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# Effects of Entomopathogenic Nematodes on *Meloidogyne javanica* on Tomatoes and Soybeans

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*Abstract:* Two Hawaiian isolates of *Steinernema feltiae* MG-14 and *Heterorhabditis indica* MG-13, a French isolate of *S. feltiae* SN, and a Texan isolate of *S. riobrave* TX were tested for their efficacy against the root-knot nematode, *Meloidogyne javanica,* in the laboratory and greenhouse. Experiments were conducted to investigate the effects of treatment application time and dose on *M. javanica* penetration in soybean, and egg production and plant development in tomato. Two experiments conducted to assess the effects of entomopathogenic nematode application time on *M. javanica* penetration demonstrated that a single application of $10^4$ *S. feltiae* MG-14 or SN infective juveniles per 100 $cm^3$ of sterile soil, together with 500 (MG-14) or 1,500 (SN) second-stage juveniles of *M. javanica,* reduced root penetration 3 days after *M. javanica* inoculation compared to that of a water treatment. Entomopathogenic nematode infective juveniles applied to assess the effects on *M. javanica* egg production did not demonstrate a significant reduction compared to that of the water control treatment. There was no dose response effect by *Steinernema* spp. on *M. javanica* root penetration or egg production. *Steinernema* spp. did not affect the growth or development of *M. javanica*-infected plants, but *H. indica* MG-13-treated plants had lower biomass than untreated plants infected with *M. javanica.* Infective juveniles of *S. riobrave* TX, *S. feltiae* SN, and MG-14 but not those of *H. indica* MG-13 were found inside root cortical tissues of *M. javanica*-infected plants. Entomopathogenic nematode antagonism to *M. javanica* on soybean or tomato was insufficient in the present study to provide a consistent level of nematode suppression at the concentrations of infective juveniles applied.

*Key words:* behavior, *Heterorhabditis, Meloidogyne javanica,* root penetration, *Steinernema,* suppression.

Plant-parasitic nematodes (PPNs) account for worldwide yield losses of between 5% and 12% annually in various crops (Barker and Koenning, 1998), with root-knot nematodes, *Meloidogyne* spp., being a major cause of such losses (Sasser and Freckman, 1987). Tropical and sub-tropical climates provide ideal conditions for PPN populations and, consequently, the damage caused by them. Management of PPNs in such climates is a challenge because few control measures are effective (Schmitt and Sipes, 1998). Chemical nematicides can be effective, but they are often highly toxic synthetic pesticides and are available only to commercial growers. These products are limited to use on particular crops and usually must be purchased and applied by a licensed pesticide applicator.

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are obligate parasites of a wide range of insects (Mason and Wright, 1997). The infective stage, known as the infective juvenile (IJ), carries a symbiotic bacterium that is released following infection of the insect host. Steinernematids are associated with *Xenorhabdus* spp., and heterorhabditids are associated with *Photorhabdus* spp. Once infection has occurred, the bacteria multiply and kill the insect host within 48 hours. The bacteria release anti-microbial agents that help prevent colonization of the insect cadaver by contaminating fungi and bacteria, and act as a food source for the developing EPNs (Kaya and Gaugler, 1993). The bacteria also produce stilbene and indole metabolites that are nematicidal to a range of nematode species, including some plant parasites (Hu et al., 1995, 1996, 1999).

EPNs have been used successfully to control a number of compost and soil insect pests (Gouge and Hague, 1995; Klein, 1993; Long et al., 2000; Midturi et al., 1994; Scheepmaker et al., 1994). These same nematodes have shown some potential as antagonists to PPNs. Applications of EPNs to soil have reduced a number of important PPN species, including *Meloidogyne* spp. (Grewal et al., 1997; Ishibashi and Choi, 1991; Ishibashi and Kondo, 1987; Smitley et al., 1992), *Belonolaimus* spp. (Grewal et al., 1997), *Tylenchorhynhcus* spp. (Smitley et al., 1992), and Criconematidae (Grewal et al., 1997; Ishibashi and Kondo, 1987). EPNs tested in laboratory (Bird and Bird, 1986; Grewal et al., 1999) and greenhouse (Gouge et al., 1994) studies, and applied to tomato plants inoculated with *Meloidogyne* spp., reduced nematode penetration and egg production. Perry et al. (1998) reported a reduction of *Globodera rostochiensis* penetration in potato tubers treated with *S. carpocapsae* in greenhouse and outdoor trials.

A number of interactive effects may be involved in suppression of PPNs by EPNs. Bird and Bird (1986) proposed that spatial competition at the mutually attractive root tip may affect root-knot nematode penetration. Ishibashi and Kondo (1986) suggested increased numbers of predators from the application of additional nematode biomass. Grewal et al. (1999) found no suppression of PPNs by living EPNs, but did find that applications of dead *S. feltiae* and *S. riobrave* temporarily suppressed root penetration by *M. incognita.* They argued that allelochemicals released at the death of the nematode affected root penetration by *M. incognita.*

Our objective was to determine the effectiveness of EPNs on the suppression of *M. javanica* for potential use in Hawaiian agriculture. The study analyzed Hawai-

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ian isolates from the island of Maui and two isolates from France and Texas.

## Materials and Methods

*Source and maintenance of nematodes:* Isolates of *Steinernema feltiae* from Hawaii (MG-14) and France (SN), *S. riobrave* from Texas (TX), and a Hawaiian *Heterorhabditis indica* isolate (MG-13) were tested for their efficacy against *Meloidogyne javanica.* Each nematode isolate was cultured at 25 °C in last instar *Galleria mellonella.* Infective juveniles were harvested in modified White traps and washed by sedimentation in three changes of tap water (Dutky *et al.,* 1964). Harvested IJs were stored at 15 °C and used within 3 weeks of emergence. *Meloidogyne javanica* were maintained on 'Pixie' tomato in a greenhouse. *Meloidogyne javanica* eggs were collected by NaOCl extraction (Hussey and Barker, 1973) and hatched on a 25-µm-pore sieve in aerated water at 25 °C. Second-stage juveniles (J2s) of *M. javanica* collected within the first 24 hours were discarded, and those hatching over the following 48 hours were used as inoculum.

*General methodology:* Soybean seedlings were used in each nematode penetration assay and tomato plants for egg production studies. Soybeans were soaked in water for 1 hour, transferred to moist tissue paper, covered, and left to germinate at 25 °C. Seedlings with a 2-cm-long radicle were used for experiments. Seedlings were transplanted to 120-ml plastic beakers filled with 100 $cm^3$ of sterilized soil:sand 1:1 mix screened through a 650-µm-pore sieve. Tomato seeds were germinated in a 10-cm-diam. pot filled with vermiculite and transplanted after 3 weeks into 10-cm-diam. pots containing 450 $cm^3$ of sterilized soil:sand 1:1 mix. Tomato plants were watered daily but were not fertilized. Soybean seedlings were not watered or fertilized over the 6-day period of the penetration experiments. Entomopathogenic nematode and control treatment applications were made the day after seedling transplant in 2 ml of water per plant. *Meloidogyne javanica* J2s were applied in 10 ml of water per plant. Greenhouse experiments were conducted at ambient temperature (21 to 29 °C) and humidity. Roots were stained using the method of Daykin and Hussey (1985) 3 days after *M. javanica* inoculation. *Meloidogyne javanica* eggs were collected after a minimum of 30 days by NaOCl extraction (Hussey and Barker, 1973). All experiments were arranged in a randomized complete block design.

*Statistical analysis:* Data were subjected to square root transformation and analyzed using 3-way ANOVA of treatment, application time or concentration, and replicate (SAS Institute, Cary, NC). Treatment means were separated by Duncan's multiple-range test if treatments were significant by ANOVA at $P \leq 0.05$. Treatments applied in the absence of *M. javanica* were excluded from the analysis for egg production effects. Regression analyses compared nematode penetration with IJ application concentration, and correlation analyses compared EPN and root-knot nematode penetration at each IJ application rate.

*Effect of* Steinernema feltiae *application time on* M. javanica *penetration in soybean:* Two greenhouse experiments were conducted in June and July 1999 to determine the effect of *S. feltiae* on root-knot nematode penetration. Each experiment comprised 6 treatments. In the June experiment, 10,000 IJs *S. feltiae* SN (100 IJs/$cm^3$) or a water treatment was applied 2 days prior, same day, or 2 days after the inoculation of 500 *M. javanica* J2s. In the July experiment, 10,000 IJs *S. feltiae* MG-14 (100 IJs/$cm^3$) or a water treatment were applied 2 days prior, same day, or 2 days after the inoculation of 1,500 *M. javanica* J2s. Each experiment was conducted in 120-m1 plastic beakers. One day after the last treatment application, seedlings were stained with acid fuschin to assess nematode penetration.

*Efficacy of entomopathogenic nematode concentrations on nematode penetration in soybean:* One experiment was conducted in the greenhouse in November 1999 and the second in the laboratory to determine the effect of EPN concentration on root-knot nematode penetration. In the greenhouse experiment, four concentrations of *S. riobrave* TX at 50,000, 10,000, 1,000, and 0 IJs (500-0 IJs/$cm^3$) were applied immediately after the inoculation of 1,000 *M. javanica* J2s to soybean in 120-ml plastic beakers. Each treatment was replicated 10 times. Seedlings were harvested 3 days after *M. javanica* inoculation and stained with acid fuschin to assess nematode penetration.

In the laboratory study, a randomized complete block design consisting of four treatments and seven replicates per treatment was employed. Soybean seedlings with a 2-cm-long radicle were placed in 6-cm-diam. × 7-cm-long glass jars filled with 50 ml of 4% (w/w) moistened sterilized sand (150 to 420-µm grain size). Four concentrations of *S. feltiae* MG-14 at 20,000, 10,000, 5,000, and 0 IJs (200-0 IJs/$cm^3$) were applied after the inoculation of 1,000 *M. javanica* J2s in 1 ml of water to the plants. Jars were covered with a petri dish. The experiment was carried out in a polyurethane box at room temperature (25 ± 1 °C). The experiment was run for 3 days after which the seedlings were removed and the roots stained with acid fuschin to assess nematode penetration.

*Effect of* Heterorhabditis indica *MG-13 application time on* M. javanica *egg production and tomato growth:* Preliminary greenhouse trials demonstrated *S. feltiae* MG-14 to be ineffective in reducing *M. javanica* egg production or affecting plant growth. A factorial experiment using *Heterorhabditis indica* MG-13 was conducted in October 1999 under greenhouse conditions. Ten thousand IJs *Heterorhabditis indica* MG-13 (22 IJs/$cm^3$) or a water treatment were applied 2 days prior, same day, or 2 days after the inoculation of 3,000 *M. javanica* J2s. Each

treatment was replicated 8 times. The number of leaves was counted and the height of the plants measured 30 days after *M. javanica* inoculation. *Meloidogyne javanica* eggs were collected, and root and stem dry weights were determined for total biomass.

*Effect of concentration and dual treatment application on* M. javanica *egg production and tomato growth:* Two concurrent experiments were conducted from January to February 2000 in a greenhouse on 'Pixie' tomato. In the first experiment, three concentrations of *S. riobrave* TX at 20,000, 10,000, and 0 IJs (44-0 IJs/cm$^3$) were applied in the presence or absence of *M. javanica*. In the second experiment, a single or dual application of *S. riobrave* TX and a control treatment of water were applied in the presence or absence of *M. javanica*. Both experiments used eight replicates per treatment. A suspension of 500 J2s in 10 ml of water was inoculated to all plants on day 1. In experiment 1, *S. riobrave* TX was applied in 2 ml of water on day 1. In experiment 2, *S. riobrave* TX was applied at a concentration of 20,000 IJs (44 IJs/cm$^3$) in 2 ml of water at day 1 or two concentrations of 10,000 IJs (22 IJs/cm$^3$) on day 1 and 30. At the time of the second *S. riobrave* TX treatment, all other treatments received a similar volume of water. The 30-day treatment targeted newly emerged *M. javanica* from the initial J2 inoculation. Thirty days after transplant for experiment 1 and 60 days after transplant for experiment 2, the plants were removed, number of leaves counted, and height of the plants measured. *Meloidogyne javanica* eggs and galls were counted, and fresh root weight and root and stem dry weights were determined.

## Results

*Effect of* Steinernema feltiae *application time on* M. javanica *penetration in soybean: S. feltiae* SN ($F = 9.13$; $df = 1, 59$; $P = 0.0046$; Fig. 1A) and *S. feltiae* MG-14 ($F = 6.65$; $df = 1, 59$; $P = 0.0133$; Fig. 1B) application reduced *M. javanica* penetration. Time of treatment application and the interaction between treatment and time were not factors in EPN suppression of *M. javanica* ($P > 0.05$). *Steinernema feltiae* SN and MG-14 IJ were recovered intercellularly within the root, between root cortical cells, but not in the root pericycle or vascular tissue. Entomopathogenic nematode root penetration was not observed in *M. javanica* uninfected soybean.

*Efficacy of entomopathogenic nematode concentrations on nematode penetration in soybean:* The number of *M. javanica* penetrating the root was not affected by the concentration of *S. feltiae* MG-14 or *S. riobrave* TX applied. The number of EPNs penetrating the roots escalated with increasing concentrations of applied IJ; this relationship was described by linear equations for *S. feltiae* MG-14 ($R^2 = 0.4513$; $P < 0.0001$; Fig. 2A) and *S. riobrave* TX ($R^2 = 0.4633$; $P < 0.0001$; Fig. 2B). A negative correlation ($r = -0.9257$, $P = 0.0010$) between *S. feltiae* MG-14 and *M. javanica* root penetration was observed at an application rate of 50,000 IJ per plant (500 IJ/cm$^3$). There was no relationship between *S. feltiae* MG-14 and *M. javanica* root penetration at lower concentrations, or between *S. riobrave* TX and *M. javanica*. No IJ were recovered from *M. javanica* uninfected roots.

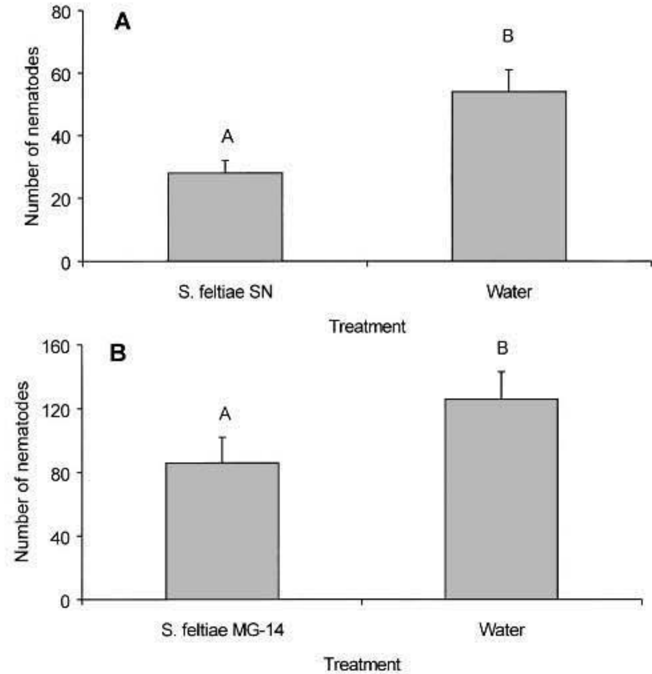


FIG. 1. Mean number and standard error of *Meloidogyne javanica* recovered from soybean roots treated with *Steinernema feltiae* infective juveniles (IJ). Ten thousand *S. feltiae* SN IJ and 500 *M. javanica* juveniles (J2) were applied in experiment A, and 10,000 *S. feltiae* MG-14 and 1,500 *M. javanica* J2 were applied in experiment B. Bars with the same letters are not different among the treatments according to Duncan's multiple range test ($P \leq 0.05$) (A: *S. feltiae* SN; $F = 9.13$; $df = 1, 59$; $P = 0.0046$; B: *S. feltiae* MG-14; $F = 6.65$; $df = 1, 59$, $P = 0.0133$).

*Effect of* Heterorhabditis indica *MG-13 application time on* M. javanica *egg production and tomato growth: Heterorhabditis indica* MG-13 treatment had minimal effect on root-knot nematode egg production in *M. javanica*-infected tomatoes; mean egg production from water-treated plants was 21,257 ± 4,623 per g dry root compared to 16,800 ± 3,851 eggs per g dry root from IJ-treated plants ($P > 0.05$). Leaf number was greater in plants that did not receive *M. javanica* ($F = 17.70$; $df = 3, 83$; $P < 0.0001$; Fig. 3A) but was unaffected by *H. indica* MG-13 treatment of *M. javanica*-inoculated plants ($P > 0.05$). Biomass was lowest in *H. indica* MG-13-treated, root-knot nematode -infected tomatoes ($F = 5.64$; $df = 3, 86$; $P = 0.0016$; Fig. 3B). Biomass of water-treated, root-knot nematode-infected tomato was comparable to non-infected plants. Time of treatment application and the interaction of treatment and time were not factors affecting leaf number or plant biomass ($P > 0.05$). Plants treated 2 days prior to *M. javanica* inoculation were taller than plants treated on the same day, or 2 days after root-knot application ($F = 3.32$; $df = 2, 93$; $P = 0.0418$; Table 1).

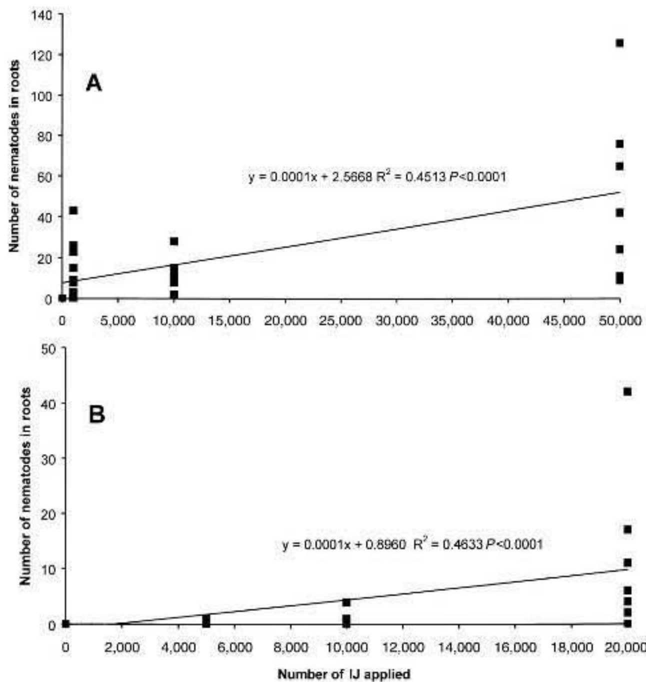


FIG. 2. Relationship between number of *Steinernema* spp. recovered from *Meloidogyne javanica*-infected soybean roots and concentration of *S. feltiae* MG-14 (A) or *S. riobrave* TX (B) applied. Experiment A was conducted in a greenhouse and experiment B in the laboratory.

*Effect of concentration and dual treatment application on* M. javanica *egg production and tomato growth: Steinernema riobrave* TX concentration did not affect egg production or root galling at 30 days post-*M. javanica* application ($P > 0.05$). Higher concentrations of *S. riobrave* ($F = 3.85$; $df = 2, 42$; $P = 0.0318$) and the absence of *M. javanica* ($F = 30.24$; $df = 1, 43$; $P < 0.0001$) increased leaf number in tomato (Table 2). *Meloidogyne javanica* reduced root length ($F = 45.24$; $df = 1, 43$; $P < 0.0001$; Table 2) and plant biomass ($F = 17.82$; $df = 1, 43$; $P = 0.0002$; Table 2) of infected plants, but *S. riobrave* TX at the applied dosage rates had no effect on these parameters ($P > 0.05$). Plant height increased by *S. riobrave* TX treatment in the absence of *M. javanica*, but not in *M. javanica*-infected plants ($F = 4.04$; $df = 5, 42$; $P = 0.0054$; Fig. 4).

Dual application of *S. riobrave* TX had no effect on tomato growth, root-knot nematode egg production, or root galling at 60 days post-*M. javanica* inoculation ($P > 0.05$). *Meloidogyne javanica* reduced leaf number ($F = 9.89$; $df = 1, 45$; $P = 0.0034$), plant height ($F = 37.57$; $df = 1, 45$; $P < 0.0001$), root length ($F = 10.04$; $df = 1, 45$; $P = 0.0032$), and biomass ($F = 7.71$; $df = 1, 45$; $P = 0.0089$) of tomato (Table 3). There was no interaction between *S. riobrave* TX and *M. javanica* on these parameters ($P > 0.05$).

## DISCUSSION

Entomopathogenic nematodes reduced *M. javanica* root penetration in soybean 3 days after J2 inoculation.

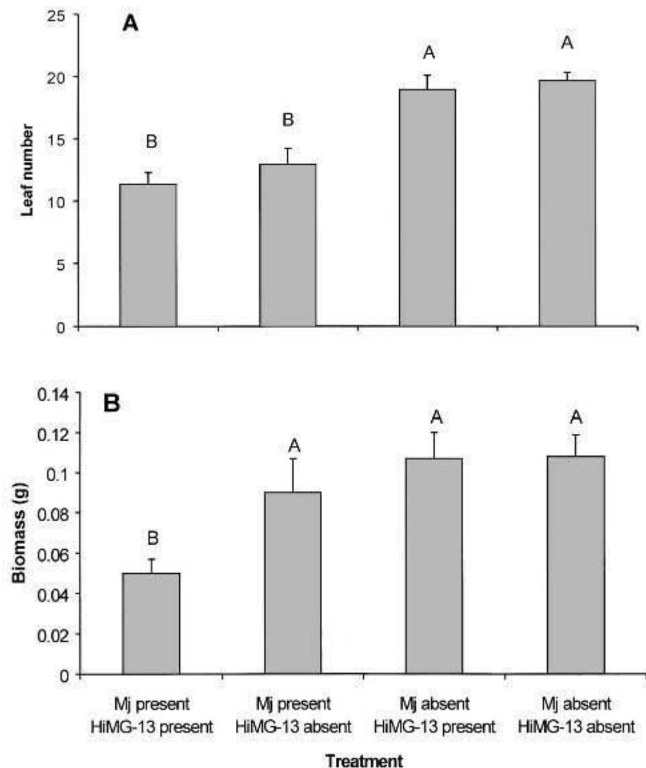


FIG. 3. Mean and standard error of tomato leaf number (A) and biomass (B) of 30-day-old plants treated with *Heterorhabditis indica* MG-13 infective juveniles (IJ). Ten thousand *H. indica* MG-13 IJ and 3,000 *Meloidogyne javanica* juveniles were applied to tomato seedlings. Bars with the same letters are not different among the treatments according to Duncan's multiple-range test ($P \leq 0.05$) (A: Leaf number; $F = 17.70$; $df = 3, 83$; $P < 0.0001$; B: Biomass; $F = 5.64$; $df = 3, 86$; $P = 0.0016$).

No significant effects were observed on *M. javanica* egg production in tomato irrespective of treatment application time or IJ concentration, at 30 or 60 days. Perry et al. (1998) had demonstrated similar short-term effects on PPN suppression by EPNs; suppression of *G. rostochiensis* penetration in potatoes 4 weeks after inoculation by *S. carpocapsae* did not lead to reduced cyst numbers after 16 weeks. Increased plant growth in *M. javanica*-uninfected tomato by *S. riobrave* TX treatment was not observed in root-knot nematode infected plants. In contrast, *M. javanica*-infected tomatoes treated by *H. indica* MG-13 had lower biomass than water-treated, infected tomatoes. There was no dose

TABLE 1. Height of 30-day-old tomato plants.

| Time of treatment application to *M. javanica* inoculation | Plant height (cm)[1] |
|---|---|
| 2 days prior | 10.46 ± 0.29 A[2] |
| Same day | 9.36 ± 0.27 B |
| 2 days after | 9.38 ± 0.29 B |

[1] $F = 3.32$; $df = 2, 93$; $P = 0.0418$.

[2] Values within a column followed by the same letter are not different among the treatments according to Duncan's post-hoc analysis ($P \leq 0.05$). Each treatment had 33 replicates.

TABLE 2. Leaf number, plant height, root length, and biomass of 30-day-old *Steinernema riobrave* TX-treated tomatoes after application of three concentrations of infective juveniles (IJ), with or without 500 *Meloidogyne javanica.*

| Class effect | n[1] | Leaf number[2] | Height (cm)[3] | Root length (cm)[4] | Biomass (g)[5] |
|---|---|---|---|---|---|
| *M. javanica* present | 21 | 9.6 ± 1.1 A[6] | 4.42 ± 0.33 | 13.6 ± 1.9 A[6] | 0.03 ± 0.01 A[6] |
| *M. javanica* absent | 24 | 13.8 ± 1.1 B | 4.55 ± 0.34 | 25.2 ± 2.3 B | 0.09 ± 0.02 B |
| 20,000 IJ | 15 | 13.2 ± 1.5 A[5] | 4.47 ± 0.33 | 22.3 ± 2.7 | 0.06 ± 0.02 |
| 10,000 IJ | 14 | 12.0 ± 1.5 AB | 4.71 ± 0.26 | 18.8 ± 3.4 | 0.07 ± 0.03 |
| 0 IJ | 16 | 10.4 ± 1.0 B | 4.28 ± 0.30 | 18.2 ± 2.7 | 0.05 ± 0.01 |

[1] Number of replicates per treatment class effect.
[2] *M. javanica:* $F = 30.24$; $df = 1, 43$; $P < 0.0001$; IJ: $F = 3.85$; $df = 2, 42$; $P = 0.0318$.
[3] *M. javanica:* $F = 0.25$; $df = 1, 43$; $P = 0.6210$; IJ: $F = 1.47$; $df = 2, 42$; $P = 0.2442$.
[4] *M. javanica:* $F = 45.24$; $df = 1, 43$; $P < 0.0001$; IJ: $F = 2.20$; $df = 2, 42$; $P = 0.1278$.
[5] *M. javanica:* $F = 17.82$; $df = 1, 43$; $P = 0.0002$; IJ: $F = 1.07$; $df = 2, 42$; $P = 0.3540$.
[6] Values within a column followed by the same letter are not different among the treatments according to Duncan's post-hoc analysis ($P \leq 0.05$).

response effect on *M. javanica* root penetration by *S. feltiae* SN or *S. riobrave* TX, or effect on egg production by *S. riobrave* TX at the concentrations tested.

In our study, we have documented the behavior of root penetration by EPN IJ. Of the nematodes tested, this phenomenon has been observed for *S. feltiae, S. riobrave* and *S. glaseri* but not for *S. carpocapsae* or *H. indica.* This phenomenon was found to occur in the plant families Fabeacae and Cruciferacea but not those of the Graminacea, Solanaceae, or Apiaceae (unpubl.). EPNs were found intercellularly, in the root cortex or at the site of lateral root branchings. It may be that IJ followed penetrating root-knot nematodes. Only isolated instances of individual IJ were found in healthy (non-*M. javanica* infected) roots (unpubl.). Bird and Bird (1986) and Ishibashi and Choi (1991) have demonstrated root attraction by EPNs, and it may be from this attraction that IJ penetrated into the root. Westcott and Barker (1976) previously reported penetration of root tissue by a nonstylet-bearing nematode. In their study, the microbivorous nematode, *Acrobeloides buetschlii,* invaded the nodular tissue and, to a lesser extent, the cortex of *Pisum sativum* roots. *Acrobeloides buetschlii* inhibited $N_2$ fixation by feeding on the *Rhizobium* in the nodules, but this did not affect growth in 8- and 10-week-old plants. Increased concentrations of applied IJ resulted in greater numbers of IJ entering the root. This behavior may have resulted in increased root-knot penetration at higher IJ application levels as the potential for negative impacts by IJ penetration on the root increase.

The quantities of EPNs used in this study were high relative to the economic rate for insect control. A rate of 7.5 IJ/cm$^3$ of media (Lewis et al., 2001) or 15,000

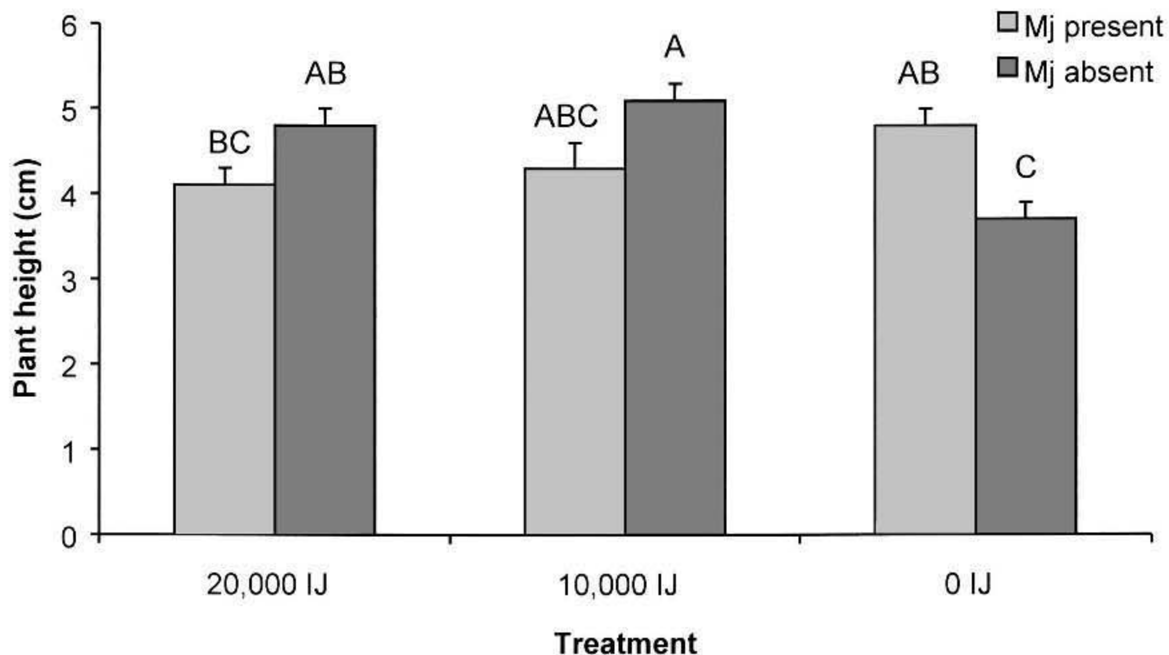


FIG. 4. Mean and standard error of tomato height of 30-day-old plants treated with three concentrations of *Steinernema riobrave* TX infective juveniles (IJ). Twenty thousand, 10,000, or zero *S. riobrave* TX IJ and 500 *Meloidogyne javanica* juveniles were applied to tomato seedlings. Bars with the same letters are not different among the treatments according to Duncan's multiple-range test ($P \leq 0.05$) ($F = 4.04$; $df = 5, 42$; $P = 0.0054$).

TABLE 3. Leaf number, plant height, root length, and total biomass of 60-day-old *Steinernema riobrave* TX-treated tomatoes.

| Class effect | n[1] | Leaf number[2] | Height (cm)[3] | Root length (cm)[4] | Biomass (g)[5] |
|---|---|---|---|---|---|
| *M. javanica* present | 23 | 15.6 ± 2.0 A[6] | 5.40 ± 0.49 A[6] | 21.4 ± 3.3 A[6] | 0.66 ± 0.15 A[6] |
| *M. javanica* absent | 24 | 19.8 ± 1.9 B | 7.46 ± 0.42 B | 30.6 ± 3.4 B | 1.01 ± 0.15 B |
| Two IJ applications | 15 | 18.1 ± 1.7 | 6.72 ± 0.40 | 28.2 ± 3.8 | 0.89 ± 0.13 |
| One IJ application | 16 | 18.8 ± 1.8 | 6.50 ± 0.53 | 25.8 ± 4.6 | 0.96 ± 0.18 |
| No IJ applied | 16 | 18.5 ± 1.9 | 6.61 ± 0.61 | 24.6 ± 3.7 | 0.90 ± 0.19 |

[1] Number of replicates per treatment class effect.
[2] *M. javanica*: $F = 9.89$; $df = 1, 45$; $P = 0.0034$; IJ: $F = 0.24$; $df = 2, 44$; $P = 0.7892$.
[3] *M. javanica*: $F = 37.57$; $df = 1, 45$; $P < 0.0001$; IJ: $F = 0.80$; $df = 2, 44$; $P = 0.4567$.
[4] *M. javanica*: $F = 10.04$; $df = 1, 45$; $P = 0.0032$; IJ: $F = 0.65$; $df = 2, 44$; $P = 0.5263$.
[5] *M. javanica*: $F = 7.71$; $df = 1, 45$; $P = 0.0089$; IJ: $F = 0.05$; $df = 2, 44$; $P = 0.9530$.
[6] Values within a column followed by the same letter are not different among the treatments according to Duncan's post-hoc analysis ($P \leq 0.05$).

IJ/10-cm-diam. pot (Perry et al., 1998) approximates a field rate considered sufficient for insect control (Georgis, 1990). A single application of 10,000 *S. feltiae* MG-14 or *S. feltiae* SN per plant reduced *M. javanica* penetration in soybean after 3 days, but was ineffective at reducing egg production in tomato after 30 days (unpubl.). Lewis et al. (2001) reported *S. feltiae* applied at 7.5 IJ/cm$^3$ suppressed *M. incognita* egg production, egg hatch, and galling in tomato 30 days after nematode inoculation, but was ineffective at rates of 20 and 100 IJ/cm$^3$. The failure of 10,000 EPN IJ to reduce *M. javanica* egg production may, in part, be attributed to differences in the density of IJ per volume media in the 10-cm-diam. pots, compared to the volume of media used in the penetration experiments. However, there was no effect on *M. javanica* penetration in soybean by *S. feltiae* SN applied at 0 to 200 IJ/cm$^3$ or *S. riobrave* TX applied at 0 to 500 IJ/cm$^3$, suggesting that application rate is not the primary factor responsible for PPN suppression by IJ.

The interaction between root-knot nematodes and the entomopathogenic nematode bacterium complex is incompletely understood. Grewal et al. (1999) found temporary suppression of *M. incognita* penetration when dead IJ of *S. riobrave* or *S. feltiae* were applied, but found no effect when living nematodes were used. They suggested that allelochemicals released upon the death of inundatively released IJ could contribute to plant-parasitic nematode suppression in studies where living nematodes were used. The nematode symbiotic bacterium, *Xenorhabdus*, produces metabolites that are toxic to plant-parasitic nematodes (Hu et al., 1999), repulse or immobilize *Meloidogyne* sp. J2 (Grewal et al., 1999), and inhibit egg hatch (Grewal et al., 1999; Samaliev et al., 2000). However, *Xenorhabdus* has not been reported to exist long in the soil in the absence of its nematode host (Burnell and Stock, 2000). Root-penetrating EPNs may release small quantities of nematode antagonistic metabolites upon their death, and the death of the bacterial symbiont, that disperse through neighboring root tissue, protecting the root from further penetration by plant-parasitic nematodes or antagonize plant-parasitic nematodes present in the root. Such a localized effect would confer only limited protection to the plant. This may explain the variation in the efficacy of entomopathogenic nematode treatments between the experiments. Entomopathogenic nematode antagonism to *M. javanica* was found to be insufficient in the present study to provide a consistent level of plant-parasitic nematode suppression at the dosages applied. Further research is needed to elucidate the relationships involved.

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