

## VIRULENCE OF ENTOMOPATHOGENIC NEMATODES AGAINST *DIAPREPES ABBREVIATUS* IN AN OXISOL

DAVID A. JENKINS<sup>1</sup>, DAVID SHAPIRO-ILAN<sup>2</sup> AND RICARDO GOENAGA<sup>1</sup>

<sup>1</sup>USDA-ARS, Tropical Agricultura Research Station, 2200 Ave. P.A. Campos, Mayaguez, Puerto Rico 00680-5470

<sup>2</sup>USDA-ARS, Southeast Fruit and Tree Nut Research Lab, Byron, GA 31008

*Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) is an insect whose host range includes more than 270 species of plants, including many economic species (Martorell 1976; Simpson et al. 1996; Wolcott 1936). Damage to roots by larvae can reduce yield and impact the long term health of host plants. There is a need to identify biocontrol options for this pest that are efficacious in Puerto Rico.

Entomopathogenic nematodes in the families Heterorhabditidae and Steinernematidae are lethal parasites of insects (Poinar 1990) and have proven effective against *D. abbreviatus* in Florida (McCoy et al. 2000; Shapiro-Ilan et al. 2002, 2005). However, these assays were conducted in the sandy soils typical of the regions where citrus is cultivated in Florida. For infective juvenile nematodes to successfully infect a host they must be able to move through the soil. Therefore, soil physical properties, such as those typical of sandy soils (porous and aerated), should facilitate nematode infectivity than denser soils, such as clays. Indeed a number of researchers have noted that the clay content of a soil is inversely proportional to the ability of nematodes to disperse in that soil (Georgis & Poinar 1983; Barbercheck & Kaya 1991; Barbercheck 1992). However, recent research has shown that the role of soil physical properties in nematode dispersal and survival is more complex, varying with species of nematode (Portillo-Aguilar et al. 1999; Koppenhöfer & Fuzy 2006). Additionally, research shows that nematode virulence to *D. abbreviatus* can be significantly higher in certain high clay content soils (Shapiro et al. 2000). Greenhouse assays with *Steinernema feltiae* (= *Neoaplectana carpocapsae*) conducted in Puerto Rico against *D. abbreviatus* revealed limited mortality, but results were ambiguous and the soil type assayed was not identified (Román & Figueroa 1985). Another study of the virulence of *S. feltiae* against *D. abbreviatus* in soils from various regions of Puerto Rico indicated that infection rates were higher in soils from regions that had a higher sand content, suggesting that increased infectivity was positively correlated with the porosity of the soil (Román & Beavers 1983).

Oxisols are representative of the ultimate stages of soil development in the tropical and subtropical regions to which they are restricted. They are characterized by extremely weathered, acidic red clay completely lacking in weatherable miner-

als and bases (Beinroth 1971). Although Oxisols occupy a relatively small percentage of Puerto Rico's surface, tropical fruit crops are commonly grown in this soil type and sustain significant damage from *D. abbreviatus*. The objective of this study was to assay the virulence of a number of nematode species and strains against *D. abbreviatus* larvae in an Oxisol.

Thirty kg of soil were collected from a fruit orchard at the USDA-ARS Experiment Station in Isabela, PR. The soil was oven-dried for 2 d and shipped to the USDA Research Laboratory in Byron, GA. A sample of the soil was analyzed for nutrient content and physical properties at USDA-ARS-TARS in Mayaguez, PR. The soil was identified as belonging to the Cotito series in the Oxisol order. The soil composition, determined by the hydrometer method, was 13.26% sand, 12.86% silt, and 73.87% clay, with a pH of 5.85. The percent of total nitrogen in the soil, determined by the micro-Kjeldahl method, was 0.22. The concentrations of other critical elements in the soil, determined by atomic absorption spectroscopy (K, Ca, Mg, Cu, Fe, Mn, and Zn) or the Bray II Method (P), were as follows: P = 39 µg/g; K = 558 µg/g; Ca = 1,136 µg/g; Mg = 122 µg/g; Cu = 0.98 µg/g; Fe = 9 µg/g; Mn = 301 µg/g; and Zn = 4 µg/g.

Larvae of *D. abbreviatus* were obtained from the Biological Control Mass Rearing Facility of the Florida Division of Plant Industry. Nine species/strains of nematodes were assayed against larvae of *D. abbreviatus*: *Steinernema riobrave* Cabanillas, Poinar & Raulston (strains 355, 7-12, 3-8b, and TP), *S. feltiae* (Filipjev) (SN strain), *S. rarum* (Doucet) (J. Levy strain), *S. diaprepesi* Nguyen & Duncan, *Heterorhabditis indica* Poinar, Karunakar & David (HOM1 strain), and *H. megidis* Poinar, Jackson & Klein (UK211 strain). Before experimentation, nematodes were reared in and transferred from no more than 5 live last-instar greater wax moth larvae, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). Nematodes were reared at approximately 25°C according to procedures described in Kaya & Stock (1997). After harvesting, nematodes were stored in tap water at 13°C (Kaya & Stock 1997) for up to 2 weeks prior to use. Viability of all nematodes was >95% at the time of application.

Bioassay methods were based on those described by Shapiro & McCoy (2000a, b). Experimental units consisted of plastic pots (10.5 cm diam., 6.5 cm deep). Each pot contained 400 grams

of soil (dry weight), 10 *D. abbreviatus* larvae, and 10 pieces of carrot. The final soil moisture in each pot was 22%, which was determined to be field capacity for this soil (graduated cylinder method). Larvae and carrot pieces were placed 1 cm from the bottom of the pot on a bed of soil and were covered with 5.5 cm of soil. Nematodes were applied in 1 mL of tap water (using a 1 mL pipette) at a rate of 40 infective juveniles/cm<sup>2</sup> 24 h after the larvae were placed in the pots. Controls received only water. The pots were tightly covered with plastic lids after application of the nematodes and stored at 25°C. After 14 d, the pots were emptied and the number of surviving larvae was recorded. Each treatment was replicated 3 times and the experiment was repeated the following day.

Analysis of variance (PROC GLM) and Student-Newman-Keuls multiple range test (SAS 2003) were used to analyze the effect of nematode species or strain on the survival of *D. abbreviatus* larvae. Analyses were performed on arcsin transformed data (proportion surviving).

There was not a significant interaction between the treatment and trial effects ( $F = 0.98, P = 0.4713, df = 9, 39$ ) so the data from the two trials were combined. Nor was there a significant effect attributable to the trial ( $F = 2.35, P = 0.1332, df = 1, 39$ ). Analysis indicated that all of the nematode treatments had lower mean survival than the control treatments ( $F = 5.98, P < 0.0001, df = 1, 39$ ) (Table 1). Three strains of *S. riobrave* (3-8b, TP, and 7-12) had lower mean survivorship than all other treatments except *H. indica* (HOM1), and *S. riobrave* (355), with zero *D. abbreviatus* larvae surviving in 2 of the treatments (strains TP and 7-12), and all *S. riobrave* strains caused

lower *D. abbreviatus* survival than *S. feltiae* or *S. rarium*; no other treatment differences were detected (Table 1).

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation nor endorsement by the U.S. Department of Agriculture. We thank Kathy Halat, Wanda Evans, and Rebeckah Long. We thank Jorge Lugo, NRCS, Mayaguez, PR, for classifying soil samples, and Ulises Chardon, USDA-ARS-TARS, Mayaguez, PR, for soil analysis, and Drs. Wayne Hunter, David Hall, and two anonymous reviewers for comments on an earlier version of this manuscript.

#### SUMMARY

We evaluated the virulence of 9 species/strains of entomopathogenic nematode against *D. abbreviatus* in a high clay content soil typical of fruit growing regions of Puerto Rico. All nematode species and nematode strains provided significant mean mortality as compared to a water control. Strains of *Steinernema riobrave* performed particularly well, with some strains resulting in 100% mortality. These laboratory and field tests indicate that some species/strains of entomopathogenic nematodes may be suitable for the control of *D. abbreviatus* in Puerto Rican soils of high clay content.

#### REFERENCES CITED

- BARBERCHECK, M. E. 1992. Effect of soil physical factors on biological control agents of soil insect pests. *Florida Entomol.* 75: 539-548.
- BARBERCHECK, M. E., AND H. K. KAYA. 1991. Effect of host condition and soil texture on host finding by the entomogenous nematodes *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) and *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *Environ. Entomol.* 20: 582-589.
- BEINROTH, F. H. 1971. The general pattern of the soils of Puerto Rico, pp. 225-230 *In* Transcripts of the Fifth Caribbean Geological Conference, Geol. Bull. No. 5. Queens College Press.
- GEORGIS, R., AND G. O. POINAR, JR. 1983. Effect of soil temperature on the distribution and infectivity of *Neoalectana carpocapsae* (Nematoda: Steinernematidae). *J. Nematol.* 15: 308-311.
- KAYA, H. K., AND S. P. STOCK. 1997. Techniques in insect nematology *In* L. A. Lacey [ed.], *Manual of Techniques in Insect Pathology*. Academic, San Diego, CA.
- KOPPENHÖFER, A. M. AND EUGENE M. FUZY. 2006. Effect of soil type on infectivity and persistence of the entomopathogenic nematodes *Steinernema scabaei*, *Steinernema glaseri*, *Heterorhabditis zealandica*, and *Heterorhabditis bacteriophora*. *J. Invertebr. Pathol.* 92: 11-22.
- MARTORELL, L. F. 1976. Annotated food plant catalog of the insects of Puerto Rico. Agric. Exp. Stn., Univ. Puerto Rico, Department of Entomology. 303 pp.

TABLE 1. PROPORTION OF *DIAPREPES ABBREVIATUS* LARVAE SURVIVING (ANALYSIS PERFORMED ON ARCSINE TRANSFORMED DATA) AFTER 14 D EXPOSURE TO NEMATODES IN AN OXISOL FROM PUERTO RICO. MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (SNK,  $P \leq 0.05$ ). THE NUMBER OF ALL TREATMENTS WAS 6, EXCEPT FOR THE CONTROL, WHICH WAS 5.

Treatment	Proportion surviving ± SEM
Control	0.78 ± 0.04 a
<i>Steinernema feltiae</i> (SN)	0.50 ± 0.12 b
<i>S. rarium</i> (J. Levy)	0.45 ± 0.12 b
<i>S. diaprepesi</i>	0.33 ± 0.13 bc
<i>S. riobrave</i> (355)	0.08 ± 0.03 cd
<i>S. riobrave</i> (3-8b)	0.02 ± 0.02 d
<i>S. riobrave</i> (TP)	0.00 ± 0 d
<i>S. riobrave</i> (7-12)	0.00 ± 0 d
<i>Heterorhabditis indica</i> (HOM1)	0.15 ± 0.06 cbd
<i>H. megidis</i> (UK211)	0.25 ± 0.06 bc

- MCCOY, C. W., D. I. SHAPIRO, L. W. DUNCAN, AND K. NGUYEN. 2000. Entomopathogenic nematodes and other natural enemies as mortality factors for larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *Biol. Control* 19: 182-190.
- POINAR, G. O. 1990. Biology and Taxonomy of Steinernematidae and Heterorhabditidae, pp 23-62 *In* R. Gaugler and H. K. Kaya [eds.], *Entomopathogenic Nematodes in Biological Control*. CRC, Boca Raton, FL.
- PORTILLO-AGUILAR, C., M. G. VILLANI, M. J. TAUBER, C. A. TAUBER, AND J. P. NYROP. 1999. Entomopathogenic nematode (Rhabditida: Heterorhabditidae and Steinernematidae) response to soil texture and bulk density. *Environ. Entomol.* 28: 1021-1035.
- ROMÁN, J., AND J. B. BEAVERS. 1983. A survey of Puerto Rican soils for entomogenous nematodes which attack *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae). *J. Agric. Univ. Puerto Rico* 67: 311-316.
- ROMÁN, J., AND W. FIGUEROA. 1985. Control of the larva of the sugarcane rootstalk borer, *Diaprepes abbreviatus* (L), with the entomogenous nematode *Neoplectana carpocapsae* Weiser. *J. Agric. Univ. Puerto Rico* 69: 153-158.
- SAS. 2003. Version 9.1. SAS Institute, Cary, NC.
- SHAPIRO, D. I., AND C. W. MCCOY. 2000a. Susceptibility of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae to different rates of entomopathogenic nematodes in the greenhouse. *Florida Entomol.* 83: 1-9.
- SHAPIRO, D. I., AND C. W. MCCOY. 2000b. Virulence of entomopathogenic nematodes to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in the laboratory. *J. Econ. Entomol.* 93: 1090-1095.
- SHAPIRO, D. I., C. W. MCCOY, A. FARES, T. OBREZA, AND H. DOU. 2000. Effects of soil type on virulence and persistence of entomopathogenic nematodes in relation to control of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *Environ. Entomol.* 29: 1083-1087.
- SHAPIRO-ILAN, D. I., D. H., GOUGE, AND A. M. KOPPENHÖFER. 2002. Factors affecting commercial success: case studies in cotton, turf and citrus, pp. 333-355 *In* R. Gaugler [ed.], *Entomopathogenic Nematology*. CABI, New York, NY.
- SHAPIRO-ILAN, D. I., L. W. DUNCAN, L. A. LACEY, AND R. HAN. 2005. Orchard crops, pp. 215-230 *In* P. Grewal, R.U. Ehlers, and D. Shapiro-Ilan [eds.], *Nematodes as Biological Control Agents*. CABI Publishing, New York, NY.
- SIMPSON, S. E., H. N. NIGG, N. C. COILE, AND R. A. ADAIR. 1996. *Diaprepes abbreviatus* (Coleoptera: Curculionidae): Host plant associations. *Environ. Entomol.* 25: 333-349.
- WOLCOTT, G. N. 1936. The life history of *Diaprepes abbreviatus* at Rio Piedras, Puerto Rico. *J. Agr. Univ. Puerto Rico* 20: 883-914.