2013

Survey of entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in Colima, México

Isaac Zepeda-Jazo
Universidad de la Cie´nega del Estado de Michoaca´n de Ocampo, Universidad de Colima

Jaime Molina-Ochoa
Universidad de Colima, University of Nebraska-Lincoln, jmolina18@hotmail.com

Roberto Lezama-Gutiérrez
Universidad de Colima

Steven R. Skoda
USDA-ARSKBUSLIRL Screwworm Research Unit

John E. Foster
University of Nebraska-Lincoln, john.foster@unl.edu

International Journal of Tropical Insect Science Vol. 34, No. 1, pp. 53–57, 2014 doi:10.1017/S1742758413000416

Short Communication

Survey of entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in Colima, México

Isaac Zepeda-Jazo[1,2], Jaime Molina-Ochoa[3,4]*, Roberto Lezama-Gutiérrez[2], Steven R. Skoda[5] and John E. Foster[4]

[1]Universidad de la Ciénega del Estado de Michoacán de Ocampo, Trayectoria Genómica Alimentaria, Av. Universidad 3000, Col. Centro, Sahuayo, Michoacán 59000, Mexico; [2]Facultad de Ciencias Biológicas y Agropecuarias, Universidad de Colima, Apartado Postal 36, Tecomán, Colima 28100, Mexico; [3]Universidad de Colima, Coordinación General de Investigación Científica, Centro Universitario de Investigación y Desarrollo Agropecuario, Km. 40, Autopista Colima-Manzanillo, Tecomán, Colima 28930, Mexico; [4]Insect Genetics Laboratory, Department of Entomology, University of Nebraska Lincoln, 3B Entomology Hall, Lincoln, NE 68583-0816, USA; [5]USDA-ARS-KBUSLIRL Screwworm Research Unit, Kerrville, TX 78028, USA

(Accepted 21 November 2013)

Abstract. A survey of entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae was conducted in three municipalities on the Pacific coast of the State of Colima, México, to determine their occurrence and recovery frequency and predominant plant species in cultivated and non-cultivated habitats. Nineteen soil samples were collected: seven from non-cultivated habitats and 12 from habitats or areas cultivated mostly with fruit and grain crops and grasses. Of the 19 soil samples, 14 were positive for EPNs; the total prevalence was 73.7%. From the 14 positive soil samples, 12 steinernematid isolates (85.7%) and two heterorhabditid isolates (14.3%) were recovered. Irrespective of the locations, EPNs from the genus *Steinernema* were recovered from the three municipalities; EPNs from the genera *Steinernema* and *Heterorhabditis* were recovered from Armería and Ixtlahuacán. Only steinernematid isolates were recovered from non-cultivated habitats. Most of the isolates were recovered from cultivated habitats, and our results suggest that there is a higher prevalence of EPNs in cultivated soils.

Key words: *Heterorhabditis*, *Steinernema*, México, natural habitats, survey

*E-mail: jmolina18@hotmail.com; jmolina@ucol.mx

Introduction

Entomopathogenic nematodes (EPNs) from the families Heterorhabditidae and Steinernematidae are microbial obligate pathogens that infect a wide range of insects in the laboratory. In the field, they mainly infect the soil-dwelling forms of insects belonging to the orders Lepidoptera, Coleoptera and Diptera and a few other soil arthropods (Mráček *et al.*, 1999). EPNs from the family Heterorhabditidae are mutualistically associated with bacteria of the genus *Photorhabdus* and those from the family Steinernematidae with bacteria of the genus *Xenorhabdus* (Burnell and Stock, 2000).

The third-stage infective juvenile nematodes (IJs), also known as dauers, are potentially useful as agents for the biological control of numerous insect pests (Hominick *et al.*, 1995) These IJs are naturally found in the soil, where they are attracted to suitable hosts by the hosts' faeces or CO_2. IJs enter the host through the natural openings, and once they invade the haemocoel through the midgut wall, they release their symbiont bacteria that rapidly multiply, killing the host within 24–48 h of septicaemia (Hazir *et al.*, 2003).

The natural distribution of biological control organisms is influenced by the insect host's age and by habitat, soil type, pesticide use, agricultural practices and location (Fuxa, 1982; Mietkiewski *et al.*, 1997). EPNs are found in broadly diverse soil habitats and exhibit considerable variations in terms of host range, reproduction, infectivity and survival conditions (pH, organic matter, temperature, soil moisture, etc.) (Stock *et al.*, 1999). Colima has a unique geographical location, surrounded by mountains and the Pacific Ocean, with a diversity of habitats that may contribute to the diversification of the distribution of EPNs.

EPNs have unique attributes ideal for their use as biological control agents: they have a broad host range, can be mass-produced, are environmentally safe, can be easily applied or sprayed, are compatible with most chemical pesticides, etc. (García del Pino and Palomo, 1996). Owing to these characteristics, they have been successfully applied to control insect pests in row crops, vegetables and orchards in many countries.

In México, the Universidad de Colima has conducted studies to determine the potential of exotic EPNs to control insect pests, evaluating them in laboratory and field conditions against fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Molina-Ochoa *et al.*, 1996, 1999), Mexican fruit flies, *Anastrepha* spp. (Lezama-Gutiérrez *et al.*, 1996), and agave weevil, *Scyphophorus acupunctatus* Gyllenhaal (Molina-Ochoa *et al.*, 2004). A native steinernematid strain, *Steinernema diaprepesi*, was isolated from grassland in Colima, México, and evaluated against engorged cattle ticks and was found to cause significant adult tick mortality (Molina-Ochoa *et al.*, 2009).

In the State of Colima, several steinernematid and heterorhabditid nematodes have been recovered from agricultural areas (Lezama-Gutiérrez *et al.*, 2001; Molina-Ochoa *et al.*, 2003). However, no survey has been conducted to determine the occurrence of EPNs in cultivated or natural/non-cultivated habitats. Herein, we report the results of a survey conducted to determine the occurrence and recovery frequency of EPNs and the predominant plant species in the cultivated and non-cultivated habitats of the coast of the State of Colima.

Materials and methods

Soil samples were collected from the municipalities of Manzanillo, Armería and Ixtlahuacán, located in the coast of the State of Colima, México, from March to May 2004.

In Manzanillo, three soil samples were collected from non-cultivated habitats with predominant vegetation locally named barcino in Spanish (*Cordia elaeagnoides* DC.), palma de cayuco or babassu (*Orbignya cohune* (Martius) Dahlgren ex Standley) and red mangrove (*Rhizophora mangle* L.). Three soil samples were collected from cultivated habitats planted with bananas (*Musa paradisiaca* L.), as well as associated forage crops of sorghum/coconut palms (*Sorghum bicolor* L. (Moench.) × *Sorghum halepense* Piper (Stapf.)/*Cocos nucifera* L.) and corn (*Zea mays* L.).

In Armería, only one soil sample was collected from a non-cultivated habitat where the predominant vegetation was sage (*Salvia* spp.), and six soil samples were collected from a Mexican lime grove (*Citrus aurantifolia* (Swingle) Tanaka), a corn field (*Z. mays* L.), grassland of African star grass (*Cynodon nlemfuensis* Vanderyst), an associated Mexican lime/coconut palm orchard (*C. aurantifolia*/*C. nucifera*), a Jalapeño hot pepper orchard (*Capsicum annuum* L.) and an associated banana/coconut palm orchard (*M. paradisiaca*/*C. nucifera*).

In Ixtlahuacán, three soil cores were collected from non-cultivated habitats where the predominant vegetation was habillo (*Hura polyandra* Baill.), barcino (*C. elaeagnoides*), nopal cactus (*Opuntia* spp.) and columnar cacti (*Stenocereus* spp.), and three soil samples were collected from areas cultivated with corn (*Z. mays* L.), muskmelon (*Cucumis melo* L.) and gamba grass (*Andropogon gyanus* Kunth.).

Each soil sample, weighing approximately 1 kg, was a composite of five random subsamples collected at least 100 m apart at each site at a depth of 10–20 cm in an area of 20 m^2.

Soil samples were placed in polyethylene bags to avoid the loss of moisture and then kept in coolers containing refrigerant gel packs during the transit to the laboratory and stored at 15 °C (Stock *et al.*, 1999) in the Entomopathogenic Nematology Laboratory of the Universidad de Colima at Tecomán, Colima, México.

The stored soil samples were processed within 1 week of collection. To bait the soils for the recovery of EPNs and entomopathogenic fungi (EPF), each sample of 1 kg weight was thoroughly mixed and a subsample of ca. 240 cc was placed in a 250 cc plastic container. Then, five last instar larvae of the greater wax moth (GWM), *Galleria mellonella* L., were placed in the soil sample and the container was covered with a lid and inverted (Kaya and Stock, 1997). The container was held at room temperature (20 ± 3 °C) for a period of 7–8 days.

GWM larvae were infected and killed by EPNs and EPF during the 15-day baiting period. Cadavers of GWM were recovered from the baited traps, disinfected with a solution of sodium hypochlorite (1%) for 3 min and rinsed with distilled water three times. They were collected at 3-day intervals over 15 days after set-up and transferred to White traps to collect the emerging IJs (Kaya and Stock, 1997).

IJs that emerged were pooled from each sample and used to infect fresh last instars of GWM to verify their pathogenicity and allow the production of progeny for identification at the genus level, considering the characteristic colour of the GWM cadavers (Kaya and Stock, 1997). Using Sabouraud dextrose yeast extract agar, with 500 ppm of chloramphenicol (Lezama-Gutiérrez *et al.*, 2001), EPF were isolated from the GWM cadavers and identified by microscopic inspection of morphological characteristics (Brady, 1979). Soil samples that were negative for EPNs and EPF during the first round were baited again with the last instars of GWM. All the soil samples were maintained at room temperature.

Results and Discussion

Current research efforts were focused on isolating EPNs and EPF that may be ubiquitous in the cultivated and non-cultivated soils of the State of Colima, México. Nineteen soil samples were collected from three municipalities (Table 1): seven from non-cultivated habitats and 12 from habitats or areas cultivated mostly with fruit and grain crops and grasses. EPNs were recovered from 14 of the 19 soil samples with a total prevalence of 73.7%; EPF were recovered from two of the 19 soil samples with a total prevalence of 10.5%.

From the 14 positive soil samples, 12 steinernematid isolates (85.7%) and two heterorhabditid isolates (14.3%) were recovered from all the three municipalities. Stock *et al.* (1999) reported a similar proportion of recovery frequency of steinernematid isolates (80%) in a survey conducted in California, USA; however, the proportion of heterorhabditid isolates reported by them was higher than that reported by us (20%).

Table 1. Predominant plant species and recovery frequency of the entomopathogenic nematodes and fungi in the coast of the State of Colima, México

Sample	Municipality	Predominant species	Altitude (m)	Recovery frequency	Nematodes or fungus genera
1	Manzanillo	*Musa paradisiaca*	14	1/5	*Steinernema*
2	Manzanillo	*Cordia elaeagnoides*	90	4/5	*Steinernema*
3	Manzanillo	*Sorghum/Cocos nucifera*	18	5/5	*Steinernema*
4	Manzanillo	*Orbignya cohune*	47	3/5	*Steinernema*
5	Manzanillo	*Zea mays*	29	1/5	*Steinernema* and *Metarhizium*
6	Manzanillo	*Rhizophora mangle*	6	5/5	*Steinernema*
7	Armería	*Citrus aurantifolia*	90	5/5	*Steinernema*
8	Armería	*Z. mays*	64	2/5	*Steinernema* *Heterorhabditis*
9	Armería	*Cynodon nlemfuensis*	65	3/5	*Steinernema*
10	Armería	*C. aurantifolia/C. nucifera*	41	2/5	*Steinernema*
11	Armería	*Salvia* sp.	77	2/5	*Steinernema* and *Beauveria*
12	Armería	*Capsicum annuum*	10	0/5	–
13	Armería	*M. paradisiaca/C. nucifera*	3	2/5	*Steinernema*
14	Ixtlahuacán	*Hura polyandra*	373	2/5	*Steinernema*
15	Ixtlahuacán	*Z. mays*	136	0/5	–
16	Ixtlahuacán	*Opuntia* sp./*Stenocereus* sp.	59	0/5	–
17	Ixtlahuacán	*C. elaeagnoides*	121	0/5	–
18	Ixtlahuacán	*Cucurbita melo*	147	1/5	*Heterorhabditis*
19	Ixtlahuacán	*Andropogon gyanus*	131	0/5	–

EPNs from the genus *Steinernema* were recovered from the three municipalities. EPNs from the genera *Steinernema* and *Heterorhabditis* were recovered from Armería and Ixtlahuacán. Other important entomopathogenic microorganisms isolated were the fungus *Metarhizium* sp. in a Manzanillo soil sample cultivated with corn and in a non-cultivated area where the predominant vegetation was red mangrove and the fungus *Beauveria* sp. from an area in Armería with sage as the predominant vegetation. The isolation of EPF and other microorganisms from cultivated soils has also been reported in surveys conducted previously in Mexico (Lezama-Gutiérrez *et al.*, 2001; Molina-Ochoa *et al.*, 2003).

Most (64.3% = 9/14) of the soil samples positive for the recovery of EPNs were from cultivated habitats and only 35.7% (5/14) were from non-cultivated habitats. No nematodes were recovered from soil samples collected from areas cultivated with hot peppers in Armería or from those cultivated with corn and grasslands cultivated with gamba grass in Ixtlahuacán. However, negative results were also obtained for non-cultivated areas where the predominant vegetation was nopal cactus, columnar cacti and barcino (Table 1).

In terms of species diversity, the associations of sorghum, Mexican lime and banana/coconut palms were the richest cultivated habitats, yielding three of the eight *Steinernema* isolates recovered, but *Heterorhabditis* isolates were recovered from corn and muskmelon orchards (Table 1). Isolates from the genus *Steinernema* were recovered in all the positive non-cultivated habitats, but three of the five positive soil samples were obtained from Manzanillo (Table 1). Our results differ from those reported by Stock *et al.* (1999), because the majority of their positive samples were recovered from woodlands and coniferous and oak forests, considered as non-cultivated areas; in their study, negative results were obtained for chaparral habitats, redwood forest and desert habitats.

The occurrence of EPN and EPF isolates in cultivated and non-cultivated soils in Colima is demonstrated for the first time in this survey. The EPNs were differentially distributed in cultivated and non-cultivated soils and their isolates were recovered in 73.7% of the soils sampled.

Most of the EPN isolates were recovered from cultivated habitats. Our results suggest that there is a higher prevalence of EPNs in cultivated habitats or cultivated soils. We speculate that the environmental conditions, particularly soil moisture, favoured the recovery of EPNs, because we conducted the survey during the spring and early summer. The rainy season increased the soil moisture in the non-cultivated habitats and also the cultivated habitats were under irrigation. Lezama-Gutiérrez *et al.* (2001) emphasized the role of rainfall in influencing the parasitism of entomopathogenic microorganisms; this point of view supports our speculation.

Conclusion

Steinernematid nematodes were more frequently recovered than heterorhabditid nematodes from both cultivated and non-cultivated habitats. The associations of sorghum, Mexican lime and banana/coconut palms were the richest cultivated habitats, yielding three of the eight *Steinernema* isolates recovered. Steinernematid nematodes were also recovered without distinction in annual or perennial crops. Heterorhabditid nematodes were isolated only from soils cultivated with corn and muskmelon. The higher frequency of recovery in the Colima soils observed in this survey suggests the adaptability of the steinernematids to a wider range of habitats in comparison with the heterorhabditids, as only a couple of EPF species were recovered: *Metarhizium* sp. and *Beauveria* sp.

Acknowledgements

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture (USDA). USDA is an equal-opportunity provider and employer.

References

Brady B. L. K. (1979) *CMI Descriptions of Pathogenic Fungi and Bacteria. Sets 61 and 62 (Nos. 601–620).* Commonwealth Mycological Institute, Egham.

Burnell A. M. and Stock S. P. (2000) *Heterorhabditis*, *Steinernema* and their bacterial symbionts – lethal pathogens of insects. *Nematology* 2, 31–42.

Fuxa J. R. (1982) Prevalence of viral infections in populations of fall armyworm, *Spodoptera frugiperda*, in southeastern Louisiana. *Environmental Entomology* 11, 239–242.

García del Pino F. and Palomo A. (1996) Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Spanish soils. *Journal of Invertebrate Pathology* 68, 84–90.

Hazir S., Keskin N., Stock P. S., Kaya H. K. and Özcan S. (2003) Diversity and distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Turkey. *Biodiversity and Conservation* 12, 375–386.

Hominick W. M., Reid A. P. and Briscoe B. R. (1995) Prevalence and habitat specificity of steinernematid and heterorhabditid nematodes isolated during soil surveys of the UK and the Netherlands. *Journal of Helminthology* 69, 27–32.

Kaya H. K. and Stock S. P. (1997) Techniques in insect nematology, pp. 281–324. In *Manual of Techniques in Insect Pathology* (Edited by L. A. Lacey). Academic Press, New York.

Lezama-Gutiérrez R., Hamm J. J., Molina-Ochoa J., López-Edwards M., Pescador-Rubio A., González-Ramírez M. and Styer E. L. (2001) Occurrence of entomopathogens of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Mexican States of Michoacán, Colima, Jalisco and Tamaulipas. *The Florida Entomologist* 84, 23–30.

Lezama-Gutiérrez R., Molina-Ochoa J., Contreras-Ochoa O. L., González-Ramírez M., Trujillo-De la Luz A. and Rebolledo-Domínguez O. (1996) Susceptibilidad de larvas de *Anastrepha ludens* (Diptera: Tephritidae) a diversos nematodos entomopatógenos (Steinernematidae y Heterorhabditidae). *Vedalia* 3, 31–33.

Mietkiewski R. T., Pell J. K. and Clark S. J. (1997) Influence of pesticide use on the natural occurrence of entomopathogenic fungi in arable soils in the UK: field and laboratory comparisons. *Biocontrol Science and Technology* 7, 565–575.

Molina-Ochoa J., Hamm J. J., Lezama-Gutiérrez R., Bojalil-Jaber L. F., Arenas-Vargas M. and González-Ramírez M. (1996) Virulence of six entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) on immature stages of *Spodoptera frugiperd*a (Lepidoptera: Noctuidae). *Vedalia* 3, 25–29.

Molina-Ochoa J., Hueso-Guerrero E. J., Lezama-Gutiérrez R., Farías-Larios J., Radillo-Juárez F., García-Berber A. and Fallad-Chávez J. (2004) Virulence of native and exotic entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) against larvae of the agave weevil, *Scyphophorus acupunctatus* (Coleoptera: Curculionidae). *HortScience* 39, 832.

Molina-Ochoa J., Lezama-Gutierrez R., González-Ramírez M., López-Edwards M., Rodríguez-Vega M. A. and Arceo-Palacios F. (2003) Pathogens and parasitic nematodes associated with populations of fall armyworm (Lepidoptera: Noctuidae) larvae in Mexico. *The Florida Entomologist* 86, 244–253.

Molina-Ochoa J., Lezama-Gutiérrez R., Hamm J. J., Wiseman B. R. and López-Edwards M. (1999) Integrated control of fall armyworm (Lepidoptera: Noctuidae) using resistant plants and entomopathogenic nematodes (Rhabditida: Steinernematidae). *The Florida Entomologist* 82, 263–271.

Molina-Ochoa J., Nguyen K. B., González-Ramírez M., Quintana-Moreno M. G., Lezama-Gutiérrez R. and Foster J. E. (2009) *Steinernema diaprepesi* (Nematoda: Steinernematidae): its occurrence in western Mexico and susceptibility of engorged cattle ticks *Boophilus microplus* (Acari: Ixodidae). *The Florida Entomologist* 92, 661–663.

Mráček Z., Bečvář S. and Kindlmann P. (1999) Survey of entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in the Czech Republic. *Folia Parasitologica* 46, 145–148.

Stock S. P., Pryor B. M. and Kaya H. K. (1999) Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California, USA. *Biodiversity and Conservation* 8, 535–549.