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Short Communication

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

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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

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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 CO₂. 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, Mexico, 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.), palm 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* BAIL.), 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 gyanos* 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 m².

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 Tecoman, Colima, Mexico.

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-Gutierrez 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, Mexico. 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, Mexico

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

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