

# Chapter 10

## Entomopathogenic Nematodes



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### 10.1 Introduction

Insect-associated nematodes are filiform non-segmented organisms that belong to the Nematoda phylum, which includes more than 30 families. These organisms may have forethic associations or parasitism with insects, which occurs with entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae), considered pathogenic to different orders of insects (Kaya and Stok 1997). The family Heterorhabditidae comprises the genus *Heterorhabditis*, with 19 species (Nguyen 2017a). The family Steinernematidae includes the genus *Neosteinerinema*, with a described species, and the genus *Steinerinema*, with 84 described species (Nguyen 2017b).

In Latin America, studies on entomopathogenic nematodes have advanced in terms of species description, identification of isolates, isolation of native species, in vivo and in vitro multiplication, virulence evaluation, and pest management of economic importance, especially in Argentina, Bolivia, Brazil, Colombia, Cuba, Mexico, Peru, and Venezuela. However, the use of these organisms is still incipient.

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Despite the already developed research and climatic conditions favorable to the implementation of the control of agricultural pests by entomopathogenic nematodes, this practice has been rarely commercially carried out in Latin America (Leite 2011).

In the case of Brazil, in the last two decades, studies have been conducted with emphasis on the isolation of native nematodes, showing excellent characteristics regarding virulence patterns and adaptation to environmental conditions. This has enabled the development of studies related to taxonomic identification, biology, application technology applied with other microorganisms (such as entomopathogenic fungi), and compatibility with phytosanitary products, besides the control of insects of economic importance (Dolinski et al. 2017).

Therefore, the objective of this chapter is to show the main results of studies associated with the bioecology of entomopathogenic nematodes in Latin America in order to compile the information obtained so far and to disseminate these results. Moreover, researchers from different parts of the world might adapt it considering their respective conditions.

## 10.2 Diversity of Entomopathogenic Nematodes

Entomopathogenic nematodes are found in all continents with a great diversity of species adapted to different environmental conditions, which improves pest control in specific niches. Many of the studies developed in Brazil have been related to the survey of the native fauna of these organisms and include morphological, taxonomic, biological, and ecological studies, tests on target pests, and research involving *in vivo* and *in vitro* preservation and production.

The number of native isolates in Latin America does not reflect the reality of the amount of species, since it is assumed that several other species are yet to be discovered, especially considering the recognized fauna and flora diversity in the different ecosystems and areas of native vegetation. The use of native populations in biological control programs may lead to higher mortality rates of target insects than when using exotic nematodes due to the greater adaptation of species to the local conditions.

The first occurrence of a nematode of the genus *Heterorhabditis* was recorded in Brazil more than 80 years ago by Pereira (1937), and it was described as *Rhabditis hambletoni*, a parasite of “broca-do-algodoeiro” *Gasterocercodes brasiliensis* Hambleton (Curculionidae) (currently named *Eutinobothrus brasiliensis*). Pizano et al. (1985) recorded the occurrence of infective juveniles (JIs) of *Neoalectana glaseri* Steiner (currently called *Steinernema glaseri*) in the egg of *Migdolus fryanus* (Westwood) (Cerambycidae) at the Amália Plant, São Paulo, Brazil. In 1990, Poinar (1990) recorded the occurrence of *Heterorhabditis bacte-*

*riophora* Poinar in the state of Pernambuco. Del Valle et al. (2005) obtained an isolate of *Heterorhabditis baujardi* Phan, Subbotin, Nguyen, & Moens in the state of Rondônia. Machado et al. (2005) isolated *S. glaseri* in soil samples in the Araras region, São Paulo, and *Heterorhabditis indica* Poinar, Karunakar, & David in Itapetininga, São Paulo.

In Amazonas state, Brazil, Andaló et al. (2006) isolated a new species of the genus *Heterorhabditis* in soil samples from Benjamin Constant using larvae of *Galleria mellonella* L. (Pyralidae), which was named *Heterorhabditis amazonensis*. Molina et al. (2005) isolated three nematode populations (JPM3, JPM3.1, and JPM4) in Lavras, Minas Gerais state, which was later identified as *H. amazonensis* by Andaló et al. (2013). In a survey carried out in Lavras, MG, Andaló et al. (2009) obtained other populations of the same species from sorghum (*Sorghum* sp.) and garlic (*Allium sativum*). Nguyen et al. (2010) conducted a study using soil samples from native vegetation areas in the state of Mato Grosso do Sul, in Brazil, and recorded a new species called *Steinernema brazilense*, which has not yet been found in other regions of the country. Barbosa-Negrisoni et al. (2009) isolated, in the state of Rio Grande do Sul, in Brazil, *Steinernema rarum* (Doucet) Mamiya, until then found only in Argentina; *H. bacteriophora*, *Steinernema feltiae* (Filipjev) Wouts, Mracek, Gerdin, & Bedding; and *Steinernema riobrave* Cabanillas, Poinar, & Raulston.

In other Latin American countries, efforts have also been made to isolate entomopathogenic nematodes. In the Southern Cone region, the Argentine Pampas revealed a great diversity of nematodes such as *Steinernema carpocapsae* (Weiser) Wouts, Mracek, Gerdin, & Bedding, *S. feltiae*, *Steinernema scapterisci* Nguyen & Smart, *H. bacteriophora*, and *Heterorhabditis argentinensis* Stock (Leite 2011). In Colombia, *H. bacteriophora* strains were commonly found associated with the cassava stink bug *Cyrtomenus bergi* Froeschner (Cydnidae) (Caicedo and Bellotti 1996) and, in the coffee region of that country, a new species *Steinernema colombiense* was also described by López-Núñez et al. (2008). *Steinernema cubanum* Mracek was isolated from the soil of citrus orchards in Cuba. Similarly, two *H. bacteriophora* (Tetuan and P2M) isolates were identified parasitizing *Cylas formicarius* (Fabricius) (Brentidae) and *Pachnaeus litus* (Germar) (Curculionidae) (Rodríguez et al. 1996). In the states of Jalisco, Colima, Michoacán, and Tamaulipas, in Mexico, different *Steinernema* and *Heterorhabditis* isolates have been found (Lezama-Gutierrez et al. 2001).

Thus, new *Heterorhabditis* and *Steinernema* species have been recorded for Latin America and native isolates have been obtained, providing greater potential of these organisms as pest control agents in this region.

## 10.3 Bioecology

### 10.3.1 Determination of Life Cycle in Native Nematodes

The life cycle and pathogenicity of entomopathogenic nematodes can vary between genera, species, and populations of the same species. This variability must be detected in order for nematodes to be successfully used in biological control programs. Molina et al. (2005) and Andaló et al. (2009) studied the population characteristics of *H. amazonensis* isolated from different locations in Brazil and detected a great variability among them, such as life cycle and insect-host pathogenicity, which underscores the importance of the knowledge of the biological and ecological characteristics of each population. The description of new native species requires the determination of biology to understand its life cycle, which may be short (one generation) or long (2–3 defined generations). An example of a complete life cycle of an entomopathogenic nematode is presented in the study of Molina et al. (2004) for *H. amazonensis* populations JPM3, JPM3.1, and JPM4, isolated in Lavras, Minas Gerais state, Brazil.

One of the factors influencing the life cycle of native isolates is the amount of inoculum used for the infection. *Heterorhabditis amazonensis* JPM4 infecting *G. mellonella* larvae may develop a short life cycle with nematode generation, which includes three development stages: egg, juvenile (J), and adult, with the juvenile phase consisting of four morphologically distinct stages (J1, J2, J3–IJ, and J4). In the adult phase, the first generation consists of hermaphrodite females and the second generation of females and amphimictic males. These first-generation of infective juveniles (IJs) are highly virulent, especially those that abandon the larvae in the first 3 days. The J3 that continue the development within the *G. mellonella* larvae determine a long life cycle, up to 456 hours (19 days), from the beginning of the initial infection to the total exhaustion of the host, determining three generations of nematode production. However, these nematodes of second and third generation are less virulent (Molina et al. 2005).

### 10.3.2 Agricultural Pests Susceptible to the Effect of Entomopathogenic Nematodes

An efficient evaluation of native and exotic *Steinernema* spp. and *Heterorhabditis* spp. nematodes for the control of insect pests in Latin America is applied to several orders, especially Coleoptera, aiming at the larval stages in the soil. However, studies on the action of nematodes on other orders, such as Diptera, Lepidoptera, and Hemiptera, and even other classes of arthropods and phyla have also been developed (Table 10.1).

**Table 10.1** Pest/crop and entomopathogenic nematodes evaluated in laboratory and/or field experiments in Latin America

Pest/crop	Biocontrol agent (country) <sup>a</sup>	Reference
<b>Lepidoptera</b>		
<i>Grapholita molesta</i> – Peach	<i>H. bacteriophora</i> RS33 (BR)	Barbosa-Negrisoni et al. (2013)
<i>Bonagota salubricola</i> – Apple	<i>H. bacteriophora</i> RS107 (BR)	Barbosa-Negrisoni et al. (2010)
<i>Spodoptera frugiperda</i> – Maize	<i>H. amazonensis</i> RSC02 (BR)	Andaló et al. (2010)
<i>Diatraea saccharalis</i> – Sugarcane	<i>H. amazonensis</i> JPM4 + <i>Metarhizium anisopliae</i> LPP39 (BR)	Molina et al. (2008), Bellini and Dolinski (2012)
<b>Coleoptera</b>		
<i>Conotrachelus psidii</i> – Guava	<i>H. bacteriophora</i> LPP30 (BR) <i>H. indica</i> IBCB05 (BR)	Silva et al. (2010), Dolinski et al. (2012)
<i>Sphenophorus levis</i> – Sugarcane	<i>S. braziliense</i> (BR)	Leite et al. (2012), Gionetti et al. (2011), Tavares et al. (2007, 2009)
<i>Alphitobius diaperinus</i> – Aviary	<i>Steinernema arenarium</i> (BR)	Alves et al. (2005, 2012), Rodrigues et al. (2009)
<i>Diabrotica speciosa</i>	<i>H. amazonensis</i> RSC01 (BR)	Santos et al. (2011)
<i>Rhynchophorus palmarum</i>	<i>Heterorhabditis</i> spp. and <i>Steinernema</i> spp. (BR)	Sabino (2014)
<i>Cosmopolites sordidus</i> – Banana	<i>S. carpocapsae</i> , <i>S. glaseri</i> , and <i>S. feltiae</i> (PR)	Figuerola (1990)
<i>Premnotrypes vorax</i> – Potato	<i>Steinernema</i> sp. (CO) and <i>Steinernema carpocapsae</i>	Garzon et al. (1996)
<i>Hypothenemus hampei</i> – Coffee	<i>Heterorhabditis</i> sp. and <i>Steinernema</i> (CO)	Molina and Lopez (2009)
<b>Diptera</b>		
<i>Anastrepha fraterculus</i> – Peach	<i>H. bacteriophora</i> RS88 (BR)	Barbosa-Negrisoni et al. (2009)
<i>Ceratitis capitata</i>	<i>S. carpocapsae</i> all, <i>Heterorhabditis</i> sp. JPM4, and <i>S. carpocapsae</i> + extracts (BR)	Rohde et al. (2013), Rohde et al. (2012a, b), Silva et al. (2010)
<i>Bradysia</i> spp. (Sciaridae)	<i>Heterorhabditis</i> spp. and <i>Steinernema</i> spp. (BR)	Leite et al. (2007), Tavares et al. (2012)
<b>Hemiptera</b>		
<i>Dysmicoccus texensis</i>	<i>H. amazonensis</i> JPM3 (BR)	Alves et al. (2009a, b)
<i>Mahanarva</i> spp. – sugarcane and pastures	<i>S. carpocapsae</i> Mexican and <i>Heterorhabditis</i> spp.	Georgis and Hom (1992), Batista and Auad (2010), Batista et al. (2011a, b, 2014)
<i>Cyrtomenus bergi</i> – Cassava	<i>H. bacteriophora</i> LFR 92 and SQC 92 (CO)	Barberena and Bellotti (1998)

(continued)

**Table 10.1** (continued)

Pest/crop	Biocontrol agent (country) <sup>a</sup>	Reference
Isoptera		
<i>Cornitermes cumulans</i>	<i>Steinernema carpocapsae</i> (BR)	Rosa et al. (2007, 2008)
Arachnida		
<i>Rhipicephalus microplus</i>	<i>Heterorhabditis bacteriophora</i> HP88 <i>H. indica</i> LPP1 <i>H. amazonensis</i> RSC5 + associations (extracts, miticides, and fungi) (BR)	Monteiro et al. (2012, 2013, 2014a, b), Silva et al. (2012)
Plant-parasitic nematodes		
<i>Meloidogyne mayaguensis</i>	<i>H. baujardi</i> LPP7, <i>S. feltiae</i> Sn, and <i>H. amazonensis</i> JPM4 (BR)	Molina et al. (2008, 2010a, 2010b)

<sup>a</sup>BR Brazil, CO Colombia, PR Puerto Rico

### 10.3.3 Factors Influencing the Efficacy of Entomopathogenic Nematode Control

Entomopathogenic nematodes are vulnerable to a number of environmental conditions, as they suffer from the action of biotic and abiotic factors. Unfavorable conditions, such as low moisture, solar radiation, soil texture, and natural enemies, can affect the success in pest control due to reduced activity or death of IJs (Cutler and Webster 2003).

The behavioral characteristics of entomopathogenic nematodes are closely related to the success of the use of these organisms as control agents. Therefore, studies on ecology and biology must first be conducted in order to ensure that IJ will survive according to the biotic and abiotic factors of the environment in which they develop and thus be able to locate the host and cause its death (Lewis 2002).

In the soil, nematodes actively search for their host, and their low mobility may favor the exposure to unfavorable biotic and abiotic factors, reducing the chance of finding and reproducing the target host (Portillo-Aguilar et al. 1999).

#### 10.3.3.1 Biotic Factors

Natural enemies, such as predatory fungi capable of producing mycelial structures to capture nematodes, are included among the unfavorable biotic factors. Andaló et al. (2008) studied the susceptibility of *H. amazonensis* to the fungi *Arthrobotrys oligospora* Fresenius, *A. conoides* Drechsler, and *Duddingtonia flagrans* (Duddington) Cooke by evaluating their predatory capacity. The authors observed the occurrence of predation after 8 days of contact with IJs, concluding that these fungi are capable of preying on *H. amazonensis*. However, an initial contact period was required to stimulate not only the vegetative growth of the fungus, but also the formation of traps. Thus, it is important to consider that possible antagonistic fungi

in the soil should be analyzed in pest control programs with the use of nematodes since many JIs can be predated.

Molina and Lopez (2009) attributed the nondevelopment of *S. cubanum* IJs in late-instar larvae of *Bombyx mori* L. (Bombycidae) to the low efficiency of the multiplication of their symbiotic bacterium, *Xenorhabdus* spp., which does not produce sufficient quantities of antibiotics, such as xenocoumacine, bacteriocin, and xenorababine, in the infection process.

Mertz et al. (2014) studied the forethric dispersion of entomopathogenic nematodes in larvae and adults of *Calosoma granulatum* Perty (Carabidae). Since entomopathogenic nematodes have limited ability to travel long distances, they can use strategies to target hosts in different locations, such as attaching to an organism that will act as a dispersing agent from one location to another. Thus, the authors verified that *C. granulatum* is a good dispersing agent of *H. amazonensis*, and the transport of IJs at distances greater than 40 cm was observed. The ability of *C. granulatum* to transport the nematode to even longer distances is important, since this carabid is a predator of caterpillars inhabiting the soils of agroecosystems in Brazil.

Valle et al. (2008) evaluated the dispersion of *H. baujardi* LPP7 in field guava culture and found nematodes up to 90 cm from the point of release of the entomopathogenic nematode-containing cadavers and up to 10 cm deep. Andaló et al. (2012) evaluated the horizontal and vertical displacement of *H. amazonensis* RSC2 in the search of *Spodoptera frugiperda* (Smith) (Noctuidae) and verified that insect mortality was inversely proportional to the distance between it and the IJs; the nematodes were able to locate and kill the host at distances up to 60 cm (horizontal) and 20 cm (vertical). The authors emphasized the importance of understanding the behavior of entomopathogenic nematodes in the host search, since several factors are involved in this process and behavioral studies are essential to ensure the success of control programs using entomopathogenic nematodes.

### 10.3.3.2 Abiotic Factors

There are several critical abiotic factors for soil nematodes, such as texture, moisture, temperature, and agrochemical residues (McCoy et al. 2002). Dolinski et al. (2010) tested the horizontal displacement of *H. baujardi* LPP7 in soil with different textural classes and found that the highest rates of host mortality and infectivity occurred at a distance of 30 cm for the sandy and sandy clay loam classes, in which mortality was also observed at a distance of 60 and 90 cm. No mortality was observed in the clay loam at 60 and 90 cm. For the clay class, no infection was found for any of the distances evaluated. The authors concluded that *H. baujardi* LPP7 showed better host-seeking behavior on sandy texture substrates, confirming that soil class may be an important indicator of the presence of nematodes and that with increasing clay content, the dispersion, persistence, and efficacy of entomopathogenic nematodes are generally reduced.

Soil temperature is another factor that influences the behavior of nematodes. Kung et al. (1991) stated that entomopathogenic nematodes tolerate temperatures between 3 °C and 35 °C. Low temperatures (5 °C to 10 °C) may affect the occurrence of some entomopathogenic species in the soil, such as *H. bacteriophora*, *S. glaseri*, and *S. carpocapsae*, but it does not affect *S. feltiae* (Brown and Gaugler 1997). Kaya (1990) found that *S. feltiae* could infect its host in the soil at temperatures between 2 °C and 30 °C. Finally, saline soils and soils with high aluminum contents affect the cellular structure and viability of entomopathogenic nematodes (Glazer 1996). Another important factor in the infection and survival of entomopathogenic nematodes is soil pH. Extreme pH values (3 or 11) limit infection capacity, but not its infectivity (Glazer 1996). Andaló et al. (2018) observed that moisture, organic matter, and the levels of phosphorus in the soil are factors that influence the presence of entomopathogenic nematodes in the soil and that knowing these factors is fundamental to enable greater persistence and efficacy of their populations in the field, providing greater chances of pest control.

## 10.4 Final Considerations

The diversity of entomopathogenic nematodes is a subject not fully explored in the ecological studies conducted in Latin American countries, which may be due to the few specialists who are dedicated to the subject, since there is a vast potential research in Latin America, in view of the biodiversity existing in its various biomes.

Many species of entomopathogenic nematodes marketed worldwide are massively produced in vitro in laboratories in the United States, Germany, and the Netherlands. However, since they are species from other regions, in addition to the high costs for production and importation and the regulatory measures of some countries that prevent the entry of exotic agents, their massive use in biological control programs in Latin America becomes unfeasible. Thus, despite the efforts made in the last three decades, which have contributed to the progress in the isolation and perspectives of the use of these organisms against pests of economic importance with potential results, it is necessary to invest in studies related to in vitro production and formulation of commercial native species, since many have high virulence against pest insects.

We believe that it is only through a greater involvement of partnership experts through Latin American research networks, coupled with both government and private initiatives, that we can advance our knowledge of the bioecology of these control agents. Therefore, it is of great importance that more research ought to be carried out in laboratory and field conditions worldwide.



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