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FORESTRY SCIENCE

Steinernema diaprepesi Nguyen & Duncan (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Brazil

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Abstract: Entomopathogenic nematodes (EPNs) can control pests due to their mutual association with bacteria. The use of these biological control agents is increasing worldwide due to advances in research about its control efficiency, range of action and mass production. The identification of EPNs adapted to specific environmental and climatic conditions is important for sustainable pest suppression in integrated management (IPM) programs. The objective is to report, for the first time, the occurrence of the *Steinernema diaprepesi* in Brazil. Steel mesh traps with *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) larvae were buried in red latosol cultivated with *Eucalyptus*. Infective juveniles (IJs) were isolated from dead larvae and multiplied in healthy ones of this host to confirm its pathogenicity and to start a laboratory population from the strain found in the field. The DNA of the IJs was extracted and amplified using PCR technique with the universal primers D2A and D3B. The detection of *S. diaprepesi* is the first report of this nematode in Brazil, increasing the knowledge about its distribution in the world and the diversity of EPNs that must be considered as agents of biological pest control in the country.

Key words: Biological control, crop protection, *Galleria mellonella*, infective juveniles, PCR.

INTRODUCTION

Steinernematidae nematodes can control important pests in the world (Javal et al. 2019, Salgado-Morales et al. 2019, Mbata et al. 2018). Entomopathogenic nematodes (EPNs) live in symbiotic association with bacteria of the genus *Xenorhabdus* (Poinar & Grewal 2012), responsible for host death and decomposition of their tissues which are used as food for the development of EPNs (Nermut et al. 2019). The use of these biological agents is increasing due to advances in the mass production, application technology and their range of action (Dolinski et al. 2012, Lacey & Georgis 2012, Gumus et al. 2015).

Research on EPNs in most South American countries is scarce (Nguyen et al. 2010, De Brida et al. 2017). Furthermore, many studies are published in non-English journals as thesis dissertations, conference proceedings and other non-readily available sources (San-Blas et al. 2019), limiting the access and comparison of data.

Steinernema glaseri (Steiner 1929), a species originally reported in North America, was the first Steinernematidae reported in Brazil, isolated from the eggs of *Migdolus fryanus* Westwood (Coleoptera: Cerambycidae) in the São Paulo state (Pizano et al. 1985). *Steinernema brazilense*

Nguyen, a species native to Brazil, was identified in 2010 (Nguyen et al. 2010). *Steinernema carpocapsae* (Hominick 2002) and *Steinernema rarum* (De Brida et al. 2017) were isolated from collections in agricultural areas in the country.

The identification of EPNs adapted to local environmental and climatic conditions is important because some factors may interfere on their survival, behavior and development, such as predators, ultraviolet radiation, temperatures, soil moisture and texture, osmotic stress and pesticides (Andaló et al. 2018, Glazer 2002, Brown & Gaugler 1997) that can affect sustainable pest suppression in integrated pest management programs (De Brida et al. 2017).

The objective of this study is to report, for the first time, the occurrence of the entomopathogenic nematode, *Steinernema diaprepesi* Nguyen & Duncan (2002) in Brazil.

MATERIALS AND METHODS

Nematode survey at field

Twenty steel mesh traps, with two seventh instar *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae each, were buried in Red Latosol at 5 cm depth in a commercial plantation (22º 59' 49'' S, 48º 29' 57'' W, 870 m) of *Eucalyptus grandis* x *Eucalyptus urophylla* clones. The relative humidity, atmospheric and soil temperature were measured with thermo hygrometer and soil thermometer during the period on what the traps were installed in the field and these factors ranged from 50 to 98%, 15 to 36°C and 20 to 25°C, respectively. After seven days, the traps were removed from the soil, packed in plastic bags and transported to the FCA/UNESP Nematology Laboratory for isolation and identification of entomopathogenic nematodes from the field. Dead *G. mellonella* larvae were removed from the traps, washed in sodium hypochlorite solution (1%) and transferred to White traps (White 1927) and maintained in an incubator chamber (B.O.D.) at 25 °C (mean soil temperature at which the nematodes were collected). The IJs emergence from the White traps, multiplied from the primary infection by EPNs in the field, was observed daily during 21 days in laboratory. The live larvae were observed on until adult emergence to confirm non-infection by EPNs.

Multiplication of the isolates

Infective juveniles (IJs) obtained from dead *G. mellonella* larvae of field traps were multiplied in healthy larvae of this host in Petri dishes (9 cm in diameter x 1.5 cm in height) coated with filter paper moistened with a suspension of 500 IJs/cm². *Galleria mellonella* larvae dead inoculated with IJs were transferred to White traps (White 1927) and maintained in an incubator chamber (B.O.D.) at 25 °C for three days to establish the first population of these nematodes in the laboratory (LP1).

Molecular identification

The genomic DNA of 50 IJs from LP1 was extracted in 50 µl of 0.85% NaCl solution by Worm Lysis Buffer (WLB) extraction method (Carvalho et al. 2018, Williams et al. 1992). These samples were left at -70 °C for 15 minutes, incubated for one hour at 60 °C, another 15 minutes at 95 °C and stored at -20°C.

The DNA extracted was amplified using the polymerase chain reaction (PCR) technique with the universal primers D2A and D3B for the expansion of the 28S rDNA sequence (Al-Banna et al. 2004). The reactions were performed in an INFINIGEN thermal cycler (model TC-96CG) with 12.5 µl of Polymerase Mix Master Red (Neobio), 7.5 µl of NucleaseFreeWater (Promega), 1 µl of each primer (10 mM) and 3 µl of genomic DNA per sample, totaling 25 µl of solution. The cycles for the universal primers D2A and D3B were

done (Mracek et al. 2006). Negative controls with 3 µl of water were added in the assays to check for possible PCR reagent contaminations. PCR amplification products were visualized by 1% agarose gel electrophoresis with marker (Norgen) and UV light transilluminator (Major Science).

The PCR product was purified according to the recommendations of the Celcco PCR Purification Kit (Qiagen, Cat#14400) and sequenced in a DNA Sanger automated sequencer (Model: ABI 3500, Applied Biosystems) at the Institute of Biotechnology (IBTEC-UNESP). Sequences were compared on BLAST, with data deposited at GenBank (http://www.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Galleria mellonella dead larvae were found in four traps in the field with EPNs infection symptoms (Figure 1). The mortality of this host at the concentration 1.000 of JIs was 100% after three days of inoculation. The IJs emergence from larvae of this host was observed confirming the virulence of this nematode.

The nucleotide sequences obtained were 100% similar to those of *Steinernema diaprepesi* (Rhabditida: Steinernematidae) (accession number GU173994.1) (Figure 2).

The pathogenicity of *S. diaprepesi* was previously reported important pests as *Diabrotica virgifera* virgifera LeConte (Coleoptera: Chrysomelidae) (Geisert et al. 2018), *Diaprepes*

Figure 1. Dead *Galleria mellonella* larvae collected with steel mesh trap showing symptoms of entomopathogenic nematode infection.

abbreviates Linnaeus (Coleoptera: Curculionidae) (Nguyen & Duncan 2002, El-Borai et al. 2007, Ali et al. 2010) and *Helicoverpa gelotopoeon* Dyar (Lepidoptera: Noctuidae) (Caccia et al. 2017). High virulence of this nematode was confirmed for *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) with mortality greater than 93% when exposed to doses of at least 50 IJs/insect (Caccia et al. 2017). This insect is one of the most important pest in Brazilian maize fields (Garcia et al. 2019) causing significant economic losses. The virulence of the nematode *S. diaprepesi* is higher for lepidopteran larvae than for some species of the orders Coleoptera and Diptera, and null for Orthoptera (Del Valle et al. 2014). The *S. diaprepesi* efficacy against pests is attributed to its symbiotic bacterium, *Xenorhabdus doucetiae* Tailliez et al. (2006) (Caccia et al. 2017), with capacity to develop and reproduce at temperatures above 35 ºC (Tailliez et al. 2006).

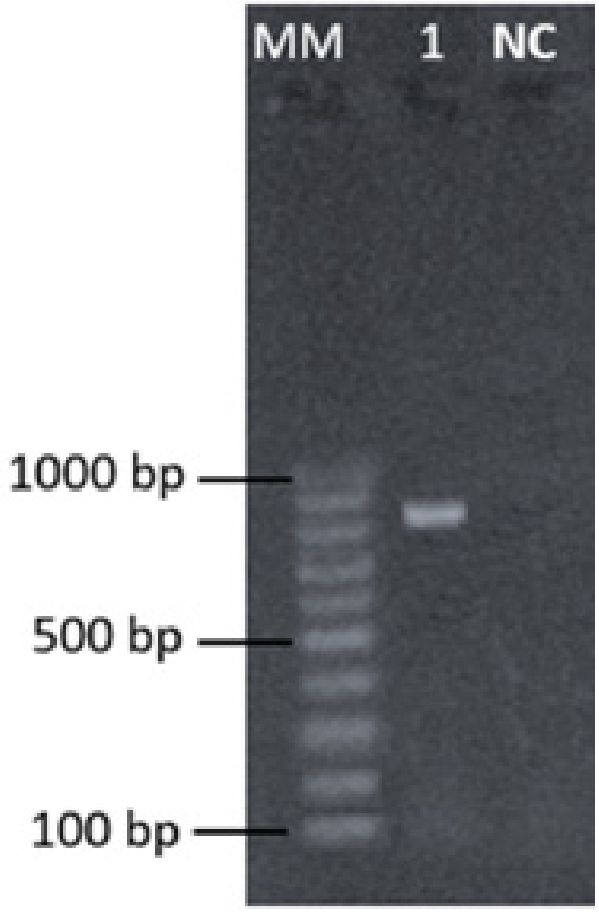

Figure 2. 1% agarose gel. MM= 100 bp molecular weight, 1: amplification of the 28S rDNA region (800 bp) using primers D2A and D3B, NC: Negative control.

The detection of *S. diaprepesi* in *Eucalyptus* plantations is the first report of this nematode species in Brazil. This species was first described in Florida, USA, isolated from *Diaprepes abbreviatus* Linnaeus (Coleoptera: Curculionidae) (Nguyen & Duncan 2002) and later in Venezuela (Spiridonov et al. 2004) in the Caribbean islands Martinique and Guadeloupe (Tailliez et al. 2006), in Mexico (Molina-Ochoa et al. 2009) and in Argentina (Caccia et al. 2017). The diversity of hosts and predators, the incidence of ultraviolet radiation on the soil surface, temperature, soil moisture and texture, osmotic stress and insecticides may interfere with the survival, behavior and development of nematodes (Brown & Gaugler 1997, Glazer 2002, Andaló et al. 2018). Therefore, detection of new EPNs species, which can be used as biological control agents, adapted to the different environments and edaphoclimatic conditions are fundamental for the IPM success programs (Andaló et al. 2014, Brown & Martin 2014).

The *S. diaprepesi* detection in Brazil increases the knowledge about the distribution of this nematode in the world and the diversity of EPNs that should be considered as agents of biological control of pests. Future studies will show the potential of *S. diaprepesi* as new alternative biological agent into the IPM programs in Brazil.

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