Journal of Nematology 34(2):171–174. 2002.


# Use of Entomopathogenic Nematodes to Suppress *Meloidogyne incognita* on Greenhouse Tomatoes

Enrique E. Pérez and Edwin E. Lewis[1]

*Abstract:* Tomato seedlings in a growth chamber were inoculated with 150 *Meloidogyne incognita* eggs and 25 infective juveniles (IJ)/cm$^2$ of *Steinernema feltiae, S. riobrave,* or *Heterorhabditis bacteriophora.* With the exception of seedling roots treated with *H. bacteriophora,* all seedlings treated with entomopathogenic nematodes had fewer *M. incognita* juveniles inside roots and produced fewer eggs than the control seedlings. Tomato plants in the greenhouse were infested with 4,000 *M. incognita* eggs and treated 2 weeks before, 1 week before, at the same time, 1 week after, or 2 weeks after with 25 or 125 IJ/cm$^2$ of *S. feltiae, S. riobrave,* or *H. bacteriophora.* Plants with pre- and post-infestation applications of *S. feltiae* or *S. riobrave* suppressed *M. incognita.* Plants treated with *H. bacteriophora* 1 week before and at the time of infestation suppressed *M. incognita.* Increasing the rate of *H. bacteriophora* and *S. feltiae* from 25 to 125 IJ/cm$^2$ improved *M. incognita* suppression.

*Key words:* biological control, entomopathogenic nematodes, *Heterorhabditis bacteriophora, Lycopersicum esculentum, Meloidogyne incognita, Steinernema feltiae, S. riobrave,* suppression, tomato.

Limited nematicide availability and high costs of nematicide development have created a need to discover alternative methods for plant-parasitic nematode management. One of the top priorities for the future of nematology is developing alternatives to hazardous chemical nematicides (Barker et al., 1994). Despite the need and potential markets, there are no biological control products for plant-parasitic nematodes commercially available (Grewal et al., 1997).

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* and their associated bacteria *Xenorhabdus* spp. and *Photorhabdus* sp., respectively, are commercially available to control soil insect pests (Georgis and Manweiler, 1994). Entomopathogenic nematode infective juveniles (IJ) search for and infect the insect host (Poinar, 1979). Upon infection, the nematodes release their associated bacteria, and toxins produced by the nematode and bacteria kill the insect within 2 to 3 days (Akhurst and Boemare, 1990; Burman, 1982).

During the last two decades, studies have reported an antagonistic interaction between entomopathogenic and plant-parasitic nematodes. Soil in sealed containers where entomopathogenic nematodes had been added had fewer plant-parasitic nematodes than the control soil (Ishibashi and Kondo, 1986). Additional research has documented plant-parasitic nematode suppression by entomopathogenic nematodes on chemically treated soil and other crops (Bird and Bird, 1985; Grewal et al., 1997; Ishibashi and Kondo, 1987; Smitley et al., 1992).

The literature does not report a standard rate of entomopathogenic nematodes or application method to suppress plant-parasitic nematodes in field and greenhouse experiments. For example, one application of 5 × 10$^6$ or 10 daily applications of 5 × 10$^5$ *S. glaseri* (Steiner) IJ/potted plant suppressed *M. javanica* (Treub) on tomato (*Lycopersicum esculentum* Mill.) in greenhouse experiments (Bird and Bird, 1986). In turf, Smitley et al. (1992) used a mix of 2.47 × 10$^9$ *Heterorhabditis bacteriophora* Poinar and 2.08 × 10$^9$ *S. carpocapsae* (Weiser) IJ/ha, whereas Grewal et al. (1997) used a single application of 2.47 × 10$^9$ *S. riobrave* Cabanillas, Poinar and Raulston IJ/ha and suppressed plant-parasitic nematodes in field experiments. Tsai and Yeh (1995) found that suppression varies with plant-parasitic nematode species and host plant species.

In the laboratory, a rate of 2.5 × 10$^9$ *S. feltiae* (Filipjev) IJ/ha scaled to a small container applied simultaneously with *M. incognita* (Kofoid and White) suppressed the latter on tomato seedlings grown in sterilized sand (Lewis et al., 2001). However, this rate has not been tested with other species of entomopathogenic nematodes, and there is no information on pre- and post-infestation applications of entomopathogenic nematodes to suppress *M. incognita* on tomato. The objective of this research was to test *M. incognita* suppression on tomato using three species of entomopathogenic nematodes at the rates of 2.5 × 10$^9$ IJ/ha and 12.5 × 10$^9$ IJ/ha applied before and after *M. incognita* infestation.

## Materials and Methods

The Greater wax moth *Galleria mellonella* (L.) was used as the host insect to rear *S. feltiae, S. riobrave,* and *H. bacteriophora* (Kaya and Stock, 1997). Infective juveniles were harvested from White traps (White, 1927). The infective juveniles were stored in water at 25 ± 1 °C. Live infective juveniles were used within 21 days of emergence from their host cadaver. *Meloidogyne incognita* cultures were maintained on tomato 'Rutgers' in the greenhouse. Eggs of *M. incognita* to be used as inoculum were extracted using a sodium hypochlorite method (Hussey and Barker, 1973).

Received for publication 22 May 2001.
[1] Research Associate and Assistant Professor, Entomology Department, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.
Supported in part by a grant from the U.S. Department of Agriculture Fund for Rural America and the Virginia Agriculture Experiment Station.
The authors thank Lydia Ingrassia for technical assistance and Jon Eisenbank and Jeffrey Bloonquist for reviewing this manuscript.
E-mail: eperez@vt.edu
This paper was edited by Parwinder Grewal.

*Growth chamber experiments:* Tomato seeds 'Rutgers' were planted individually in 6-cm-diam. cups filled with 100 g of sterilized sand. Tomato seedlings approximately 10 cm in height were inoculated with 150 *M. incognita* eggs in aqueous suspension. Immediately thereafter, *S. feltiae, S. riobrave,* or *H. bacteriophora* were applied at a rate of 25 IJ/cm$^2$ to each tomato seedling. A completely randomized experimental design was used. Each treatment was replicated 18 times (6 + 12, see below). Two weeks after *M. incognita* infestation, six tomato seedlings from each treatment were removed and the entire root was stained with acid fuchsin. After staining, the entire root system was pressed between two glass plates and *M. incognita* juveniles inside the roots were counted under a dissecting microscope. Five weeks after *M. incognita* infestation, the remaining 12 tomato seedlings were removed from each treatment and nematode eggs were extracted from the root systems with sodium hypochlorite (Hussey and Barker, 1973). Eggs were counted with a dissecting microscope. The experiment was repeated twice. Data were subjected to analysis of variance for a completely randomized design (Montgomery, 1991) using SAS (SAS Institute, Inc., Cary, NC). Treatment mean differences against the control were tested using Dunnett's test.

*Greenhouse experiments:* Experiments in the greenhouse were conducted to test pre- and post-infestation applications of entomopathogenic nematodes on *M. incognita* suppression. Tomato seedlings 'Rutgers' were transplanted 1 week after germination into 12-cm-diam. pots containing approximately 900 cm$^3$ of potting soil Pro-Mix (Premier Horticulture, Red Hill, PA) and sterilized sand (1:1) mixture. Plants were allowed to grow for 4 weeks, and approximately 4,000 *M. incognita* eggs in aqueous suspension were placed in depressions around the plant.

Six experiments were conducted separately. Each experiment used one entomopathogenic nematode species (*S. feltiae, S. riobrave,* or *H. bacteriophora*) applied at a rate of either 25 IJ/cm$^2$ or 125 IJ/cm$^2$. Each entomopathogenic nematode species and rate combination was applied 2 weeks before, 1 week before, at the same time, 1 week after, or 2 weeks after tomato plants were infested with *M. incognita* eggs. In each experiment treatments were replicated 18 times (6 + 12, see below). Plants were watered as needed, and no fertilizer was added. Two weeks after *M. incognita* infestation, six tomato plants were removed from each treatment and the root was stained by the acid fuchsin method. After staining, *M. incognita* juveniles were counted as previously described. Five weeks after *M. incognita* infestation, 12 tomato plants were removed from each treatment and nematode eggs were extracted and counted as previously described. Data from each experiment were subjected to analysis of variance for a completely randomized design using SAS (SAS Institute, Inc., Cary, NC). In each experiment, nematodes inside the root and egg counts in treatments were compared to those for the control using Dunnett's test.

## Results

*Growth chamber experiments:* Fewer *M. incognita* juveniles were found in roots of tomato seedlings treated with *S. feltiae* and *S. riobrave* ($P \leq 0.05$) than in the controls (Fig. 1A). Treatment with *H. bacteriophora* did not affect *M. incognita* juvenile numbers in roots (Fig. 1A). Fewer *M. incognita* eggs ($P \leq 0.05$) were recovered from tomato seedlings treated with *S. feltiae, S. riobrave,* or *H. bacteriophora* than from the control seedlings (Fig. 1B).

*Greenhouse experiments: Meloidogyne incognita* juveniles inside tomato roots were fewer at all application times ($P \leq 0.05$) in plants treated with 25 IJ/cm$^2$ of *S. feltiae* than those in the control plants (Table 1A). Fewer *M. incognita* eggs ($P \leq 0.05$) were recovered from plants treated with 25 IJ/cm$^2$ of *S. feltiae* than from the control plants at all application times, except for plants treated 2 weeks after *M. incognita* infestation (Table 1A). Fewer juveniles and *M. incognita* eggs ($P \leq 0.05$) were recovered from plants treated with 25 IJ/cm$^2$ of *S. riobrave* than from the control plants at all application times (Table 1A). Plants treated with 25 IJ/cm$^2$ of *H. bacteriophora* at the time of *M. incognita* infestation had fewer ($P \leq 0.05$) *M. incognita* juveniles inside the roots than

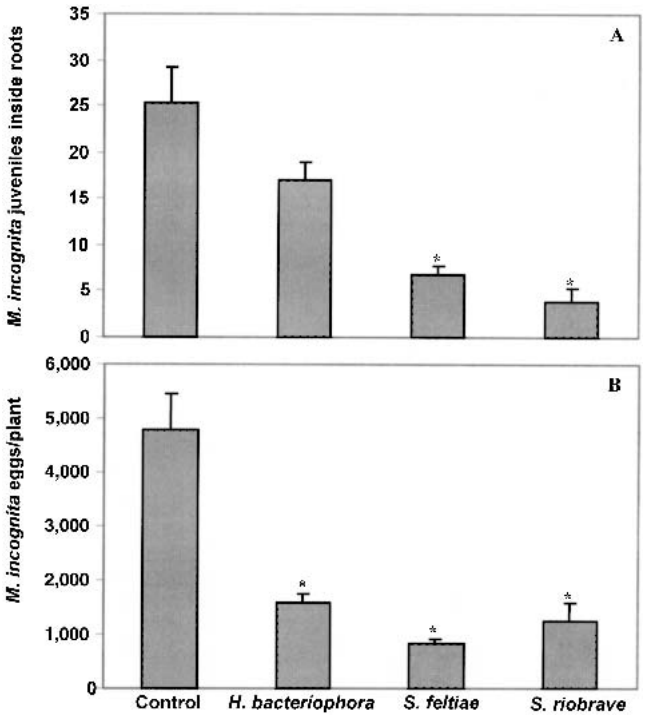


FIG. 1. *Meloidogyne incognita* penetration and egg production in tomato seedlings treated with 25 infective juveniles/cm$^2$ of *Steinernema feltiae, Heterorhabditis bacteriophora, S. riobrave,* and control. A) Number of juveniles in roots 2 weeks after infestation ($n = 12$). B) Number of eggs recovered from roots 5 weeks after infestation ($n = 24$). Bars indicate standard error of the mean. *Significantly different ($P \leq 0.05$) from the control according to Dunnett's test.

TABLE 1. *Meloidogyne incognita* root penetration and egg production on tomato plants treated at different times with *Steinernema feltiae, S. riobrave,* or *Heterorhabditis bacteriophora.* A) Application rate of 25 infective juveniles/cm$^2$. B) Application rate of 125 infective juveniles/cm$^2$.

| | Application times | | | | | |
|---|---|---|---|---|---|---|
| | 2 WBI[a] | 1 WBI | 0 WBI | 1 WAI[b] | 2 WAI | Control |
| A. 25 infective juveniles/cm$^2$ | | | | | | |
| | *M. incognita* juvenile numbers inside roots/plant ± SEM ($n$ = 6) | | | | | |
| *S. feltiae* | 65* ± 12 | 58* ± 19 | 50* ± 25 | 86* ± 16 | — | 242 ± 58 |
| *S. riobrave* | 148* ± 57 | 121* ± 23 | 178* ± 28 | 200* ± 19 | — | 483 ± 73 |
| *H. bacteriophora* | 277 ± 49 | 366 ± 78 | 128* ± 31 | 289 ± 63 | — | 321 ± 74 |
| | *M. incognita* egg numbers/tomato plant ± SEM ($n$ = 12) | | | | | |
| *S. feltiae* | 16,873* ± 3,464 | 10,968* ± 1,430 | 7,739* ± 1,616 | 11,740* ± 4,388 | 23,213 ± 4,453 | 35,783 ± 4,964 |
| *S. riobrave* | 3,166* ± 1,199 | 3,405* ± 1,649 | 5,705* ± 1,964 | 9,391* ± 2,220 | 6,426* ± 2,964 | 25,346 ± 5,470 |
| *H. bacteriophora* | 25,835 ± 5,860 | 18,621* ± 4,276 | 15,883* ± 5,487 | 26,361 ± 4,937 | 26,000 ± 6,053 | 30,607 ± 5,001 |
| B. 125 infective juveniles/cm$^2$ | | | | | | |
| | *M. incognita* juvenile numbers inside roots/plant ± SEM ($n$ = 6) | | | | | |
| *S. feltiae* | 289* ± 62 | 180* ± 65 | 187* ± 45 | 225* ± 74 | — | 551 ± 81 |
| *S. riobrave* | 99* ± 32 | 83* ± 15 | 55* ± 14 | 139* ± 26 | — | 387 ± 42 |
| *H. bacteriophora* | 380 ± 70 | 363 ± 57 | 206* ± 47 | 245* ± 22 | — | 440 ± 52 |
| | *M. incognita* egg numbers/tomato plant ± SEM ($n$ = 12) | | | | | |
| *S. feltiae* | 31,897* ± 2,284 | 26,968* ± 3,915 | 12,392* ± 2,562 | 12,593* ± 1,070 | 21,670* ± 3,947 | 58,643 ± 3,460 |
| *S. riobrave* | 11,608* ± 2,037 | 16,536* ± 3,060 | 9,169* ± 1,505 | 15,418* ± 2,560 | 17,158* ± 3,485 | 48,521 ± 4,738 |
| *H. bacteriophora* | 28,960 ± 4,631 | 24,181* ± 2,372 | 14,994* ± 2,350 | 28,752 ± 5,295 | 31,916 ± 4,805 | 37,423 ± 3,538 |

[a] WBI = Weeks before *M. incognita* infestation.
[b] WAI = Weeks after *M. incognita* infestation.
* Significantly different ($P \leq 0.05$) from the control according to Dunnett's test.

those in the control plants (Table 1A). Fewer *M. incognita* eggs ($P \leq 0.05$) were recovered from plants treated with 25 IJ/cm$^2$ of *H. bacteriophora* 1 week before and at the time of *M. incognita* infestation than from the control plants (Table 1A).

Fewer juveniles and *M. incognita* eggs ($P \leq 0.05$) were recovered from plants treated with 125 IJ/cm$^2$ of *S. riobrave* or *S. feltiae* than from the control plants at all application times (Table 1B). *Meloidogyne incognita* juveniles inside plants treated with 125 IJ/cm$^2$ of *H. bacteriophora* 1 week after and at the time of infestation with *M. incognita* eggs were fewer ($P \leq 0.05$) than those in the control plants (Table 1B). Fewer *M. incognita* eggs ($P \leq 0.05$) were recovered from plants treated with 125 IJ/cm$^2$ of *H. bacteriophora* 1 week before and at the time of infestation with *M. incognita* than from the control plants (Table 1B).

## Discussion

The rate of 25 IJ/cm$^2$ of *H. bacteriophora, S. riobrave,* and *S. feltiae* suppressed *M. incognita* on tomato plants in growth chamber and greenhouse experiments. Our findings for *S. feltiae* agree with those of Lewis et al. (2001) and showed that the same rate of *H. bacteriophora* and *S. riobrave* also suppressed *M. incognita* on tomato in both growth chamber and greenhouse trials.

Pre- or post-*M. incognita* infestation applications of 25 IJ/cm$^2$ of *H. bacteriophora, S. riobrave,* or *S. feltiae* suppressed *M. incognita* on tomato plants grown in the greenhouse. This finding agrees with the report of long-term plant-parasitic nematode suppression on turf with a single application of *S. riobrave* (Grewal et al., 1997).

Both the low and high rates of *S. riobrave* suppressed *M. incognita* at all application times, which suggests that a rate increase may not improve suppression. Grewal et al. (1997) reported that increasing the rate of *S. riobrave* from 1 billion to 6 billion nematodes per acre did not improve plant-parasitic nematode suppression on turf. However, the high rates of *H. bacteriophora* applied 1 week after or *S. feltiae* applied 2 weeks after *M. incognita* infestation suppressed *M. incognita* penetration and eggs, respectively, whereas the low rate did not.

The symbiotic bacteria, *Xenorhabdus* spp. for *Steinernema* and *Photorhabdus* sp. for *Heterorhabditis,* may be a factor for *M. incognita* suppression. Grewal et al. (1999) reported repellency, toxicity, and egg hatch reduction of *M. incognita* exposed to *Xenorhabdus* spp. cell-free extract. They suggested allelochemicals produced by *Xenorhabdus* spp. as the cause of antagonism to *M. incognita.* In our experiments, *M. incognita* suppression using *Heterorhabditis* was less consistent than *M. incognita* suppression using *Steinernema. Photorhabdus* sp. cell-free extracts have not been tested, yet their metabolites may also be antagonistic to *M. incognita.*

*Meloidogyne incognita* suppression by entomopathogenic nematodes may vary with *M. incognita* initial infestation density, crop, and soil type. Further experiments that account for these factors are needed. We found that pre- and post-infestation applications sup-

pressed *M. incognita* on greenhouse tomatoes. Testing of application times to suppress plant-parasitic nematodes under field conditions is warranted.

## Literature Cited

Akhurst, R., and N. E. Boemare. 1990. Biology and taxonomy of *Xenorhabdus*. Pp. 75–87 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

Barker, K. R., R. S. Hussey, L. R. Krusberg, G. W. Bird, R. A. Dunn, H. Ferris, V. R. Ferris, D. W. Freckman, C. J. Gabriel, P. S. Grewal, A. E. MacGuidwin, D. L. Riddler, P. A. Roberts, and D. P. Schmitt. 1994. Plant and soil nematodes: Societal impact and focus for the future. Journal of Nematology 26:127–137.

Bird, A. F., and J. Bird. 1986. Observations on the use of insect parasitic nematodes as means of biological control of root-knot nematodes. International Journal for Parasitology 16:511–516.

Burman, M. 1982. *Neoplectana carpocapsae:* A toxin production by axenic insect parasitic nematodes. Nematologica 28:62–70.

Georgis, R., and S. Manweiler. 1994. Entomopathogenic nematodes: A developing biological control technology. Agricultural and Zoological Reviews 6:63–94.

Grewal, P. S., E. E. Lewis, and S. Venkatachari. 1999. Allelopathy: A possible mechanism of suppression of plant-parasitic nematodes by entomopathogenic nematodes. Nematology 1:735–743.

Grewal, P. S., W. R. Martin, R. W. Miller, and E. E. Lewis. 1997. Suppression of plant-parasitic nematode populations in turfgrass by application of entomopathogenic nematodes. Biocontrol Science and Technology 7:393–399.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025–1028.

Ishibashi, N., and E. Kondo. 1986. *Steinernema feltiae* (DD-136) and *S. glaseri*: Persistence in soil and bark compost and their influence on native nematodes. Journal of Nematology 18:310–316.

Ishibashi, N., and E. Kondo. 1987. Dynamics of the entomogenous nematode *Steinernema feltiae* applied to soil with and without nematicide treatment. Journal of Nematology 19:404–412.

Kaya, H. K., and S. P. Stock. 1997. Techniques in insect nematology. Pp. 281–322 *in* L. Lacey, ed. Manual of techniques in insect pathology. New York: Academic Press.

Lewis, E. E., P. S. Grewal, and S. Sardanelli. 2001. Interactions between the *Steinernema feltiae-Xenorhabdus bovienii* insect pathogen complex and the root-knot nematode *Meloidogyne incognita*. Biological Control 21:56–62.

Montgomery, D. C. 1991. Design and analysis of experiments, 3rd ed. New York: John Wiley.

Poinar, G. O., Jr. 1979. Nematodes for Biological Control of Insects. Boca Raton, FL: CRC Press.

Smitley, D. R., F. W. Warner, and G. W. Bird. 1992. Influence of irrigation and *Heterorhabditis bacteriophora* on plant-parasitic nematodes in turf. Supplement to the Journal of Nematology 24:637–641.

Tsai, B.-Y., and H.-L. Yeh. 1995. Effect of *Steinernema carpocapsae* Weiser on the infectivity of *Pratylenchus coffeae* (Zimmermann) Filipjev and Schuurmans Stekhoven and *Meloidogyne javanica* (Treub) Chitwood. Plant Pathology Bulletin 4:106–110.

White, G. F. 1927. A method for obtaining infective nematode larvae from cultures. Science 66:302–303.