penetrate the roots is unknown. The roots grew continuously throughout the experiment and had extended past the point of nematode application by the end of the experiment. The density of *H. rhos-*

*siliensis* that we tested is within the high range of densities found in naturally infested field soils (13). Higher densities may be achieved by adding formulated hyphae along with the potato-seed piece at planting. Alginate pellets containing macerated *H. rhossiliensis* hyphae suppressed the number of *Heterodera schachtii* infecting cabbage roots by up to 95% in small vials (10).

Reduction in the population of *P. penetrans* during the growing season of the current crop will result in lower nematode densities for the next susceptible crop. When we tested the effect of *H. rhossiliensis* on the nematode population after they had entered potato roots, we documented a 20% suppression of the nematode population by the fungus after 60 days. A small proportion of *P. penetrans* were found in the soil. Nematodes that leave roots during plant growth may eventually reenter the roots or remain outside and feed ectoparasitically (19). Some of the individuals that entered the soil in *Hirsutella*-infested pots may have become infected by the fungus and died before reentering the roots. An alternative explanation may be that the fungus killed some of the nematodes within the roots. However, no infected nematodes were observed within the roots, and *H. rhossiliensis* can not grow saprophytically on or in unsterilized roots (7).

Our results demonstrate the potential of *H. rhossiliensis* for reducing root penetration and population increase of *P. penetrans* on potato. A weakness of our study was that we killed most of the resident organisms in the soil before adding the fungus. We don't know what influence these organisms may have had on *H. rhossiliensis*. Further research should be done to determine the ability of the fungus to control the nematode under field conditions and to enhance its effectiveness.

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