

# Ecological characterisation of the Colombian entomopathogenic nematode *Heterorhabditis* sp. SL0708

Mejía-Torres, MC.<sup>a\*</sup> and Sáenz, A.<sup>b</sup>

<sup>a</sup>Laboratorio de Control Biológico, Pontificia Universidad Javeriana – HPUJ,  
Cra 7a No 43-82 Ed 54 (Jesús Emilio Ramírez)

<sup>b</sup>Unidad de Ecología y Sistemática – UNESIS, Departamento de Biología, Pontificia Universidad Javeriana – HPUJ,  
Cra 7a No 43-82 Ed 54 (Jesús Emilio Ramírez), Of. 200

\*e-mail: mariacamila30@gmail.com

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(With 5 figures)

## Abstract

The entomopathogenic nematode *Heterorhabditis* sp. SL0708 (Rhabditida: Heterorhabditidae) isolated from soil in Alcalá, Valle del Cauca (Colombia) was characterised ecologically using *Galleria mellonella* larvae (L) (Pyralidae: Galleriinae) as hosts. The effect of temperature on the viability, infectivity and reproduction, and of moisture on infectivity and storage in liquid were evaluated in infective juveniles (IJs). Significant differences were found in the viability, infectivity and reproduction of the IJs at different temperatures. No nematodes were recovered at 5 °C and 10 °C, and at 35 °C no infectivity was observed. Average daily nematode recovery was best at 25 °C, and survival of the IJs was low in substrates presenting 13% moisture. The optimal storage temperature for *Heterorhabditis* sp. SL0708 was between 20 °C and 30 °C, keeping its infectivity for up to 8 weeks.

**Keywords:** Rhabditida, infective juveniles, Heterorhabditidae, temperature, moisture, storage.

## Caracterização ecológica do nematoide entomopatogênico colombiano *Heterorhabditis* sp. SL0708

### Resumo

O nematoide entomopatogênico colombiano *Heterorhabditis* sp. SL0708, isolado do solo de Alcalá, Valle del Cauca (Colombia), foi caracterizado ecologicamente utilizando-se como hospedeiro lagartas de *Galleria mellonella* (L) (Pyralidae:Galleriinae). O efeito da temperatura na viabilidade, na infecção e na reprodução, e da umidade na infecção e do armazenamento em líquido foi avaliado em juvenis infectantes (JIs). Diferenças significativas na viabilidade, na infecção e na reprodução dos JIs foram observadas nas diferentes temperaturas. Não foram recuperados nematoides a 5 °C e 10 °C, e não se observou infecção a 35 °C. A maior média diária de recuperação de nematoides foi a 25 °C. Por outro lado, a sobrevivência dos JIs foi baixa nos substratos com unidades de 13% e a melhor temperatura de armazenamento para SL0708 foi entre 20 °C e 30 °C, mantendo a infecção até oito semanas.

**Palavras-chave:** Rhabditida, juvenis infectantes, Heterorhabditidae, temperatura, umidade, armazenamento.

### 1. Introduction

Entomopathogenic nematodes (NE) are organisms suitable for pest control (Sáenz, 2005), and compared to other methods of insect control their use has recently increased. Most species of *Heterorhabditis* and *Steinernema* have been isolated from soil samples using *Galleria mellonella* larvae (L), (Lepidoptera: Pyralidae), in general, little information is provided on their biology and ecology and they are not complemented in subsequent studies, this may hinder their use as biological control agents in integrated pest management systems despite being obligated pathogens of a wide range of hosts (Hazir et al., 2004, Morton and Garcia-del -Pino, 2009), having a mutualistic relationship

with bacteria of the *Photorhabdus* and *Xenorhabdus* genera (Adams et al., 2006), high virulence and specific ecological characteristics (Lewis et al., 2006; Rohde et al., 2010; Strauch et al., 2000).

In the present study, we provide the ecology of the entomopathogenic nematode *Heterorhabditis* sp. SL0708, consistent with recommendations by Koppenhöfer and Kaya (1999). *Heterorhabditis* sp. SL0708 was isolated from bamboo-soil in the town of Alcalá - Valle del Cauca, Colombia. The life cycle of this nematode is known; (Sáenz and López, 2011), its virulence in *G. mellonella*, *Plutella xylostella* L (Lepidoptera: Yponomeutidae), *Delia platura*

Meigen (Diptera: Anthomyiidae), *Collari scenica* Stal (Hemiptera: Miridae) and *Conatrachellus psidii* Marshall (Coleoptera: curculionidae) (data in press) as well as its biology (Mejia and Sáenz, 2013). However, the effect of environmental conditions and of the storage method on the viability, reproductive capacity and the infectiousness of infective juveniles (IJs) are unknown, although this information is fundamental for the use of *Heterorhabditis* sp. SL0708 as a biological control agent.

## 2. Material and Methods

### 2.1. General test conditions

The tests were conducted at the Pontificia Universidad Javeriana's (PUJ) biological control laboratory at a temperature of 25 °C and in darkness. Bioagro S.A., last instar larvae of *G. mellonella* weighing 250-300 mg were used in the reactivation and multiplication of the IJs, and in the development of bioassays. To carry out the different environmental performance tests, IJs were recovered between 5 and 7 days, and White traps were recovered and stored in sterile distilled water at 10 °C.

### 2.2. IJs tolerance to different temperatures

To establish a temperature range in which *Heterorhabditis* sp. SL0708 remains viable, 100 IJs were inoculated into ½ oz plastic containers filled with 8g of sterile river sand at field capacity. 60 containers were incubated at 5, 10, 20, 25, 30 and 35 °C for 2, 4, 6, 8, 10 and 12 hours. Once the time of exposure to the different temperatures elapsed, the containers were kept at 25 °C for 24 h. To establish IJs viability, Baermann funnels were set-up for each sand sample. After a period of 4 hours a count of the IJs was conducted using a stereomicroscope.

### 2.3. The effect of temperature on infectivity

To determine the optimal temperature range in which *Heterorhabditis* sp. SL0708 can infect last instar larvae of *G. mellonella*, 20 individual larvae were exposed to 50 IJs in ½ oz plastic containers with 2 g of sterile river sand and incubated at 5, 10, 20, 25, 30, 35 °C. Larvae mortality was assessed every 24 hours. Dead larvae were placed in Petri dishes on filter paper at 25 °C (Table 1). After 48 hours, the dead larvae were washed with sterile distilled water and dissected to determine the number of nematodes established in each dead larva.

### 2.4. The effect of temperature on reproduction

To establish the optimal temperature range for *Heterorhabditis* sp. SL0708 reproduction, 50 larvae were

**Table 1.** Range of temperature and infectivity incubation period for *Heterorhabditis* sp. SL0708.

Range of temperature used (in °C)	Incubation period (in days)
5-10	3
15-20	2
25-37	1

exposed to 50 IJs in ½ oz plastic containers filled with 2 g of sterile river sand at field capacity and were kept until death was recorded. The dead larvae were placed in White traps and incubated at 5, 10, 20, 25 and 30 °C until the emergence of the IJs.

### 2.5. IJs tolerance to different levels of soil water content

To determine the range of soil water potential in which IJs of *Heterorhabditis* sp. SL0708 can seek and infect the host, 50 plastic, ½ oz containers with 2 g of river sand were inoculated with 1000 IJs in 10 µl of sterile distilled water. A *G. mellonella* larva was exposed in each container for a period of 48 hours. The potential soil moisture (% w/w soil water content) assessed was -5.5 kPa (13%), -14 kPa (33%), -43 kPa (100% field capacity), -58 kPa (133%) and 73 kPa (166%). After the incubation period, the larvae were dissected so as to count the number of nematodes established in the host and the latter's mortality rate. Furthermore, the number of living IJs obtained from the Baermann funnels from each sand sample was also calculated.

### 2.6. The effect of storage period

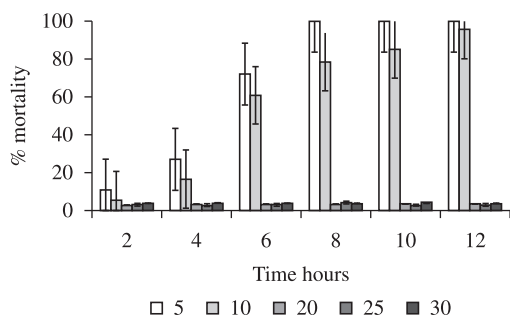
To determine the optimal storage conditions for *Heterorhabditis* sp. SL0708, a suspension in sterile distilled water of 2000 IJs /mL for one day with recovered dead *G. mellonella* larvae, was placed in 72 tissue culture flasks of 50 ml and exposed to temperatures of 5, 10, 15 and 25 °C. Subsequently, three flasks per treatments were sampled randomly every 1, 2, 4, 8 and 16 weeks to evaluate the viability and infectivity of the IJs. Viability was established by counting the mobile IJs using a stereomicroscope and determining the survival percentage. In order to evaluate infectivity, 10 individual larvae were exposed to 25 IJs for a period of three days in plastic containers of ½oz filled with 3g of sterile river sand. After the incubation period, the dead larvae were washed, dissected and the number of nematodes in them was counted. 10 larvae placed with 25 newly retrieved IJs were used as a control.

### 2.7. Data analysis

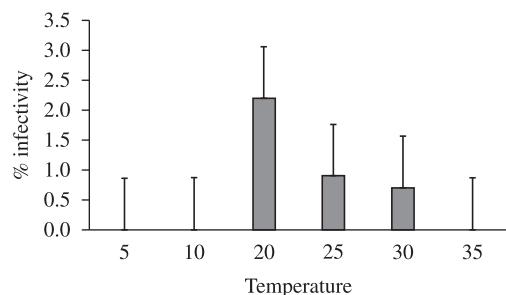
Excluding the storage test, all the experiments were conducted twice. In all cases, the results of both tests were similar and were combined for analysis. The variance analysis and Tukey's HSD mean separation test ( $P < 0.05$ ) were performed using SPSS 18 (SPSS, 2009).

## 3. Results

Exposing the *Heterorhabditis* sp. SL0708 IJs to different temperatures, significant differences in their viability ( $df = 5, 54; F = 60.123, P = 0.0002$ ; Figure 1) and infectivity ( $df = 5, 114; F = 123, P = 0.0002$ ; Figure 2) were found. Upon completing the maximum exposure time, the percentage of survival and infectivity was between 0 and 3% at temperatures of 5 and 10 °C. At 35 °C, *G. mellonella* presented no evidence of infection. Similarly, reproduction is affected by temperature ( $df = 4, 45; F = 6.215, P = 0.0004$ ). At 5 and 10 °C the life cycle was not completed. The recovery of IJs was similar in all



**Figure 1.** *Heterorhabditis* sp. SL0708 viability at five temperatures. The error bars were calculated using the standard deviation of each treatment.

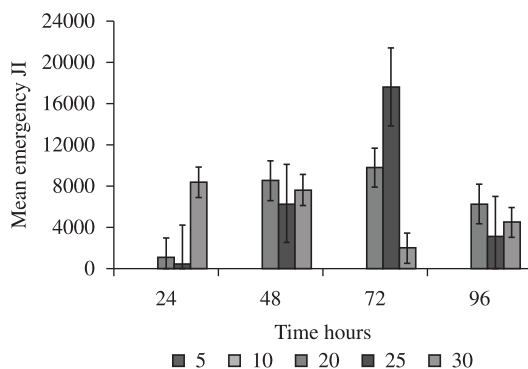


**Figure 2.** *Heterorhabditis* sp. SL0708 infectivity on *Galleria mellonella* exposed to six temperatures. The error bars were calculated using the standard deviation of each treatment.

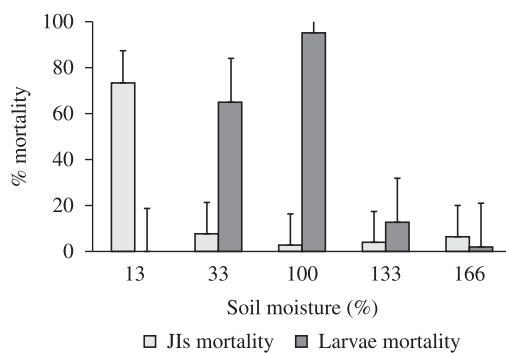
the three treatments at temperatures of 20, 25 and 30 °C (Figure 3). IJs tolerance tests at different moisture levels presented significant differences between treatments ( $df = 4, 45; F = 180.971, P = 0.0002$ ), a mortality of 73.45% was obtained in IJs exposed to 13% moisture after 48 hours (Figure 4). In 100% humidity, a mortality of 95% in *G. mellonella* larvae exposed to these IJs was recorded (Figure 4). Finally, in evaluating *Heterorhabditis* sp. SL0708 IJs temperature and storage time, significant differences ( $df = 3,68; F = 132.140, P = 0.0001$ ) were found in the nematodes' viability. The IJs survive up to eight weeks at temperatures between 20 and 30 °C (Figure 5). Infectivity was reduced over time in all treatments; however at 25°C infectivity exceeded 50% until the eighth week (Table 2).

#### 4. Discussion

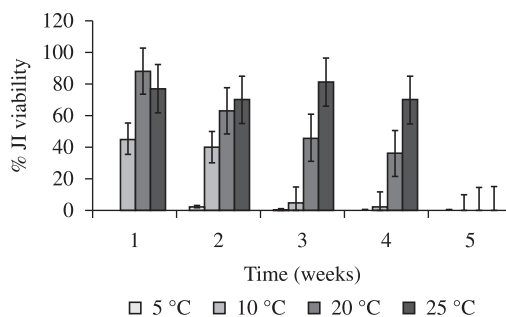
To study the ecology of entomopathogenic nematodes, tests must be conducted to evaluate the behaviour of IJs in different temperatures and humidity. It is recognised that temperature is an important factor in the life cycle of entomopathogenic nematodes (Griffin, 1993). Varying temperatures have effects on the viability of the IJs and their ability to reproduce, these effects are observed mainly in extreme temperatures (0 and 40 °C), which are lethal (Rohde et al., 2010; Susurluk, 2008; Morton and Garcia-del-Pino, 2009). As observed in *Heterorhabditis* sp. SL0708, low temperature reduces the IJs' mobility and persistence.



**Figure 3.** *Heterorhabditis* sp. SL0708 IJs emergence at five temperatures. The error bars were calculated using the standard deviation of each treatment.



**Figure 4.** *Heterorhabditis* sp. SL0708 IJs mortality and *Galleria mellonella* infection at different moistures. The error bars were calculated using the standard deviation of each treatment.



**Figure 5.** *Heterorhabditis* sp. SL0708 suspension viability under four storage temperatures. The error bars were calculated using the standard deviation of each treatment.

The results obtained with *Heterorhabditis* sp. SL0708 at temperatures between 20 and 35 °C, show the persistence of IJs in time, this is consistent with Koppenhöfer and Kaya (1999), who state that these temperatures are suitable for nematode pathogenicity, infectivity and reproduction. The survival of IJs in this temperature range indicates that it is optimal for this Colombian isolate and should not be generalised to other species, as the temperature ranges

**Table 2.** Percentage of *Heterorhabditis* sp. SL0708 infection at different temperatures.

Temperature (°C)	% Infection			
	1	2	4	8*
5	0	0	0	0
10	80-100	70-90	0	0
20	80-100	70-100	60-70	50
25	80-90	80-100	70-80	40-60

\* Due to nematode death, no infectivity was assessed at 16 weeks.

affecting IJs vary depending on the geographic area where they are collected (Molyneux, 1986). Specifically for *Heterorhabditis* sp. SL0708, exposure to temperatures between 20 and 30 °C does not affect their viability over time, which is within the range established for other heterorhabditidae and some steinernematidae. An example of this is *H. bacteriophora*, individuals which can be recovered at temperatures up to 37 °C while other species such as *Steinernema carpocapsae* and *Steinernema feltiae* can only be recovered at temperatures up to 35 °C (Morton and Garcia-del-Pino, 2009). The infectivity of *Heterorhabditis* sp. SL0708 IJs is affected at temperatures below 20 °C while inexistent at 5° and 10 °C. The results are similar to those obtained by Chen et al. (2003), Morton and Garcia-del-Pino (2009) and Saunders and Webster (1999), who report that at temperatures below 15 °C infection is not good, while at temperatures between 15 and 35 °C infectivity is optimal. In the case of *Heterorhabditis* sp. SL0708, greatest infectivity is presented at 20 °C, diverging from results found by Morton and Garcia-del-Pino (2009) for some species of heterorhabditidae, where 25 °C was the optimum temperature or 30 °C for *Heterorhabditis georgiana* (Shapiro-Ilan et al., 2009). The inconsistency in results regarding temperature for different species of the *Heterorhabditis* genus, shows that a single temperature cannot be assigned to the genre and that the infectivity of nematode species may also depend on the size of the larva, the depth of the host in the substrate and search behaviour of the IJs (Boff et al., 2001; Susurluk, 2008).

While each temperature affects the percentage of infection of hosts by the IJs, it also generates a different impact on the life cycle inside the host. The main difference between treatments regarding the reproduction of *Heterorhabditis* sp. SL0708 was observed with the emergence of IJs from the dead *G. mellonella*, as described by Koppenhöfer and Kaya (1999). The results obtained with *Heterorhabditis* sp. SL0708 are similar to those reported by Morton and Garcia-del-Pino (2009) who state that the first emergence of the nematodes at 20 °C occurs after 20 days, and at lower temperatures after 45 days following infection; however, although the emergence of *Heterorhabditis* sp. SL0708 came earlier at 20 °C, the number of nematodes recovered is greater at 25 °C, indicating that this is the optimum temperature and is favourable for the reproduction and recovery of IJs (Sáenz and López, 2011). This is consistent

with results for *H. bacteriophora* and *Steinernema rarum* (Koppenhöfer and Kaya 1999; Morton and Garcia-del-Pino 2009).

Soil moisture is also an important factor for nematode mobility and survival (Hominick, 1990; Rohde et al., 2010). *Heterorhabditis* sp. SL0708 had poor survival in substrates with low humidity, this is in agreement with observations by Glazer (2002), Grewal et al. (2006), Mukuka et al. (2010) and Shapiro-Ilan et al. (2005), who point out *Heterorhabditis*'s low potential to survive desiccation. According to studies by Koppenhöfer and Fuzy (2007), O'Leary et al. (2001) and Rohde et al. (2010) nematode viability is affected when suddenly exposed to dry soil, but if humidity is decreased progressively, the nematode can adapt and enter anhydrobiosis. Accordingly, the exposure of *Heterorhabditis* sp. SL0708 to low percentages of moisture may have caused the high mortality rate; therefore, future trials would be relevant to evaluate the feasibility and adaptability of the nematode in a gradual desiccation process.

Viability during storage in liquid was slightly affected after 8 weeks at 10 °C, this is despite findings indicating viability, depending on the species, for periods of 3 to 4 months and temperatures of 8 and 15 °C (Molina et al., 2006). For instance, Klingler (1990) reports for *H. bacteriophora* an optimal storage temperature of 6 °C for a period of 9 weeks with high viability and no effect on infectivity. Fitters and Griffin (2004) established for three strains of *H. megidis* a viability of over 80% after 6 weeks of storage at 20 °C. For *H. indica* and *H. bacteriophora*, Strauch et al. (2000) reported finding less than 20% of live IJs following 4 months of storage in liquid. Unlike reports for the Colombian *Heterorhabditis* sp. SL0708 nematode, which survived for periods of up to 8 weeks, no survival was established for any of these species at temperatures exceeding 20 °C. Although concentration for storage in liquid is typically 2000 JI/mL, it is possible that the concentration of nematodes per mL affected the results, given that Molina et al. (2006) found that the best concentration to store Heterorhabditidae is 1000 JI/mL for a period of 15 days and one month at a temperature between 16 and 24 °C. Additional trials utilising other methods of storage are needed to attain longer periods of viability for *Heterorhabditis* sp. SL0708.

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