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# Effect of Entomopathogenic Nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on *Meloidogyne mayaguensis* Rammah and Hirschmann (Tylenchida: Meloidoginidae) Infection in Tomato Plants

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*Abstract:* Some studies suggest that entomopathogenic nematodes (EPN) affect plant-parasitic nematode populations. Here, the effects of live and dead IJ of *Heterorhabditis bacteriophora* JPM4, *H. baujardi* LPP7, *Steinernema feltiae* SN and *S. carpocapsae* All were evaluated against eggs and J2 of the plant-parasitic nematode *Meloidogyne mayaguensis*. According to treatment, 100 IJ were applied with 350 eggs, 350 J2 or 175 eggs + 175 J2 to tomato plants. Bioassays were conducted in March to May and repeated in September to November 2005. Both experiments lasted 9 weeks, and the variable evaluated was number of galls per plant. When eggs were used for infections in the first trial, plants exhibited lower gall number compared to control when live and dead *H. baujardi* IJ and live *S. feltiae* IJ were added (9.7, 4.5, 7.3 and 85.7 galls, respectively). In the second trial, live *S. feltiae* and *S. carpocapasae* IJ influenced gall formation compared to control (14.33, 14.57 and 168.02 galls, respectively). When J2 were used for infections, plants with live *H. baujardi* IJ presented less galls when compared to control in both trials (38.3 and 355.7 galls in the first trial and 145.2 and 326.2 in the second one, respectively). Infection with a mixture of J2 and eggs resulted in fewer galls than when live *S. feltiae* IJ were present in both trials, compared to control (38.3 and 44.2 galls vs. 275.3 and 192.2 galls, respectively). We conclude that *H. baujardi* and *S. feltiae* apparently may be inhibiting egg hatching and J2 infection.

*Key words:* Entomopathogenic nematodes, nematode-nematode interaction, biological control, plant-parasitic nematode, *Meloidogyne mayaguensis*

Entomopathogenic nematodes (EPN) in the families Steinernematidae and Heterorhabditidae infect and kill insects with the aid of symbiotic bacteria carried in their intestines (*Steinernema* spp. carry *Xenorhabdus* spp., while *Heterorhabditis* spp. carry *Photorhabdus* spp.). Over the past 25 years, consideration of EPN as exclusively biological control agents of insect pests has rapidly expanded. Alternative studies in the last decade shows positive and negative interations with other soil pathogens, indicating the potential of some EPN species to supress plant-parasitic nematodes (PPN). Other studies have shown that EPN and their associated bacteria possibly may interfere with the infection and reproduction of some PPN (Grewal et al., 1999). However, their application does not always reduce PPN populations, and the outcomes of their interactions vary according to EPN species, PPN species, the crop receiving the application and the method used to evaluate the impact on PPN (Lewis and Grewal, 2006). Some nematologists are interested in determining this interaction between EPN and PPN because of the potential for PPN management. Antagonistic interactions between EPN and PPN were first shown by Bird and Bird (1986), who showed that a reduction of the infection of *Meloidogyne javanica* (Treub) (Tylenchida: Meloidogynidae) in tomato plants was caused by *Steinernema glaseri* (Steiner) in greenhouse pot tests. Similarly, *S. glaseri* DD-136 and *S. feltiae* (Filipjev) reduced populations of PPN and increased the populations of bacteriophagous rhabditids nematodes (Ishibashi and Kondo, 1986). Lewis and Grewal (2006) reviewed the literature describing these interactions and have found that, while antagonism exists in many cases, the amount of PPN reduction caused is rarely to a level that would be considered acceptable in most agricultural settings.

Lewis et al. (2001) showed that *S. feltiae* and its symbiotic bacterium, *X. bovienii*, affected the infectivity of *M. incognita* in tomato roots. On the other hand, the effect of *S. feltiae* applications on second-stage juveniles (J2) and on the penetration of these in roots were not evaluated. Recent studies performed by Shapiro-Ilan et al. (2006) showed that the number of *M. partityla* (Kleynhans) egg masses was lower compared to control when *S. riobrave* Cabanillas, Poinar and Raulston (7–12) was applied in walnut seedlings. In laboratory experiments, Grewal et al. (1999) observed that penetration of *M. incognita* in tomato seedlings was suppressed by the application of dead IJ killed by thermal shock, but not with live IJ application. Later, Jagdale et al. (2002) observed that application of both dead and live IJ of *S. carpocapsae* reduced the PPN population in boxwood. Perez and Lewis (2004) evaluated the effect of the EPN *S. feltiae*, *S. riobrave* and *H. bacteriophora* Poinar against *Meloidogyne* spp. infecting tomato and peanut seedlings and found that *S. feltiae* and S. *riobrave* reduced *M. hapla* penetration into roots.

The plant-parasitic nematode *M. mayaguensis* Rammah & Hirschmann is an important pest in guava. This nematode reduces fruit production drastically and eventually causes death of full-grown trees in a short period of time (Moreira and Henriques, 2001). *Meloidogyne mayaguensis* has been reported in different African tropical countries such as Mali, Senegal, South Africa, the Côte d'Ivoire and Burkina Fasso (Carneiro et al., 2001). The new world distribution of this species includes Brazil, Trinidad and Tobago, Puerto Rico, Cuba, Martinica and the US in different crops (Decker and

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Rodriguez, 1989; Carneiro et al., 2001; Brito et al., 2003). The first report of this nematode in Brazil was in guava by Moura and Moura in 1989 in the northeast part of this country. Lima et al. (2005) registered the presence of *M. mayaguensis* in a native tropical forest, which suggests that this species could be native to tropical regions.

Our objective in this study was to evaluate the effect of the EPN/bacterial complex in the infection of different stages of *M. mayaguensis*, initially determining the reduction of number of galls formed. A possible reduction will establish the direct effect of EPN in the infection of all or determinate stages of PPN as egg, J2 or their combination. The heat treatment was used to test the hypothesis that compounds from the nematode-bacteria complex would affect the plant-parasitic nematode infection.

## Materials and Methods

Experiments were performed in a greenhouse at Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) in Campos dos Goytacazes-RJ, Brazil. Bioassays were conducted twice, once in March to May 2005 and again in September to November 2005, corresponding to fall and spring, respectively. Both experiments lasted 9 wk.

*Entomopathogenic Nematode cultures:* Four EPN were used in the experiments: two Brazilian *Heterorhabditis* strains, *H. bacteriophora* JPM4 and *H. baujardi* LPP7, and two exotic steinernematid strains, *S. feltiae* SN and *S. carpocapsae* All. The Brazilian nematodes were chosen for being adapted to tropical temperatures. The exotic nematodes herein used were cited in other studies evaluating plant-parasitic nematodes suppresion.

Nematodes were cultured in *Galleria mellonella* (Lepidoptera: Pyralidae) larvae at 25°C according to procedures described by Woodring and Kaya (1988). *Meloidogyne mayaguensis* was originally collected from infested guava roots from São João da Barra (RJ, Brazil). Nematodes were cultured on the tomato variety Santa Cruz Kada Gigante (Top Seed, Petropolis-RJ-Brazil), since they germinate homogeneously and faster than guava seeds. For the "dead IJ treatment," the EPN were killed by heating them in a microwave oven at 1,000 W for 2 min. After standing for 6 hr at 25 ± 2°C, IJ mortality was verified in a Peters slide under stereomicroscope. The nematodes were considered dead when they were in straight position.

*Plant-parasitic nematode culture: M. mayaguensis*-infected guava roots were collected from orchards in the municipality of São João da Barra, RJ, Brazil. To establish pure nematode cultures, tomato seedlings cultivated in autoclaved soil were inoculated with egg masses extracted from mature females. The recovered females had their "M2" electrophoretic esterase phenotype determined according to Carneiro and Almeida (2001) and Carneiro et al. (2001).

*Meloidogyne mayaguensis inoculum:* To obtain nematode eggs, about 20 g of infected tomato roots were shaken in flasks with 200 ml 0.6% sodium hypochlorite aqueous solution for about 2 min. The resulting suspension was poured onto a set of two precision screens (60 and 500 mesh), and washed with tap water. The eggs retained in the latter screen were collected and counted. To obtain active J2, the egg suspension was poured onto a coarse screen covered with tissue paper and nested into petri dishes (15-cm diam.). The petri dishes were incubated under agitation for 24 hr at 25°C to stimulate egg hatching. The hatched J2 which had passed through the tissue paper and the screen were collected and counted.

*Experimental parameters:* The experimental arenas were plastic germination tubes (3 × 15 cm) containing autoclaved soil from an infested guava orchard in Cachoeiras de Macacu, RJ, Brazil (76% sand, 6% silt, 18% clay; 20% dried cow manure collected directly from pastures). Water-soaked cotton was at the bottom of the germination tube in order to absorb water and keep the substrate humid without any contamination. The tip of each germination tube was immersed in an individual plastic cup containing distilled water and maintained in a greenhouse at 30 ± 2°C. Tomato seedlings approximately 12 cm high with three pairs of leaves were transplanted to these germination tubes. Infective juveniles of the four EPN species in two different conditions (live and dead) plus a control (with PPN application only) were applied as treatments with eight replicates each, according to the following bioassays:

*Bioassay 1. Application of IJ and M. mayaguensis eggs:* Aliquots of 350 *M. mayaguensis* eggs were applied to germination tubes with a micropipette. Immediately afterwards, 25 IJ/cm$^2$ of each EPN strain, live or dead, (100 IJ) were applied topically to germination tubes. Both IJ and egg numbers applied were based on previous studies on the nematode/bacteria complex and plant-parasitic nematodes.

*Bioassay 2. Application of IJ and M. mayaguensis infective J2:* Repeating the same inoculum size as in Bioassay 1, aliquots of 350 J2 of *M. mayaguensis* were applied to germination tubes with a micropipette. Subsequently, the same number of EPN IJ as in Bioassay 1 was applied to each germination tube.

*Bioassay 3. Application of IJ and M. mayaguensis infective J2 plus eggs:* Mixtures of 175 eggs plus 175 J2 of *M. mayaguensis* were applied to germination tubes with a micropipette. Subsequently, the same number of EPN IJ as in Bioassay 1 were applied to each germination tube. In all bioassays, after 9 wk, plant height, number of galls and dry and wet root weight for each plant were measured. The control treatment was the application of *M. mayaguensis* eggs or J2 acording to the bioassay without IJ application.

*Data analysis:* Each experiment contained eight rep-

licates (germination tubes) for each treatment, arranged in a randomized block design. The two experiments were analyzed separately, but the total number of galls were compared between the trials. Analysis of variance and Duncan's test ($P \leq 0.05$) were performed using System for Statistical Analyses (SAEG) v9.0 (UFV-MG-Brazil).

## Results

Among all the variables evaluated (plant height, number of galls and dry and wet root weight) for each plant, the number of galls was the one that showed consistent results throughout all treatments and trials evaluated in relation to infection reduction of eggs, J2 and the combination of these stages of *M. mayaguensis*. The average air temperature in the greenhouse during the first trial was 25.6°C and in the second trial 22.3°C.

*Bioassay 1. Effect of IJ on M. mayaguensis egg infection:* In the first trial, number of galls was significantly affected by all treatments, with the exception of dead *S. feltiae* SN. The treatments that most affected gall formation were live and dead *H. baujardi* LPP7 and live *S. feltiae* SN compared to control (9.7 ± 3.2, 4.5 ± 2.1, 7.3 ± 2.9 vs. 85.7 ± 29.4 galls, respectively) (Fig. 1A) ($F = 5.24$; $P \leq 0.05$). In the second trial, all EPN treatments differed from the control. Dead and live *S. carpocapsae* All and live *S. feltiae* SN had fewer galls compared to control (14.6 ± 4.5, 18.3 ± 5.4, 14.3 ± 2.2 vs. 178.0 ± 18.2, respectively) ($F = 39.03$; $P \leq 0.05$) (Fig. 1B). Differences were detected in the variable number of galls between the first and second trial ($F = 13.65$; $P \leq 0.05$). In trial 1, the average number of galls in all treatments was lower than the second trial (Fig. 1).

*Bioassay 2. Effect of IJ in M. mayaguensis J2 infection:* In the first trial, only treatments live *H. baujardi* LPP7, live *S. carpocapsae* All and live *S. feltiae* SN were different from the control (162.8 ± 18.3, 180.0 ± 29.6, 232.2 ± 38.9 vs. 365.7 ± 26.5 galls) ($F = 5.09$; $P \leq 0.05$) (Fig. 2A). While in the second trial, live *H. baujardi* LPP7, live *H. bacteriophora* JPM4 and live *S. feltiae* SN were the ones that presented lower number of galls compared to control (145.2 ± 16.4, 160.5 ± 26.4, 207.1 ± 34.8 vs. 326.2 ± 23.6 galls, respectively) ($F = 4.97$; $P \leq 0.05$) (Fig. 2B). Differences were detected in the variable number of galls between the first and second trials ($F = 4.57$, $P = 0.0355$). In the first trial, the average number of galls was slightly greater than the second trial in some treatments and it was similar in the controls (Fig. 2).

*Bioassay 3: Effect of IJ in M. mayaguensis eggs and J2 infection:* In the first trial, the number of galls was significantly affected by all treatments ($F = 5.34$; $P \leq 0.05$), although live *S. feltiae* SN and live *H. baujardi* LPP7 were the treatments with lower number of galls compared to

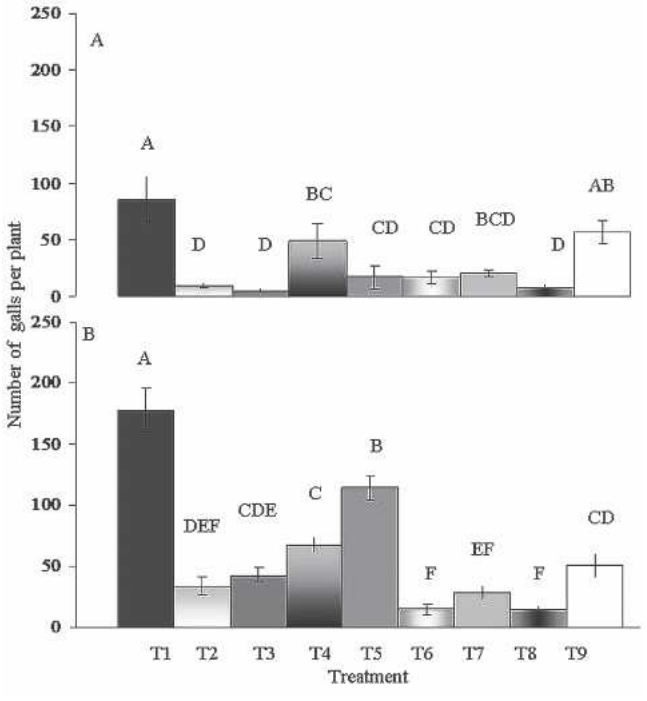


FIG. 1. Average number of galls recovered from tomato roots after *M. mayaguensis* egg and EPN application. A: Trial 1. B: Trial 2. IJ L = live infective juveniles; IJ D = dead infective juveniles. Treatments: T1: Control; T2: *Heterorhabditis baujardi* LPP7/IJ L; T3: *H. baujardi*/IJ D; T4: *H. bacteriophora* JPM4/IJ L; T5: *H. bacteriophora* JPM4/IJ D; T6: *S. carpocapsae* All/IJ L; T7: *Steinernema carpocapsae* All/IJ D; T8: *S. feltiae* SN/IJ L; T9: *S. feltiae* SN/IJ D. Columns with the same letter are not significantly different at 5%.

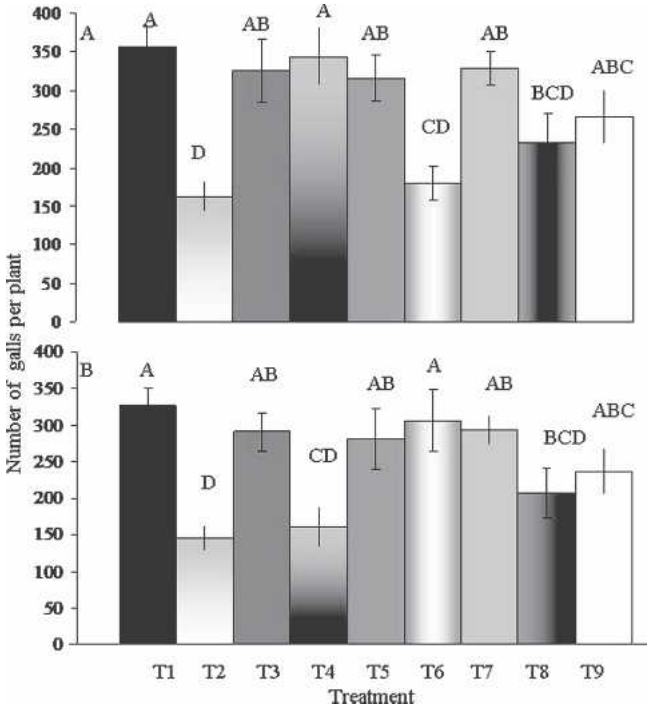


FIG. 2. Average number of galls recovered from tomato roots after *M. mayaguensis* J2 and EPN species application. A: Trial 1. B: Trial 2. IJ L = live infective juveniles; IJa D = dead infective juveniles. Treatments: T1: Control; T2: *Heterorhabditis baujardi* LPP7/IJ L; T3: *H. baujardi*/IJ D; T4: *H. bacteriophora* JPM4/IJ L; T5: *H. bacteriophora* JPM4/IJ D; T6: *Steinernema carpocapsae* All/IJ L; T7: *S. carpocapsae* All/IJ D; T8: *S. feltiae* SN/IJ L; T9: *S. feltiae* SN/IJ D. Columns with the same letter are not significantly different at 5%.

control (38.3 ± 10.8, 63.23 ± 13.8 vs. 275.3 ± 36.4 galls, respectively) ($F = 4.92$; $P \leq 0.05$) (Fig. 3A). A similar effect appeared in the second trial, in which live *S. feltiae* SN and live *H. baujardi* LPP7 had significantly fewer galls than the control (44.2 ± 4.6 and 68.3 ± 4.6 vs. 192.2 ± 4.8 galls, respectively) ($F = 21.61$; $P \leq 0.05$) (Fig. 3B). Differences were detected in the variable number of galls between the first and second trials ($F = 4.76$, $P = 0.0318$). In trial 1, the average number of galls in all treatments was similar to the second trial.

## Discussion

In this experiment, the application of either dead or live IJ to reduce *M. mayaguensis* infection expressed by gall formation was considered and assessed. Either dead or live IJ of some EPN species reduced gall formation when they were applied to eggs, and some live ones caused reduction in J2 infection. The results in infection when IJ were applied to eggs + J2 evidenced the main effect on eggs.

Shapiro-Ilan et al. (2006), after testing different strains, stated that the number of egg masses of *M. partityla* per pecan plant varied, and it was lower when *S. riobrave* 7–12 was applied. Our work confirms that EPN strains vary in terms of affecting PPN, and we point out *H. baujardi* LPP7 and *S. feltiae* SN as the most effective ones.

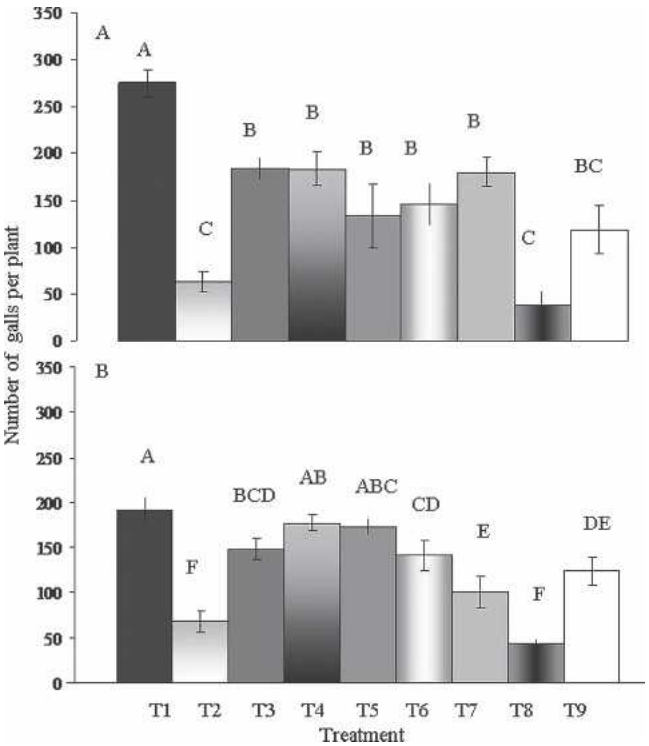


Fig. 3. Average number of galls recovered from tomato roots after *M. mayaguensis* J2 + egg and EPN species application. A: Trial 1. B: Trial 2. IJ L = live infective juveniles; IJ D = dead infective juveniles. Treatments: T1: Control; T2: *Heterorhabditis baujardi* LPP7/IJ L; T3: *H. baujardi*/IJ D; T4: *H. bacteriophora* JPM4/IJ L; T5: *H. bacteriophora* JPM4/IJ D; T6: *Steinernema carpocapsae* All/IJ L; T7: *S. carpocapsae* All/IJ D; T8: *S. feltiae* SN/IJ L; T9: *S. feltiae* SN/IJ D. Columns with the same letter are not significantly different at 5%.

According to Grewal et al. (1999), Hu et al. (1999) and Jagdale et al. (2002), alellelopathic substances produced by live or dead IJ may be toxic and/or repellent to PPN, thus reducing their population density. EPN-associated bacteria, *Xenorhabdus* spp. or *Photorhabdus* spp., produce endotoxins composed of lipopolysacarides that are toxic and could kill or affect in another way the evaluated stages (Dunphy and Webster, 1988). In this study, the dead IJ caused infection reduction when both eggs and J2 were used. Jagdale et al. (2002) stated that live and dead *S. carpocapsae* IJ reduced PPN populations 15 and 30 days after the application by more than 50%. They also suggested a chemical disturbance instead of a physical one. Our study added more evidences that bacterial thermo-resistant compound-like-toxins are responsible for inhibiting egg hatching or J2 penetration.

According to Pérez and Lewis (2004), application of *S. feltiae* to tomatoes two weeks before the release of *M. hapla* eggs and juveniles suppressed the penetration of PPN in tomato roots. Similarly, application of *S. glaseri* and *H. bacteriophora* in high concentrations diminished the penetration of *M. incognita* juveniles. They suggested that a physical disturbance (i.e., behavior or orientation) caused by the IJ could have affected J2 root penetration. Our data do not show great interference of EPN in treatment with J2, so the hypotheses of physical disturbance and direct death caused by the compound-like-toxins must be further tested.

Our data agree with Lewis et al. (2001), showing significant effect of EPN in egg hatching and gall reduction. In our study, the reduction in galling caused by IJ was less in treatments with J2 than in treatments in which eggs were used. The higher number of galls found in the control treatment in all trials suggests the direct effect of IJ in egg hatching and consequently gall formation. Therefore, it is possible that bacterial thermo-resistant compounds like toxins or nematode components were inhibiting egg hatching.

The infection reduction found in the bioassay with the mixture of *M. mayaguensis* eggs and J2, compared to the bioassay with J2 only, confirmed the main negative effect of EPN on eggs. If the bioassay with eggs and J2 alone is compared to that with the mixture stages, we notice an intermediate value in gall formation in the mixture bioassay. Our data suggest that IJ act differently on PPN stages, the egg stage being the most affected one. This is the first work that confirm this fact.

It is possible that temperature could have affected the behavior of some EPN or even *M. mayaguesis* J2 and eggs during the trials. The seasons in which the experiments were set were different, as was the average temperature, and nematode behavior and physiology are direcly influenced by temperature (Lee, 2002). Although the numbers were different, the consistently

lower number of galls in all trials reflects the EPN interference.

The hypothesis of the existence of a thermo-resistant bacterial or nematode component from dead juveniles could explain the infection reduction of *M. mayaguensis* even when IJ were applied dead. Further specific studies could test that hypothesis.

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