Biological Traits of Two Native Brazilian Entomopathogenic Nematodes
(Rhabditida: Heterorhabditidae)

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Nematóides entomopatogênicos (NEPs) causam doença e morte a vários insetos em diferentes ordens. Esses nematóides estão sendo usados como agentes de controle biológico com grande sucesso em várias culturas. Em pomares onde os insetos-praga estão concentrados sob a copa, os NEPs estão sendo aplicados na forma de insetos-cadáver. Para aumentar o controle da praga, é muito importante saber a quantidade de juvenis infectantes (JIs) produzidos e o tempo para emergência desses do inseto-cadáver de cada linhagem aplicada ao campo. Essas duas características foram avaliadas para duas linhagens nativas brasileiras: Heterorhabditis baujardi LPP7 e LPP1, nas temperaturas de 25 e 28 °C. LPP7 e LPP1 produziram em média 147,680 ± 24,126 e 165,813 ± 20,830 JIs a 25 °C e 122,350 ± 8,905 e 131,980 ± 8,774 JIs a 28 °C, respectivamente. O tempo para os JIs emergirem dos cadáveres foi de 14,7 ± 2,2; 14,4 ± 0,8; 11,4 ± 1,1 e 10,7 ± 1,7 dias, para LPP7 e LPP1 às temperaturas de 25 e 28 °C, respectivamente. A temperatura afetou significativamente ambas as variáveis. As linhagens avaliadas diferiram significativamente para o parâmetro produção de JIs a 25 °C, sendo que H. baujardi LPP1 apresentou a produção mais alta.

Palavras-chave: produção de juvenis infectantes, influência da temperatura, tempo para emergência, inseto-cadáver, Nemata.


Entomopathogenic nematodes (EPNs) cause disease and death to a number of insects in different orders. Those nematodes are being used as biological control agents with great success in various crops. In orchards where the insect pests are concentrated under the canopy, the EPNs are being applied as insect-cadavers. In order to increase the pest control, it is very important to know from each strain applied in the field the number of infective juveniles (IJs) produced and their emergence time from the insect-cadaver. Those characteristics were evaluated for two Brazilian native strains: Heterorhabditis baujardi LPP7 and LPP1, under the temperatures of 25 and 28 °C. LPP7 and LPP1 strains produced average of 147,680 ± 24,126 and 165,813 ± 20,830 IJ at 25 °C and 122,350 ± 8,905 and 131,980 ± 8,774 IJ at 28 °C, respectively. The IJ emergence time were 14.7 ± 2.2, 14.4 ± 0.8, 11.4 ± 1.1 and 10.7 ± 1.7 days, for LPP7 and LPP1 at 25 and 28 °C, respectively. The temperature significantly affected both IJ production and emergence time. The strains tested differed significantly for the variable IJ production at 25 °C, with higher production for H. baujardi LPP1.

Key words: infective nematode yield, temperature influence, emergence time, insect- cadaver, Nemata.
Introduction

Steinernematids and heterorhabditids are entomopathogenic nematodes (Nemata: Rhabditida), which are capable to kill many insect species in soil and cryptic habitats. Those nematodes are obligate pathogens that kill insects with the aid of two symbiotic bacterium carried in the intestine, Xenorhabdus spp. and Photorhabdus spp., associated respectively with the genera Steinernema spp. and Heterorhabditis spp. (Poinar, 1990; Forst & Clarke, 2002).

The infective juvenile (IJ) is soil-dwelling, non-feeding, third-stage juvenile protected by the second-stage cuticle. Once in contact with the insect, IJ penetrate the insect via natural opening or directly through the cuticle. They release the bacteria into the insect's hemocoel for its development. The bacteria then multiply, produce endotoxins that kill the insect in 24-48 hours by septicemia (Dowds & Peters, 2002). The bacteria provide nourishment for the nematodes that develop, make ecdises and become adults. In the case of steinernematids, males and females compose the first generation; they mate and produce two or three generations in the same insect-cadaver. The life cycle of heterorhabditis is similar except that the IJ develop into hermaphrodite adults capable to self-fertilization, and that their progeny are formed by males and females. In both families, after two or three generations within the host, IJ emerge from the cadaver to seek new hosts (Poinar, 1990).

Entomopathogenic nematodes can be produced in vivo or in vitro depending on the scale production needed. Small-scale production, as for small farmer and laboratories, uses in vivo cultivation, while larger farmers will need in vitro in liquid or solid culture (Ehlers, 2001). Larvae of Galleria mellonella (L.) (Pyralidae: Lepidoptera) have been used for in vivo rearing of these nematodes due to its high susceptibility to EPNs to a wider range of nematodes species (Gaugler & Han, 2002). In average 100,000-150,000 IJs may emerge from each G. mellonella larvacecadaver, depending on the nematode species and/or strain (Woodring & Kaya, 1988).

Two features that may affect the EPNs suitability as a biological control agent against specific insect-pests are their level of infectivity and reproductive capacity. Infectivity refers to the ability of nematodes to cause infection in different insects, and may vary with the insect target and the nematode species or strains, e.g. the effective control of Diaprepes abbreviatus (L.) by Steinernema riobrave (Cabanillas, Poinar & Raulston) 1994 355 (Stuart et al. 2004), Conotrachelus nenuphar (Herbst) by S. carpocapsae (Weiser) All (Shapiro-Ilan et al. 2002), and Conotrachelus psidii Marshal by H. banjardi Phan, Subbotin, Nguyen & Moens LPP7 (Dolinski et al., 2006).

The reproductive capacity and emergence time can be affected by abiotic and biotic factors. Temperature is the most important factor limiting the reproductive success of EPNs. Each strain has its own temperature range to which it is best-adapted, and an understanding of the effects of soil temperature on nematode reproduction and development helps to improve the accuracy of field applications (Kaya, 1990; Zervos et al., 1991; Choo et al. 2002). As for biotic factors, density of invading IJs (Selvan et al., 1993; Koppenhöfer & Kaya, 1995; Boff et al., 2000), as well as the insect host (Jansson, 1996; Molina et al., 2004) and nematode strain (Jansson, 1996; Zervos et al., 1991) may affect the nematode development.

In this study, we tested the influence of temperature and strain type on IJ production and emergence time. Also, the method for counting nematode progenies was detailed.

Material and Methods

Nematodes, Galleria mellonella larvae, and experimental design. The nematode strains used in this study, H. banjardi LPP7 and H. banjardi LPP1, were reared in G. mellonella larvae at 25 °C, according to procedures in Woodring & Kaya (1988). Harvested IJ were kept at 16 °C for less than one week before the tests. G. mellonella larvae were reared under laboratory conditions in plastic pots (30 x 15 x 10 cm), on a diet based on cereals, sugar cane and honey. These strains were obtained from the Tropical Forest of Montenegro (RO), Brazil, and being described elsewhere.

One thousand IJ of H. banjardi LPP7 were suspended in 0.5 ml of distilled water and distributed...
evenly onto a 9 cm-diameter plastic Petri dish with paper filter (Whatman n° 1) on the bottom. Five last-instar *G. mellonella* larvae (250-280 mg) were placed in each dish. Since at that time best IJ concentration was not known, the amount of about 200 IJs / larva was used in order to avoid overcrowding and / or sub-infections. A total of five Petri dishes with five larvae in each were placed in plastic bags and incubated in the dark at 25 ± 2 °C and 28 ± 2 °C (60 % humidity). The same procedure was repeated for *H. baujardi* LPP1. The Petri dishes were placed in plastic bags to avoid temperature oscillation during the infection period. Larval mortality was recorded after 4 days and, when dead insects showed typical symptoms (homogeneous brownish color), they were transferred individually to modified White traps (White, 1927), arranged side by side on the germination chamber shelves, and inspected daily for nematode emergence. The time required for IJs to emerge from each cadaver was also recorded. The IJs were collected from each White trap to 50 ml-erlenmeyers during one week. After that, the volume of each erlenmeyer was adjusted to 40 ml with distilled water and kept in a germination chamber at 16 °C.

**Counting.** A total of 20 erlenmeyer flasks of each strain in each temperature were used for counting nematodes, each considered a replicate. The erlenmeyer flasks containing the nematode suspensions were shaken and homogenized for 10 seconds by hand every time an aliquot was taken. The number of IJs in each harvested flask was estimated from the average number of *IsJ* present in five aliquots of 20 µl, counted on a counting slide under a stereomicroscope.

**Statistical analysis.** Average IJ production and emergence time were subjected to analysis of variance (ANOVA) using Sistema de Análises Estatísticas (SAEG, 1990). Significant differences among different temperatures and strains were determined using Tukey’s Honestly Significant Difference Test at $P = 0.05$. Data are presented as means ± standard error.

**Results and Discussion**

This study showed the influence of the temperature on the IJ production and emergence time of *H. baujardi* LPP1 and *H. baujardi* LPP7, two entomopathogenic nematodes strains.

Temperature significantly affected IJ production of both strains tested in *G. mellonella* larvae, *H. baujardi* LPP7 and *H. baujardi* LPP1. The data shows the average juvenile yield significantly different between temperatures for *H. baujardi* LPP7 ($df = 1, 38; F = 13.63; P = 0.05$), with yields at 25 °C greater than at 28 °C (147,680 ± 24,126 vs. 122,350 ± 8,905 IJ). For *H. baujardi* LPP1, the same was observed ($F = 74.53$), with average IJ production higher at 25 °C as well (165,813 ± 20,830 vs. 131,980 ± 8,774 IJ at 25 °C and 28 °C, respectively) (Figure 1).

![Figure 1 - Temperature effect on infective juveniles production (± SD) of *Heterorhabditis baujardi* LPP7 and *H. baujardi* LPP1 in *Galleria mellonella* larvae. Bars with the same letter are not significantly different ($P = 0.05$).](image-url)
Hussaini et al. (2005) showed that temperature affects the progeny production of different nematode strains in different hosts. Specifically in *G. mellonella*, the IJ production was significantly higher at 25 °C in all strains tested, including *H. indica* EN, a very close related strain of *H. baujardi*. In this present study, the temperature of 25 °C was also the most indicated temperature in both strains tested. That reassures the necessity of irrigating the soil in order to lower soil temperature before applying the nematodes in the field (Georgis and Gaugler, 1991).

*H. baujardi* LPP1 produced significantly more IJ than *H. baujardi* LPP7 at 25 °C (Figure 1). In general, it can be concluded that optimal IJ production varies within nematode strain, but also depends on the number of inoculated IJ and temperature, in this case 25 °C was the optimum temperature for both strains.

Temperature also affected significantly the emergence time of *H. baujardi* LPP7, (14.7 ± 2.2 vs. 11.4 ± 1.1 days at 25 and 28 °C, respectively; \(F = 34.77\)), and *H. baujardi* LPP1 (14.4 ± 0.8, and 10.7 ± 1.7 days at 25 and 28 °C, respectively; \(F = 73.85\)). The time for IJ to emerge from the cadavers was not different between the two strains at 25 °C (\(F = 0.31\)) nor at 28 °C (\(F = 2.10\)) (Figure 2).

The emergence day was significantly lower at 28 °C for both strains tested. This lower number of days has positive and negative views. As positive, the sooner the juveniles leave the cadaver and start searching for new hosts in the soil, the better. This characteristic also influences the nematode production, as it speeds up the process, making the cycle shorter (Ehlers, 2001). On the other hand, Schirocki and Hague (1997) mentioned that if the nematode development in the host is longer, for example under lower temperatures, that increases the nutrient absorption by the nematodes and it could be expressed as longer IJs. As a negative view, at 28°C IJs are produced faster, but they may have less nutrient reserves in their bodies, what could affect their ability to search and invade a host. This hypothesis could be tested by analyzing the IJ lipid contents of both strains in both temperatures.

Bedding et al. (1983) suggested applying EPNs at a concentration of 100 IJs / insect as a preliminary assessment of host susceptibility. It has been shown for a number of EPNs species, that when the number of penetrating IJs exceeds an optimal level, intraspecific competition occurs among the developing nematodes in the insect cadaver. This competition is mainly for nutrients and space, affecting nematodes’ fecundity, producing smaller progeny (Koppenhöfer and Kaya, 1996; Zervos et al., 1991; Boff et al., 2000). Selvan et al. (1993) showed an optimal survival and nematode reproduction at a density of approximately 100 IJs per host, agreeing with Bedding et al. (1993).

Within the genera *Heterorhabditis*, this optimal number of entering IJs also varies; as for *H. heliothidis* (a synonym of *H. bacteriophora*) NC1, the inoculum...
level that yields the highest number of IJs is 25 IJs per *G. mellonella* larvae (Zervos et al. 1991). For *H. megidis* NLH-E 87.3, the best concentration is 300 IJs (Boff et al. 2000). In this study, a tentative concentration of 200 IJs / larvae was used aiming to avoid intraspecific competition. Tests on best yields based on entering IJs of *H. baujardi* LPP7 and *H. baujardi* LPP1 are in progress (results not shown).

Several unknown factors affect nematode production *in vivo*, and these factors interact in unpredictable ways. This reflects on a high variability in the number of juveniles produced per host, which is indicated by high standard error values. Specifically in this study, we tried to correct that using more replicates (20 progenies for each treatment), but in a few treatments the standard error was still high.

These results herein presented are of interest mainly to visualize and understand what takes place in the soil when *G. mellonella* larvae infected with EPNs are applied in the field. We expect that native nematodes such as *H. baujardi* LPP7 and *H. baujardi* LPP1, that are well adapted to local climate and soil conditions, could be very effective as biological control agents against local pests.

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