Efficacy of Entomopathogenic Nematodes Versus Diaprepes abbreviatus (Coleoptera: Curculionidae) Larvae in a High Clay-Content Oxisol Soil: Greenhouse Trials With Potted Litchi chinensis

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Source: Florida Entomologist, 91(1) : 75-78

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2008)091[0075:EOENVD]2.0.CO;2

EFFICACY OF ENTOMOPATHOGENIC NEMATODES VERSUS *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) LARVAE IN A HIGH CLAY-CONTENT OXISOL SOIL: GREENHOUSE TRIALS WITH POTTED *LITCHI CHINENSIS*

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ABSTRACT

In a previous study, laboratory trials indicated that 9 strains and species of entomopathogenic nematodes (EPNs) were pathogenic against larvae of *Diaprepes abbreviatus* in an Oxisol from Puerto Rico. In this study we tested the efficacy of 5 species/strains of EPN in an Oxisol under greenhouse conditions. The nematodes were applied at 100 infective juveniles per cm^2 to 19-L pots containing a high clay-content Oxisol (69% clay) and 3 seedlings of *Litchi chinensis*. All treatments significantly reduced the mean proportion of *D. abbreviatus* larvae surviving (ranging from 0 to 36%) compared to survival in untreated controls (ranging from 80 to 86%). This suggests that EPNs might be suitable to play a role in integrated pest management strategies against *D. abbreviatus* in tropical soils with high clay content.

Key Words: *Litchi chinensis*, *Steinernema riobrave*, *Heterorhabditis megidis*, Oxisol

RESUMEN

Estudios recientes en el laboratorio demostraron que nueve especies de nemátodos entomopatogénicos (EPN) causaron patogenicidad en larvas de *Diaprepes abbreviatus* en un suelo Oxisol en Puerto Rico. En este estudio se determinó la eficacia de cinco especies de EPN en un Oxisol bajo condiciones de invernadero. Se aplicaron 100 nemátodos juveniles por cm^2 en tiestos de 19 litros conteniendo un suelo Oxisol (69% arcilla) y tres plántulas de *Litchi chinensis*. Todos los tratamientos redujeron significativamente la sobrevivencia de larvas de *D. abbreviatus* en relación al tratamiento control (porciento de sobrevivencia para todos los tratamientos vario de 0 a 36 ± 6 EEM). Esto sugiere que EPN's pueden jugar un papel importante en el manejo integrado de plagas, particularmente artrópodos en suelos tropicales con alto contenido de arcilla.

Translation provided by the authors.

Diaprepes abbreviatus (L.) (Coleoptera: Curculionidae) is a key pest of a wide variety of crops in Puerto Rico (Martorell 1976; O'Brien & Wibmer 1982). Adults feed on the foliage of fruit trees and other hosts (Simpson et al. 1996) and the larvae attack the roots, reducing yield, allowing the ingress of pathogenic organisms, or directly killing the tree by removing cortex from the roots, especially in seedlings (Knapp et al. 2000; Nigg et al. 2001; Quintela et al. 1998). *Litchi chinensis* Sonn. (Sapindaceae) is a tropical/subtropical fruit tree native to southern China (Morton 1987). Because of its marketability, some growers in Puerto Rico are attempting to cultivate orchards of *L. chinensis*, but there are currently no recommendations for dealing with the pests associated with this fruit tree in Puerto Rico, including *D. abbreviatus*. Recent surveys in Puerto Rico indicate that *L. chinensis* is a suitable host for *D. abbreviatus* and can suffer significant damage from the root-feeding larvae of this pest (Jenkins, unpublished data).

Entomopathogenic nematodes (EPNs) have a mutualistic association with bacteria found in their intestine and the nematodes are obligate parasites of insects (Poinar 1990). The infective juveniles move through the soil, either actively searching for suitable hosts or relying on the host to come to them (Lewis et al. 2006). The infective juveniles enter the host through natural openings, such as spiracles, the anus, or the mouth (Poinar 1990). Once inside the host, the nematodes release the mutualistic bacteria, which kill the host within 48 h (Poinar 1990). Two or 3 generations of nematodes are completed within the host cadaver before infective juveniles are released into the soil to search out new hosts (Poinar 1990).

Entomopathogenic nematodes have had a demonstrable effect against many soil-borne insect pests, including *D. abbreviatus* (McCoy et al. 2000; Shapiro-Ilan et al. 2002, 2005). However, it is generally thought that soils of high clay content, such as those common in Puerto Rico, reduce

the efficacy of EPNs as biocontrol agents of soil-borne insect pests (Duncan et al. 2001; McCoy et al. 2002). Indeed, several studies have indicated that nematode dispersal is hampered in soils of high clay-content (Georgis & Poinar 1983; Barbercheck & Kaya 1991; Portillo-Aguilar et al. 1999). However, virulence and persistence of *Steinernema riobrave* (Cabanillas, Poinar & Raulston) and *Heterorhabditis bacteriophora* Poinar appeared to improve in a higher clay-content Entisol when compared to a sandy Spodosol and a sandy Entisol (Shapiro et al. 2000). Furthermore, recent laboratory trials indicated that some EPNs were efficacious in high clay-content Oxisols found in Puerto Rico (Jenkins et al. 2007).

The objective of this study was to assess the efficacy of 5 species/strains of EPNs under greenhouse conditions in a larger volume of high clay-content soil than had been assayed in earlier laboratory trials. This would more rigorously test the EPN's ability to locate their host in an experiment that more closely resembles a nursery or an orchard setting.

Materials and Methods

Soil (Oxisol, Coto clay, clayey, kaolinitc isohypoerthermic Typic Hapludox) was collected from the USDA-ARS Experiment Station in Isabela, P.R. The soil composition, determined by the hydrometer method, was 29.55% sand, 13.92% silt, and 60.88% clay and had a pH of 8.07. The proportion of total nitrogen in the soil, determined by the micro-Kjeldahl method, was 0.20. The concentration of other critical elements in the soil was determined by atomic absorption spectroscopy (K, Ca, Mg, Cu, Fe, Mn, and Zn) or the Bray II Method (P) and were as follows: P = 35 µg/g; K = 170 µg/g; Ca = 2,842 µg/g; Mg = 61 µg/g; Cu = not detected; Fe = not detected; Mn = 123 µg/g; and Zn = 4 µg/g.

Soil (22-23 kg) was placed into 19-L buckets (bucket dimensions = mouth diameter of 29 cm and depth of 36 cm). Three *L. chinensis* seedlings were planted in the soil in each bucket. Ten 9th through 11th instars of *D. abbreviatus*, obtained from the Florida Division of Plant Industry, were placed in 10 holes, each 10 cm deep, made with an auger around the 3 *L. chinensis* seedlings. Holes were manually filled with soil after placement of larvae.

Five strains/species of EPNs were assayed against larvae of *D. abbreviatus*: *S. riobrave (*strains 355, 7-12, and TP), *S. diaprepesi* Nguyen & Duncan, and *Heterorhabditis megidis* Poinar, Jackson, & Klein (UK211 strain). Prior to the assays, nematodes were reared in last instars of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). All nematodes were reared at 25 ± 1°C according to procedures described in Kaya & Stock (1997). After harvesting nematodes from *G. mellonella*, the nematodes were stored in tap water at 13°C (Kaya & Stock 1997) for up to 1 week prior to the assays. Viability of all nematodes was >90% at the time of application.

Nematodes were applied 24 h after placement of *D. abbreviatus* larvae at the rate of 100 infective juveniles per cm^2 in 1 L of water (surface area of soil = 660 cm^2 × 100 infective juveniles/cm^2 = 66,000 infective juveniles / bucket). Control buckets were treated with 1 L of water poured evenly over the soil surface. Buckets were then placed in a greenhouse at the USDA-ARS Tropical Agriculture Research Station in Mayaguez, PR (mean temperature 25°C, RH 74%). Each treatment was replicated 5 times and the entire experiment was repeated in a second trial a month later. After 14 d, the experimental units were dismantled and the number of living *D. abbreviatus* larvae was recorded in each bucket. Dead larvae were inspected under the microscope to confirm that death was caused by nematodes. Nematodes reared from cadavers were placed in a Petri-dish with 5 *G. mellonella* larvae and monitored to confirm pathogenicity.

Analysis of variance (PROC GLM) and Student-Newman-Keuls multiple range test (SAS 2003) were used to analyze the effect of EPN species/strain on the survival of *D. abbreviatus* larvae. Statistical analyses were conducted on arcsine transformed data (percent surviving).

Results

Analysis indicated that survival of *D. abbreviatus* larvae was reduced by EPN (for trial 1, $F = 20.24$, $P < 0.0001$, $df = 1,5$; for trial 2, $F = 45.11$, $P < 0.0001$, $df = 1,5$) (Table 1). We detected no surviving larvae in the buckets receiving *S. riobrave* (TP strain) in Trial 1 or in buckets receiving *S. riobrave* (355 strain) in both trials (Table 1). In the first trial no species/strains of EPN could be statistically differentiated based on the proportion of surviving *D. abbreviatus* larvae (arcsine transformed), but in the second trial all *S. riobrave* strains caused significantly lower larval *D. abbreviatus* survivorship than did *S. diaprepesi* or *H. megidis* (Table 1).

Discussion

Our results demonstrate that EPNs can provide high levels of *D. abbreviatus* suppression in an Oxisol under greenhouse conditions. Entomopathogenic nematodes have been reported to control *D. abbreviatus* under greenhouse conditions previously (Shapiro & McCoy 2000a; Shapiro-Ilan et al. 2003), however, this is the first report of efficacy in potted plants in a high clay-content soil. Thus, it is clear that, under the conditions of this study, the Oxisol's texture is not a significant impediment to nematode movement

TABLE 1. PROPORTION OF *DIAPREPES ABBREVIATUS* LARVAE SURVIVING AFTER 14 D EXPOSURE TO NEMATODES. THE NEMATODES WERE APPLIED TO POTTED *LITCHI CHINENSIS* HELD IN 19 L OF AN OXISOL FROM PUERTO RICO.

Treatment: Nematode species (strain)	Proportion surviving ± SEM[a]	
	Trial 1	Trial 2
Control	0.80 ± 0.07 a	0.86 ± 0.03 a
Steinernema diaprepesi	0.32 ± 0.10 b	0.36 ± 0.06 b
*S. riobrave (*7-12)	0.26 ± 0.07 b	0.12 ± 0.05 c
S. riobrave (355)	0.00 ± 0.00 b	0.00 ± 0.00 c
S. riobrave (TP)	0.00 ± 0.00 b	0.02 ± 0.02 c
Heterorhabditis megidis (UK211)	0.24 ± 0.07 b	0.30 ± 0.12 b

[a]Means followed by the same letter are not significantly different (Student-Newman-Keuls multiple range test $P \leq 0.05$: For trial 1, $F = 20.24$, $P = <0.0001$, $df = 1, 5$; For trial 2, $F = 45.11$, $P < 0.0001$, $df = 1, 5$). Each treatment was replicated 5 times and analysis was performed on arcsine transformed data.

and infection. It may be argued that since we only placed the *D. abbreviatus* larvae 10 cm deep, the nematodes did not have far to travel. However, in every case larvae were found in the lower half of the soil column (18 cm or deeper) and more than 85% percent of the larvae were found within 10 cm of the bottom of the bucket.

Previous assays of EPNs in Puerto Rico used large numbers of infective juveniles (>1000/cm^3 of soil) and achieved only minimal control of *D. abbreviatus* (<50% survival) (Román & Figueroa 1985; Figueroa & Román 1990). Many previous evaluations in Puerto Rico were restricted to *Steinernema carpocapsae* (=*Neoaplectana carpocapsae*) (Román & Beavers 1983; Román & Figueroa 1985), a nematode that tends to inhabit the surface of the soil and would have little chance of encountering root-feeding grubs that tend to be found deeper in the soil (Lewis et al. 2006).

Although the volume and weight of soil used in this study (19 L, 22 kg) was vastly larger than in a previously conducted laboratory assay (0.087 L, 0.4 kg), the species/strains of EPNs performed similarly in both studies (Jenkins et al. 2007). The mean proportion of larval *D. abbreviatus* surviving were remarkably similar in both assays for treatments with *S. diaprepesi* (0.32-0.36 for the current study and 0.33 for Jenkins et al. 2007) and *H. megidis* (0.24-0.30 for the current study and 0.25 for Jenkins et al. 2007). This is somewhat unexpected given the larger volume of soil used in the current experiments, but we do note that the earlier laboratory assay did use a lower rate of EPNs (40 infective juveniles per cm^2) (Jenkins et al. 2007). The 7-12 strain of *S. riobrave* appeared to perform better in the laboratory assay (mean proportion surviving = 0; Jenkins et al. 2007) than in the current study (mean proportion surviving = 0.12-0.26) whereas the 355-strain of *S. riobrave* appeared to perform better in the current study (mean proportion surviving = 0 for both trials) than in the laboratory assay (mean proportion surviving = 0.08; Jenkins et al. 2007). Treatment with the TP-strain of *S. riobrave* resulted in 0 percent survival in both the current and the laboratory study (Jenkins et al. 2007).

This study provides evidence (Trial 2) that, under the conditions tested, performances of *S. riobrave* strains are superior to those of *H. megidis* and *S. diaprepesi*. Superior virulence of *S. riobrave* has been observed previously in sandy soil (Shapiro & McCoy 2000b). In a laboratory study Stuart et al. (2004) observed superior virulence in the 7-12- and TP-strains of *S. riobrave* relative to the 355-strain, but no differences were observed among *S. riobrave* strains in our study. Nor was any difference among these strains observed in Jenkins et al. (2007). Possibly the discrepancy is due to different soil types (a sandy soil was used in the study of Stuart et al. 2004). The superior efficacy of *S. riobrave* indicates that this may be the nematode of choice for control of *D. abbreviatus* in an Oxisol. However, it is conceivable that other characteristics, such as persistence, could factor into the choice of nematode to use, e.g., *S. diaprepesi* is known to be highly persistent relative to *S. riobrave*, at least in its native soil (Duncan et al. 2003).

In summary, all 5 species/strains of EPNs assayed reduced the proportion of *D. abbreviatus* larvae surviving compared with the control treatment. The second trial indicated that all strains of *S. riobrave* were more efficacious than the other EPN species assayed. Field trials are needed to assess the efficacy of these EPNs under orchard conditions.

ACKNOWLEDGMENTS

We thank Suzanne Fraser at the Florida Division of Plant Industry for providing us with *Diaprepes abbreviatus* larvae. We also thank Elkin Vargas for tremendous field and lab work and Ulises Chardon for analyzing the soil samples. Finally, we thank Robin Stuart and Wayne Gardner for reviewing earlier versions of this manuscript.

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