Potential of entomopathogenic nematodes (Rhabditida: Heterorhabditidae) to control Mediterranean fruit fly (Diptera: Tephritidae) soil stages

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HIGHLIGHTS

• Two native nematode tropical strains were more virulent than exotic strains to *Ceratitis capitata* larvae under laboratory conditions.
• *Heterorhabditis baujardi* LPP7 killed 90% of *C. capitata* larvae under laboratory conditions.
• *H. baujardi* LPP7 killed more than 80% pupae in the laboratory.
• Larval and pupal mortality were highest at 28 °C using *H. baujardi* LPP7.
• In the field, larval mortality using *H. baujardi* LPP7 was on average 87.43%.

ABSTRACT: Entomopathogenic nematodes are presented as an additional tool for *Ceratitis capitata* control, focusing on the soil stages. This study showed the importance of testing native tropical strains adapted to tropical weather conditions. We tested eight strains, six of them native to Brazil, in sand columns against 3rd instar larvae (L3) of *C. capitata*. The highest mean mortality values were reached by *Heterorhabditis* sp. LPP17, *Heterorhabditis* sp. LPP14 and *H. baujardi* LPP7 with 98.5, 95.5 and 90% mortality, respectively. These three tropical strains were also tested against *C. capitata* L3 at six different concentrations, and results show that mortality increases with IJs concentration. *H. baujardi* LPP7, at 45 IJs/3 larvae or 237 IJs/cm$^2$, caused 81.5% mortality, and 100% at 105 IJs/3 larvae or 552.6 IJs/cm$^2$.

On the other hand, pupal mortality above 80% was only observed at 155 IJs/3 pupae or 816 IJs/cm$^2$ or 197 IJs/cm$^3$; a concentration three times higher than to kill larvae. When four different temperatures were tested against L3 and one-day-old pupae, *H. baujardi* LPP7 performed better at 24 and 28°C. In a guava orchard, *H. baujardi* LPP7 was tested against *C. capitata* L3, and the average larval mortality in treated trees was significantly different in relation to the control (58.6% and 7.7%, respectively). When the experiment was repeated, the same positive tendency was reached (87.4% and 30.4%, respectively). We conclude that *H. baujardi* LPP7, as a native tropical strain, is well adapted to field conditions tested and may be one more tool for controlling *C. capitata* soil stages, as part of an integrated pest management of pests in guava. This study showed the importance of testing native tropical strains adapted to tropical weather conditions.

Keywords: biological control, Medfly, guava, Ceratitis capitata, tropical strains

Cite as

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INTRODUCTION

Brazilian fruit production is considered one of the largest in the world, related to yield and planted area. Brazil ranks seventh in the world, producing around 838 MT/year\(^{[9]}\). However, there are still many problems in producing and exporting fresh fruit, since the international market demands high fruit quality, with low pesticide levels. Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), the Mediterranean fruit fly or Medfly, is widely distributed worldwide; combined with its ability to tolerate cooler climates better than most other species of tropical fruit flies, and its broad range of hosts, it is ranked first among the economically important fruit fly species. Once the fly is established, eradication efforts may be extremely difficult and expensive. As well as reducing crop yield, infested areas have the additional expense of control measures and costly sorting processes for both fresh and processed fruit. Moreover, some countries maintain quarantine against the Medfly, including the U.S.A\(^{[5]}\). Recently, the importance of this species increased with the evidence of its ability to transmit the bacterium Escherichia coli through commercial fruits\(^{[5]}\). Other species such as Ceratitis anona (Graham), C. cosyra (Walker), C. fasciventris (Bezzi), and C. rosa Karsch have also been reported on guava fruits\(^{[6]}\).

The length of time required for the Medfly to complete its life cycle under tropical conditions is 21-30 days\(^{[7]}\). Eggs are deposited under the skin of fruit that is just beginning to ripen, often in an area where some break in the skin has occurred. Several females may use the same deposition hole, with 75 or more eggs clustered in one spot. Each female will deposit 2 to 10 eggs. Eggs hatch in 1.5-3 days in temperatures around 25°C. Larvae pass through three instars, and the larval stage may last as few as 6-10 days or as many as 14-26 days depending on temperature and host. By the time the larvae have fully developed and are ready to pupate, the fruit has usually dropped to the ground, where pupation occurs, and pupae take around 10-15 days until becoming adult\(^{[8]}\).

The application of insecticides has been the control method commonly used, but its rejection by society is increasing, due to effects on non-target organisms, water contamination, residues found in fruit and vegetables and development of resistance among insect pests\(^{[9]}\). In this context, biological control is becoming a useful alternative, as part of an integrated pest management (IPM) strategy. Entomopathogenic nematodes (EPNs) play an important role as biocontrollers\(^{[10]}\); additionally, EPNs focus on the soil stages, which are difficult to control with chemical pesticides.

Entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) are obligate insect pathogens that do not cause any damage to plants or animals. The infective juvenile (IJ) is the soil-dwelling, non-feeding, third-stage juvenile protected by the second-stage cuticle. Once in contact with the insect, the IJs invade the insect via natural openings or directly through the cuticle (only heterorhabditids). Subsequently, they release their symbiotic bacteria into the insect’s hemocoel to resume their development\(^{[11]}\). The bacteria then multiply, producing endotoxins that kill the insect in 24-48 hours by septicemia\(^{[12]}\). The bacteria provide nourishment for the nematodes, which develop, molt and become adults. In both families, after two or three generations within the host, when the food is depleted, IJs emerge from the cadaver to seek new hosts\(^{[13]}\).

Different studies have been performed using EPNs against different Medfly stages, and the results are listed in Table 1. However, there is no consensus among studies regarding which stage is the most susceptible, and researchers are still testing larvae and pupae. Also, there is a marked absence of experiments performed in the field and using tropical strains. Therefore, the goal of this study was to evaluate the potential of different tropical and commercially used EPNs (Table 2), against 3rd instar larvae (L3) and one-day-old pupae of C. capitata in the laboratory under different concentrations and temperatures. In addition, we tested Heterorhabditis baujardi Phan, Subbotin, Nguyen & Moens LPP7 against larvae in a commercial guava orchard. We hypothesize that tropical strains are best adapted to search, find and infect the larval stage before they become pupae, lowering the C. capitata adult population.

MATERIAL AND METHODS

Source of Ceratitis capitata

Ceratitis capitata was obtained from laboratory colonies maintained on an artificial diet used for larvae rearing, consisting of sugar, agar, yeast, Nipagin\(^{[14]}\), citric acid, and water\(^{[21]}\). Adults were confined in a wooden cage (50 x 50 x 50 cm) lined with nylon screen mesh (70%) and fed on distilled water and honey 20% solution. The insects were kept in an acclimatized room at 27 ± 5 °C and 12 h photophase.
The insect population was observed daily, and only 3\textsuperscript{rd} instar larvae (L3 or prepupae) and one-day-old pupae were used in the experiments. The fully grown 3\textsuperscript{rd} larval stage was 8 mm long, with a fully opaque white body. This stage was easy differentiable since it has a “jumping” habit. Pupae were cylindrical, 3 to 4 mm long, and dark reddish brown. We picked the pupae just after total cuticle sclerotization.

Table 1. Data from studies using entomopathogenic nematodes against different *Ceratitis capitata* stages.

<table>
<thead>
<tr>
<th>Nematode strain/species</th>
<th>Insect stage</th>
<th>Number of infective juveniles applied</th>
<th>Location of study</th>
<th>Average % mortality</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Heterorhabditis</em> sp. LPP17, <em>H. baujardi</em> LPP7</td>
<td>L3</td>
<td>10/insect or 57 IJs/cm(^2) or 14 IJs/cm(^3)</td>
<td>Laboratory</td>
<td>98.5</td>
<td>This study</td>
</tr>
<tr>
<td><em>S. feltiae</em> Mexican</td>
<td>L3</td>
<td>5000, 1500, 500, 150, 50/insect</td>
<td>Laboratory</td>
<td>92, 70, 52, 18 and 9</td>
<td>Lindegren &amp; Vail (^{[20]})</td>
</tr>
<tr>
<td><em>S. riobrave</em> Texas</td>
<td>L3</td>
<td>100/cm(^2)</td>
<td>Laboratory</td>
<td>82.5</td>
<td>Gazit et al. (^{[21]})</td>
</tr>
<tr>
<td>Steinernema sp. with chitosan</td>
<td>L3</td>
<td>250/insect</td>
<td>Laboratory</td>
<td>Above 90</td>
<td>Laborda et al. (^{[52]})</td>
</tr>
<tr>
<td><em>Heterorhabditis</em> sp. CB-n10, <em>H. baujardi</em> CB-n16, <em>S. glaseri</em> CCA, <em>S. arenarium</em> CCA</td>
<td>L3</td>
<td>400/insect</td>
<td>Laboratory</td>
<td>82.5, 65, 45, 22.5</td>
<td>Goulart et al. (^{[22]})</td>
</tr>
<tr>
<td><em>H. indica</em> IBCBn 05</td>
<td>L3</td>
<td>200/insect</td>
<td>Laboratory</td>
<td>76, 40</td>
<td>Almeida et al. (^{[24]})</td>
</tr>
<tr>
<td><em>S. feltiae</em> 09-31, <em>S. weiseri</em>, <em>S. carpocapsae</em> two <em>H. bacteriophora</em> strains</td>
<td>L3</td>
<td>100/cm(^2)</td>
<td>Laboratory</td>
<td>78, 50, 56, other two &lt; 50</td>
<td>Karagoz et al. (^{[25]})</td>
</tr>
<tr>
<td><em>H. bacteriophora</em> SF134</td>
<td>L3</td>
<td>200/insect</td>
<td>Laboratory</td>
<td>95</td>
<td>Malan &amp; Manrakhan (^{[26]})</td>
</tr>
<tr>
<td><em>S. carpocapsae</em> All <em>H. amazonensis</em> RSC01</td>
<td>L3</td>
<td>200/insect</td>
<td>Laboratory</td>
<td>86.3, 87.5</td>
<td>Rohde et al. (^{[28]})</td>
</tr>
<tr>
<td><em>H. baujardi</em> LPP7</td>
<td>Pupae</td>
<td>205/ insect or 1079 IJs/cm(^2) or 261 IJs/cm(^3)</td>
<td>Laboratory</td>
<td>100</td>
<td>This study</td>
</tr>
<tr>
<td><em>S. feltiae</em> All <em>S. feltiae</em> S3</td>
<td>Pupae</td>
<td>50/insect 100/ insect</td>
<td>Laboratory</td>
<td>26.6 and 33.3 30 and 40</td>
<td>Kepenekci &amp; Susurluk (^{[23]})</td>
</tr>
<tr>
<td><em>S. glaseri</em> <em>Heterorhabditis</em> sp. PI <em>Heterorhabditis</em> sp. JPM4 <em>S. feltiae</em></td>
<td>Pupae</td>
<td>200/insect</td>
<td>Laboratory</td>
<td>43.8, 43.8, 41.3, 41.2</td>
<td>Rohde et al. (^{[29]})</td>
</tr>
<tr>
<td><em>H. baujardi</em> LPP7</td>
<td>L3</td>
<td>1000/insect or 2.5/cm(^2)</td>
<td>Field</td>
<td>87.4</td>
<td>This study</td>
</tr>
<tr>
<td><em>S. feltiae</em> Mexican</td>
<td>L3</td>
<td>500/cm(^2) 5000, 1500, 500/ cm(^2)</td>
<td>Field</td>
<td>86, 95.8, 85.1 and 76.5</td>
<td>Lindegren et al. (^{[18]})</td>
</tr>
<tr>
<td><em>H. indica</em> IBCB n05</td>
<td>L3 and pupae</td>
<td>1 to 10/cm(^2)</td>
<td>Field</td>
<td>66 to 93</td>
<td>Silva et al. (^{[20]})</td>
</tr>
</tbody>
</table>
Nematode source

All nematode strains (Table 2) were multiplied in Galleria mellonella L. (Lepidoptera: Pyralidae) 7th instar larvae at 26 ± 1 °C and RH > 80% according to Woodring and Kaya[22]. After 12 days, the IJs were collected in cell culture bottles (40 mL) on alternate days using Pasteur pipettes and stored in a climate-controlled chamber at 16 ± 1 °C and RH > 80% for up to one week before testing.

Laboratory studies

Virulence of different strains against Ceratitis capitata larvae

Sand columns were used to evaluate the capability of IJs of different strains to find and kill C. capitata larvae. As sand columns we used glass test tubes (8 cm X 3 cm) each filled with 32 g of autoclaved sand at 10% w/v, with surface area of 7.1 cm². We used sand to standardize all experiments, and we did not use soil from the guava orchard because we were not certain about the area in which we would perform the field experiment. In each column, 10 C. capitata larvae were placed in the bottom of a glass tube, which was then filled with autoclaved sand. We placed the larvae at 8 cm deep to best challenge nematodes to find the larvae. A total of eight EPN strains were used, six from Brazil and two from Florida, U.S.A. (Table 2). One hundred IJs were suspended in 0.5 mL of distilled water and added to the top of each column, which gave the rate of 14 IJs/cm² or 57 IJs/cm³. There were 20 replicates for each nematode strain. Twenty tubes with the same number of larvae, but without nematodes, were used as control. The tubes were closed with plastic film, then punctured with the aid of a needle to allow aeration. They were placed in an upward position on trays lined with styrofoam and maintained in climate-controlled chambers at 28 ± 2 °C, RH= 80 ± 10% and photophase of 12 h for 14 days, or until total adult emergence in the control. The number of emerged adults were counted and mortality quantified, subtracting the emerged adults from the number of larvae added originally. We did not dissect the insect cadavers, due to the large number of replicates and fast cadaver decomposition.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Source/ Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterorhabditis baujardi Phan, Subbotin, Nguyen &amp; Moens</td>
<td>LPP7</td>
<td>C. Dolinski, Rondônia, Brazil</td>
</tr>
<tr>
<td>H. indica Poinar, Karanukar &amp; David</td>
<td>LPP1</td>
<td>C. Dolinski, Rondônia, Brazil</td>
</tr>
<tr>
<td>H. bacteriophora Poinar</td>
<td>HP88</td>
<td>R. Stuart, University of Florida, Lake Alfred</td>
</tr>
<tr>
<td>Steinernema carpocapsae (Weiser)</td>
<td>All</td>
<td>R. Stuart, University of Florida, Lake Alfred</td>
</tr>
<tr>
<td>Heterorhabditis sp.</td>
<td>LPP9</td>
<td>C. Dolinski, Rondônia, Brazil</td>
</tr>
<tr>
<td>Heterorhabditis sp.</td>
<td>LPP2</td>
<td>C. Dolinski, Rondônia, Brazil</td>
</tr>
<tr>
<td>Heterorhabditis sp.</td>
<td>LPP14</td>
<td>C. Dolinski, Rondônia, Brazil</td>
</tr>
<tr>
<td>Heterorhabditis sp.</td>
<td>LPP17</td>
<td>C. Dolinski, Rondônia, Brazil</td>
</tr>
</tbody>
</table>

Table 2. Species and strains of entomopathogenic nematodes used in this study.

Virulence of different strains at different concentrations against Ceratitis capitata larvae

For the virulence test with different concentrations, we used the three most virulent strains from the first experiment: H. baujardi LPP7, Heterorhabditis sp. LPP14 and Heterorhabditis sp. LPP17 at concentrations of 0 (control), 5, 25, 45, 65, 85 and 105 IJs/arena in 0.5 mL of distilled water and 13 replicates for each combination of concentration/nematode. Sterile test tubes (4 cm high and 0.19 cm² of surface area) containing 1.5 g autoclaved sand, dampened with distilled water at 10% w/v, formed an experimental arena of 0.78 cm³ each. The number of IJs per cm² were 26, 132, 237, 342, 448, 553. After nematode application, three C. capitata larvae were added to each test tube, and these were closed and placed in an upward position on trays lined with styrofoam and maintained in climate-controlled chambers at 28 ± 2 °C, RH= 80 ± 10% and photophase of 12 h for 14 days, or until total adult emergence in the control. The number of emerged adults were counted and mortality quantified, subtracting the emerged adults from the number of larvae added originally. As mentioned earlier, mortality was quantified by subtracting the emerged adults from the number of larvae added originally.
Virulence of different strains at different concentrations against Ceratitis capitata pupae

To test against pupae stage, *H. baujardi* LPP7 was used following the same procedure previously described, but using three one-day-old pupae instead of larvae. Again, 13 replicates were used for each treatment, which were the concentrations of 0, 5, 30, 55, 80, 105, 155, 180, and 205 IJs/arena or 0, 26, 158, 289, 421, 553, 684, 816, 947, 1079 IJs/cm². As in the first experiment, the mortality was obtained based on emerged adults subtracted from the number of pupae added to each treatment.

Virulence of *Heterorhabditis baujardi* LPP7 against *Ceratitis capitata* larvae and pupae at different temperatures

*Heterorhabditis baujardi* LPP7 was the only strain used in this test, since it had showed a good performance in previous experiments. Each arena was made up of 1000 µl-automated-pipette tips, containing 1.5 g of autoclaved sand dampened with distilled water at 10% w/v, forming an experimental arena of 1 cm³. Three *C. capitata* larvae and 70 IJs or 368 IJs/cm² were added to each arena. For the control treatment, 0.5 mL of distilled water was added without nematodes. The tips were covered by a plastic film with punctures made by a needle. Each treatment had 13 replicates. All tips were arranged in an upward position on trays lined with styrofoam and maintained in climate-controlled chambers at 16, 20, 24 or 28 ± 2 °C, RH= 80 ± 10% and photophase of 12 h for 15 days. To access the virulence towards pupae, the experiment was set up in the same way as described earlier, using three pupae and 180 IJs or 947 IJs/cm².

Field experiment

Virulence of *Heterorhabditis baujardi* LPP7 against *Ceratitis capitata* larvae

In a guava orchard, with no incidence of *C. capitata*, the experiment was set up following a completely randomized design with two treatments: with and without nematodes, and seven replicates each. Fourteen cages were constructed with nylon screen and bamboo sticks (2 x 2 x 2 m), over guava trees under production, located in Cachoeiras de Macacu, RJ, Brazil (Figure 1). The area inside the cages was

Figure 1. Cages used in the field experiment located in a guava-growing property in Cachoeiras de Macacu, RJ, Brazil.
cleared of weeds, and soil analysis was performed. The soil temperature was monitored weekly using a soil thermometer (Watchdog Data Logger, Model 450, Spectrum Technologies, Inc.).

One hundred *C. capitata* larvae were randomly dispersed on the ground in each cage. With the help of a sprayer, a 500 mL suspension with 100,000 *H. baujardi* LPP7 IJs were distributed evenly on the soil in each cage, giving a concentration of 2.5 IJs/cm². In the treatment without nematodes, the cages received only distilled water and fruit-fly larvae. In each cage a McPhail trap® with attractant made of protein hydrolysates was placed. After 15 days, the experiment was evaluated, and the control efficiency was assessed indirectly by comparing the emergence of *C. capitata* adults caught in traps in both treatments, and subtracting the total number of initial larvae population introduced into each cage. Thirty days after the first application, the entire experiment was repeated.

**Statistical analysis**

Laboratory and field data were tested for normality (Lilliefors test) and homogeneity of variance (Cochran and Bartlett tests). Percent mortality was square root transformed ($x_1 = \sqrt{(X + 0.5)}$) and submitted to ANOVA[23]. Differences in treatment means were compared using Tukey’s honestly significant difference procedure at $P \leq 0.05$. A fitted polynomial regression model was used to identify the relationship between the IJ concentration (dependent variable) and mortality (independent variable) of larvae and pupae in the concentration and temperature experiments. The program used was Excel (2007) with alpha 0.05. All experiments were repeated once (Assays 1 and 2) with a fresh batch of nematodes and fruit flies. All results are shown separately for better visualization, except the screening in the lab in which the results were combined, since the test F for the factor time was not significant.

**RESULTS AND DISCUSSION**

Laboratory screening of EPN strains in sand column showed different significant larval mortality percentages, with highest values reached by tropical native strains ($P \leq 0.05$). In addition, mortality differences were observed when different concentrations and temperatures were tested against Medfly’s larva and pupae. In the field, application of *H. baujardi* LPP7 showed greater larval mortality when compared to control.

**Laboratory studies**

**Virulence of different strains against Ceratitis capitata larvae**

Bedding et al.[24] suggested testing EPNs at a concentration of 100 IJs/insect as a preliminary assessment of host susceptibility and to begin the process of selecting nematode species or strains as potential biological control agents. Our sand column assays were conducted with this in mind, but since the host was very small we decided to use 10 instead of one insect, which gave a theoretical rate of 10 IJs/insect. Even so, all eight strains tested caused high mortality levels, greater than the control, and two native strains were significantly more virulent than the others (Figure 2). In the first Assay, the mortality ranged between 72.5 and 98.5% ($F=102.45$; $gl=8, 171$; $P \leq 0.05$). When the test was repeated with a different batch of nematodes and larvae, the results were similar, with mortality ranging from 70.5 to 98.5% ($F=105.44$, $gl=8, 171$; $P \leq 0.05$). The highest absolute mortality values were obtained by *Heterorhabditis* sp. LPP17 (98.5% in both Assays), followed by *Heterorhabditis* sp. LPP14 (96 and 93%, Assays 1 and 2, respectively) and *H. baujardi* LPP7 (89 and 91%, Assays 1 and 2, respectively). Since there was no statistic difference between Assays, we present the mean values in Figure 2.

It is well known that nematodes can successfully infect and develop in many different host species; these can be hosts in which optimal infection and development differ among nematode species and/or strains[25]. For that reason, screening several different nematode species and/or strains against a particular target pest is essential in developing any control program. The specificity that each species/strain has for a specific host is directly linked to their ability to reach and penetrate it, but it is also based on the capacity to avoid the host’s immune system using its specific symbiotic bacteria, whose virulence varies. This specificity is a very complex process depending on the host, nematode and bacteria, which explains why there is such variability in infection among strains within the same species against the same target host[26].
Nematode and host sizes play an important role in infection as pointed out by Bastidas et al., and we agree with that, since all strains tested had IJs measuring from 500 to 600 µm and they were all highly virulent. However, observing other laboratory studies, we notice that in some cases, IJs’ size might have negatively affected infectivity. When two species with large IJs were used, *S. feltiae* (Filipjev) and *S. glaseri* (Steiner), larval mortality of *C. capitata* was low in spite of high IJ concentrations applied [10, 13]. On the other hand, some studies showed the best control using small IJs, as in our work [11, 12, 14, 28]. In others, size does not seem to affect infection at all [16, 18], and other factors could have affected it. Perhaps if laboratory procedures were standardized, we could closely compare the results.

We used seven different heterorhabditids and one steinernematid nematode, and their virulence varied, even though they all caused mortality above 70%. Since we mainly tested heterorhabditids, we cannot infer that they are more virulent, as another study suggested [13]. However, among the heterorhabditids we tested, two native strains caused higher mortality than exotics. Karagoz et al. [16] also tested native Turkish strains and they concluded that *Steinernema* strains were more virulent than *H. bacteriophora* Poinar, the only *Heterorhabditis* species tested.

**Virulence of different strains at different concentrations against Ceratitis capitata larvae**

On assessing the interaction between the IJ concentrations and mortality caused by different strains, *H. baujardi* LPP7, *Heterorhabditis* sp. LPP14, and *Heterorhabditis* sp. LPP17 showed a direct correlation between mortality and IJ concentration, reaching a maximum of 100%. By means of regression, it was possible to observe a high $R^2$ value, when data were adjusted for second-order polynomial regression (Figure 3). At the concentration of 45 IJs/3 larvae or 237 IJs/cm$^2$, *H. baujardi* LPP7 reached mortality of 87% in Assay 1 and 80% in Assay 2 (Figure 3a), while the other two strains had mortality below 80% at the same concentration, but they all reached 100% at the highest concentration tested (Figures 3a-c). *H. baujardi* LPP7 achieved higher mortality with lower concentration, and because of that it was chosen to pursue other tests. After the highest concentration tested, we notice a tendency of the curve to decrease, most probably due to to high IJ concentration or intraspecific competition [27].
Among numerous studies done with EPNs against *C. capitata* larvae and pupae, we notice a wide variation of concentrations tested and results achieved, which makes comparison among them difficult (Table 1). Ideally, studies should be standardized, citing the number of IJs applied in a certain volume (IJs/cm$^3$). Lindegren & Vail$^{10}$ showed that mortality reached 92% when 5,000 IJs/insect were used, and...
only 9% when 50 IJs/insect were applied. Since we do not know the exact size of the container they used, we cannot infer if the IJ rate used was high or low. Other studies have used different concentrations of IJs/insect with various responses[12-14, 18, 28] (see Table 1).

Gazit et al.[11] evaluated 12 different species/strains against C. capitata prepupae, with S. riobrave Texas being the most effective strain, and they concluded that IJ activity is directly related to nematode species and concentration. When testing different IJ concentrations, they recorded the highest larval mortality (~85%) at 150 IJs/cm², while we reached that mortality applying around 237 IJs/cm² of surface area. We used test tubes that have narrow tops as testing arenas. In terms of volume, we applied 57 IJs/cm³, but since the containers used were different, it cannot be compared.

**Virulence of different strains at different concentrations against Ceratitis capitata pupae**

When H. baujardi LPP7 was tested against pupae, the data were also adjusted for second-order polynomial regression, but the mortality above 80% was only observed at 155 IJs or 815 IJs/cm² or 197 IJs/cm³; a concentration three times higher than to kill larvae (Figure 4). Although more IJs were needed to kill, 100% pupal mortality was achieved when 1079 IJs/cm² or 261 IJs/cm³ were used. Extrapolating for a possible field application, we would need around 10⁸ IJs/ha, which is still very reasonable for an IPM program.

Rohde et al.[18] also tested different EPNs against Medfly pupae using 200 IJs/insect and reported low mortality in general. Since our results show an increase in mortality as the IJ rate rises, we may infer that they could have tried a higher IJ concentration, different from the one used against larvae. Kepenekci & Susurluk[16] tested two Turkish strains (S. feltiae All and S. feltiae S3) against Medfly pupae and observed low mortality caused by both S. feltiae All (26.6 and 33.3%) and S. feltiae S3 (30 and 40%) with 50 IJs/insect IJs/cm³ and 100 IJs/insect, respectively. Although they doubled the IJ concentration, which increased mortality, it was below what we found in this study. We believe they used a low concentration and could have reached higher mortality if they had used higher concentrations. However, the authors suggest that those results were sufficient to use both nematodes in an IPM program against Medfly.

We can hypothesize that the general low mortality in one-day-old pupal stage is due to the cuticle sclerotization, which affects mainly the nematodes that enter through the cuticle, such as heterorhabdits. We observed that other natural apertures, such as mouth, anus and spiracles, were still open in this pupal stage. Therefore, there is a need for a higher number of IJs to overcome this problem, and the results confirm the need for three times more IJs to achieve the same larval mortality.

**Figure 4.** Mortality rate of one-day-old pupae of Ceratitis capitata following exposure to different concentrations of Heterorhabditis baujardi LPP7 infective juveniles (IJ). Each value is a mean of 13 replicates, to which were added three pupae and 0, 5, 30, 55, 80, 105, 130, 155, 180, 205 IJs/arena or 0, 26, 158, 289, 421, 553, 684, 816, 947, 1079 IJs/cm².
Virulence of *Heterorhabditis baujardi* LPP7 against *Ceratitis capitata* larvae and pupae at different temperatures

On assessing the interaction between temperature and the virulence of *H. baujardi* LPP7 at 16, 20, 24 and 28 °C by means of regression, it was possible to observe a positive regression when data were adjusted for a second-order polynomial equation (Figure 5). *H. baujardi* LPP7 was more virulent to larvae and pupae at 28 °C and less virulent at 16 °C.

It is well known that optimum temperatures for infection will vary among EPN species and strains. Some nematodes such as *H. indica* Poinar, Karunakar & David, *S. glaseri*, *H. baujardi* and *S. riobrave* are better adapted to heat, while other species, such as *H. megidis* Poinar, Jackson & Klein, *S. feltiae*, and *H. marelatus* Liu & Berry, are generally more tolerant to cooler temperatures[17].

**Figure 5.** Mortality rate of *Ceratitis capitata* following exposure to *Heterorhabditis baujardi* LPP7 infective juveniles (IJs) at different temperatures. (a) Larval mortality. Values are means of 13 replicates, to which were added three larvae and 70 IJs or 368 IJs/cm². (b) Pupal mortality. Values are means of 13 replicates, to which were added three pupae and 180 IJs or 947 IJs/cm².
The nematode *H. baujardi* LPP7 was isolated from the Amazon Forest (Monte Negro, RO, Brazil), and its biological traits have been assessed. Its optimum temperature for reproduction and infection is 28 °C, which was confirmed in this study. Gazit et al. also tested EPN different species/strains against *C. capitata* larvae at temperatures ranging from 22 to 41 °C, and they did not find any significant effect on nematode activity, although there was lower activity under cooler conditions (17 °C). Rohde & Moino Jr. also tested five different soil temperatures (19, 22, 25, 28, and 31 °C) on the infectivity of *Heterorhabditis* sp. RSC01 and *Steinernema carpocapsae* (Weiser) Wouts, Mracek, Gerdin & Bedding All against *C. capitata* larvae. The infectivity was directly proportional to temperature rise, with maximum mortality of 86.7% and 80.0% for *S. carpocapsae* and *Heterorhabditis* sp., respectively, at 31 °C. So, in these studies, the strains are adapted to warmer temperatures, having been isolated from hot climates.

**Field experiment**

**Virulence of *Heterorhabditis baujardi* LPP7 against *Ceratitis capitata* larvae**

The soil at the experimental site was 76% sand; 6% silt; 18% clay; 28.8 g/cm³ of organic matter; pH 5.5. The average soil temperature during the entire field experiment at 10-cm depth was 20.2 °C (ranging from 20.0 to 20.5 °C), and the water available in the soil during the first experiment was on average 6.9% and during the second experiment was 56.6%.

Significant differences in larval mortality were detected between treatments (F=148.87; df=1; P ≤ 0.05) (Figure 6). In the treated cages, the average mortality (58.57%) was significantly different from the control cages (7.71%). When the experiment was repeated, one month after the first application, the same pattern was obtained in the treated versus control cages (87.43% and 30.43%, respectively) (F=2039.88; df=1; P ≤0,05). We were not able to recover the larvae from soil to check the presence of nematodes, due to fast cadaver decomposition.

Gazit et al. affirmed that a biological control system for *C. capitata* using EPNs requires the presence of IJs in the soil, ready to encounter the prepupating larvae as they reach the ground and shortly after

![Figure 6. Mortality (%) of *Ceratitis capitata* larvae with and without *Heterorhabditis baujardi* LPP7 infective juveniles added at a rate of 100 IJs/cm² soil surface. Columns with a common letter do not differ significantly at P = 0.05.](http://dx.doi.org/10.4322/nematoda.02016)
that, when they are most susceptible to infection. So, we set up the experiment based on this premise, and our data confirm the high susceptibility of third instar larvae to *H. baujardi* LPP7. What stands out in this study is the low concentration of IJs used and the high mortality reached (2.5 IJs/cm² of soil and average mortality of 58.57 and 87.43%).

We noticed an increase in mortality, comparing the first application with the second, one month apart. This may have happened due to the accumulation of IJs in the soil, since we applied IJs in the same cages. This confirms their ability to survive for more than a month in the soil. Although the mortalities reached in this study were considered sufficient for IPM programs, perhaps we could try two different approaches, one applying lower concentrations (2.5 IJs/cm²) more often, and the other applying higher concentrations, aiming for higher mortalities, as other trials did. Lindegren et al.[19] applied *S. feltiae* Mexican in a papaya field, aiming at larval eradication, with an increased ratio of 500 IJs/cm², and obtained 86% mortality. In a guava orchard, Silva et al.[20] tested different EPN strains against Mediterranean fruit fly larvae and pupae and considered *H. indica* IBCB n5 the most virulent strain, applying 1 and 10 IJs/cm², causing mortality of 66 and 93%, respectively. Another field study tested *H. bacteriophora* larvae against of *Anastrepha ludens* (Loew) (Diptera: Tephritidae), and obtained mortality of 47 and 76% at concentrations of 115 and 345 IJs/cm², respectively.[21]

We could also explain the lower mortality and/or adult emergence in Assay 1 by low water content in the soil. According to Grant & Villani[22] EPNs in soils with low humidity may have their mobility and infectivity inhibited; if the dryness persists they may dehydrate and die. Several other studies have shown the influence of soil moisture on the virulence of entomopathogenic nematodes, showing, in general, a decrease in virulence according to reduction of soil moisture[30, 33]. There was no irrigation system in the area, and the rainfall measured during the first experiment was 10.6 mm, while during the repetition the rainfall measured was 90.3 mm. The dryness in the soil may have affected the IJs negatively, causing lower larval mortality. On the other hand, the excess of water may have negatively affected the Medfly larvae, which explains the high mortality in the control treatment in Assay 2.

*H. baujardi* LPP7 has been successfully used against a very important guava pest, the guava weevil, *Conotrachelus psidii* Marshal (Coleoptera: Curculionidae), and its population is very low in the areas where IPM was implemented compared to areas with conventional chemical control[34, 35]. With this study, we can include the control of Medfly as part of the IPM in guava.

### CONCLUSION

Tropical strains seemed better adapted to high temperatures, with the ability to find and kill Medfly 3rd stage larvae and one-day-old pupae at characteristic temperatures in a tropical orchard during summer. Moreover, *H. baujardi* LPP7 seemed very effective against the larval and pupal stage of *C. capitata* at different concentrations tested. Low emergences and consequent high larval mortality found in the field gave an indication that *H. baujardi* LPP7 may be used to control *C. capitata* soil stages, as well as other agricultural pests in an IPM program for guava. This study confirms the virulence of different EPNs against *C. capitata* larval stages, especially the third stage, which is the one coming out of the fruit and going to the ground for pupation. The suppression of this stage would lead to a lower number of adults in the area, especially in guava orchards affected by the use of pesticides where there is no occurrence of natural enemies. EPNs may be considered an alternative to conventional control, which is focused on the chemical control of adults. This tactic, in organic orchards, would allow less use of pesticides and would be a new alternative to integrated pest control in the culture of organic guava.

### REFERENCES


