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STEINERNEMA DIAPREPESI (NEMATODA: STEINERNEMATIDAE): ITS OCCURRENCE IN WESTERN MEXICO AND SUSCEPTIBILITY OF ENGORDED CATTLE TICKS *BOOPHILUS MICROPLUS* (ACARI: IXODIDAE)

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Entomopathogenic nematodes (EPN) belong to the families Steinernematidae and Heterorhabditidae. They infect a wide range of insect hosts and are used to control numerous species of soil borne insect pests (Grewal 2002). We identified EPN genera based on the color of cadavers of the wax moth, *Galleria mellonella* L., in which *Heterorhabditis* and *Steinernema*, produce red and tan colors, respectively, according to Woodring & Kaya (1988). We obtained an isolate of an EPN from sandy loam soil with the *Galleria* technique (Bedding & Akhurst, 1975) from grasslands in the Campus Tecomán of the Universidad de Colima, and we designated it as JMO94. In 2008, the morphometry and molecular characterization of JMO94 was conducted at the University of Florida Gainesville, Department of Entomology and Nematology under the supervision of Dr. K. B. Nguyen, and our JMO94 isolate was identified as *Steinernema diaprepesi* by morphological, morphometrical, and molecular results. Our strain is now labeled as *S. diaprepesi* Colimense strain, and represents a new record of occurrence of *S. diaprepesi* outside of the United States. DNA extraction, PCR amplification, sequencing, multiple alignments and other processes were conducted by methodology reported by Nguyen et al. (2001, 2004, 2006). PCR amplification was conducted by methodology reported by Nguyen et al. (2004) with the following exceptions: for ITS regions, the 2 internal primers for ITS regions suggested by Nguyen et al. (2001): KN58 = 5'-GTATGTTTGGTTGAAGGTC-3' and KNRV = 5'-CACGCTCATACAACTGCTC-3', were used; and for region D2/D3 regions, the primers D2F, 5'-CCTTAGTAACGGCGAGTGAAA-3' (forward) and 536, 5'-CAGCTATCCTGAGGAAAC-3' (reverse), were used as external primers to amplify the D2/D3 regions; primers 502, 5'-CAAGTACCGTGAGGGAAAGTTGC-3' (forward) and 503, 5'-CCTTGGTCCGTGTTTCAAGACG-3' (reverse), were used as internal primers for sequencing. Molecular phylogenetic relationships were obtained by maximum parsimony (MP) with PAUP, 4.0b8 (Swofford 2002). For phylogenetic analysis of the ITS regions, *Steinernema intermedium* was treated as the outgroup taxon for resolving relationships among the rest of *Steinernema* species (Nguyen et al. 2001). For D2/D3 regions, *Panagrellus redivivus* was used as the outgroup taxon (Stock et al. 2001). Branch support was estimated by bootstrap analysis (1000 replicates) based on the same parameters as the original search.

Steinernema diaprepesi has been isolated from citrus groves in Polk County, Florida, naturally attacking the citrus root weevil, *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae) (Nguyen & Duncan 2002), and has been isolated from other orchards located on the central ridge of Florida, which is characterized by deep sandy soils similar to our soils in the Agricultural Experimental Station in the Tecomán Campus.

We used the Petri dish bioassay procedure (Freitas et al. 2005; Reis-Menini et al. 2008) to evaluate the susceptibility of engorded ticks (*Boophilus=Rhipicephalus microplus* Canestrini) to concentrations of 0, 500, 1000, 2000, 4,000, 8,000, and 16,000 infective juveniles (IJ) of *S. diaprepes* Colimense strain applied in 1 mL of sterile distilled water dispensed on the surface of a couple of moistened filter papers (Whatman No. 1) in Petri

dishes. For each treatment concentration, 10 ticks were placed in the Petri dishes (60 × 15 mm) and each treatment was replicated 4 times.

Tick mortality was recorded every 24 h for 9 d (de Oliveira-Vasconcelos et al. 2004). Dead ticks were incubated and later examined to confirm the presence of nematodes. To compare tick mortality among different nematode concentrations, analysis of variance was used. Angular transformation was performed on the percent of mortality by square-root transformation before the analysis. Means were separated by the Tukey test (P = 0.05) (SAS Institute 1985).

The ANOVA showed statistical differences for all the sources of variance including the interaction concentrations x times ($P < 0.001$). The highest mortality (approx. 40%) was obtained with the concentration of 2000 IJ after 9 d post-exposure (Quintana-Moreno et al. 2007).

We observed a positive correlation between mortality of engorded ticks and the concentration of EPN and exposure time. Similar results were reported by Freitas-Rivero et al. (2005) with *Boophilus microplus*, and Samish et al. (1999) with other species of ticks. Because the cattle ticks are not specific hosts of *Steinernema diaprepesi*, we speculate that this is one of the reasons for the low cumulative mortality exhibited in our Petri dish experiment. Another possible reason is the body dimensions of the IJs; *S. diaprepesi* has a long body length of 1002 µm and a width of 34 µm. These dimensions could reduce the opportunities to penetrate the natural openings of the engorded ticks.

The potential of our strain could be determined in the future with other insect pest species in western Mexico such as the agave weevil, *Scyphophorus acucpunctatus* Gyllenhal (Coleoptera: Curculionidae), and the coconut weevil, *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae).

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Summary

A steinernematid nematode was isolated from soil of grasslands in Tecomán Colima, Mexico; it was initially designated as JMO94, and determined to be *Steinernema diaprepesi* Colimense strain based upon morphometrics and molecular data; the susceptibility of cattle engorded ticks, *Boophilus microplus,* to the nematode strain is reported. The highest tick cumulative mortality was approximately 40% after 9 d post-exposure to the nematode.

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