N O T E

Susceptibility of Adult Nut Curculio, *Curculio hicoriae* (Coleoptera: Curculionidae) to Entomopathogenic Nematodes under Laboratory Conditions[1]

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The nut curculio, *Conotrachelus hicoriae* Schoof (Coleoptera: Curculionidae), can cause serious damage to pecan, *Carya illinoinensis* (Wangenheim) K. Koch, in certain areas of the southeastern US, particularly Louisiana (Dutcher 2002, J. Entomol. Sci. 37: 259 - 269; Hall and Austin 2002, J. Entomol. Sci. 37: 293 - 299). Although overwintering adults may emerge from soil in the orchard anytime between midMay through early August, most insects emerge between midJune to midJuly (Hall and Austin 2002, J. Entomol. Sci. 37: 293 - 299). Adults feed and oviposit in nuts, which subsequently drop to the ground. Larvae emerge from the fallen nuts and burrow into the soil forming pupal cells (< 3 cm below the surface) (Calcote 1970, J. Econ. Entomol. 63: 2010 - 2011). Adults emerge from soil cells from August to October and briefly feed on shucks before entering the soil again to overwinter.

Chemical insecticides are currently used to control *C. hicoriae*. In the interest of reducing chemical inputs, and preserving natural enemies, research toward developing biological control methods is desirable. Entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* are effective biological control agents that are used for the suppression of a variety of curculionid pests in orchards and other systems (Grewal et al. 2005, Nematodes as BioControl Agents, CABI, Wallingford; Lacey and Shapiro-Ilan 2008. Annu. Rev. Entomol. 53: 121 - 144). Thus, entomopathogenic nematodes may also be capable of contributing to suppression of *C. hicoriae*. The objective of this study was to determine the pathogenicity (ability to cause disease) and virulence (relative disease-causing power) of 3 entomopathogenic nematode species to *C. hicoriae* adults. Prior to this study, the susceptibility of *C. hicoriae* to entomopathogenic nematodes had not been determined.

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The 3 entomopathogenic nematodes included in this study were *Heterorhabditis indica* Poinar, Karunakar, and David (HOM1 strain), *Steinernema carpocapsae* (Weiser) (All strain), and *Steinernema riobrave* Cabanillas, Poinar, and Raulston (355 strain). The nematodes were cultured in last-instar greater wax moth, *Galleria mellonella* (L.), based on procedures described by Kaya and Stock (1997. Pp. 281 - 324. *In* Lacey, L.A. (ed.), Manual of techniques in insect pathology. Academic Press, San Diego) and stored less than 2 wks prior to experimentation.

Adult *C. hicoriae* were collected from pecan orchards at 3 locations: the USDA-ARS SE Fruit and Tree Nut Research Laboratory, Byron, GA; the Merritt Farm in Weston, GA; and the LSU AgCenter Pecan Research-Extension Station in Robson, LA. The cultivars in the Byron, GA, orchard were a mixture of Stuart and Schley; in Weston, GA, cultivars were Desirable and Pawnee; and in Robson, LA, the orchard contained Stuart cultivar trees. The insects were captured in June and July 2008 and 2009 using wire cage cone traps and Circle traps (Hall and Austin 2002, J. Entomol. Sci. 37: 293 - 299; Mulder et al. 2003, Southwest. Entomol. Suppl. No. 27: 85 - 99); the insects were used in the experiment within 1 wk of capture.

Bioassays to determine pathogenicity and virulence of nematodes to *C. hicoriae* were based on procedures described by Shapiro-Ilan (2001, J. Entomol. Sci. 36: 325 - 328) and Shapiro et al. (2002, J. Nematol. 34: 246 - 249). Briefly, experimental units consisted of 30-ml lidded plastic cups (3 - 4 cm inner diam., 3.5 cm deep) filled with soil. The soil was a loamy sand with the percentage sand:silt:clay = 84:10:6, pH 6.1, and organic matter = 2.8% by weight. For each nematode treatment, 500 infective juveniles (IJs) were pipetted onto the soil surface of each cup. soil cups designated as controls received an equivalent amount of water without nematodes; the final soil moisture level in each cup was 16% (= estimated field capacity). After nematodes were applied, 1 insect was added to each cup. The cups were then placed in an incubator at 25°C. Percentage mortality in *C. hicoriae* was assessed 7 and 14 d posttreatment. The experiment contained 3 replicates. The first 2 replicates were conducted in 2008 and contained 10 and 5 insects per treatment, respectively. The third replicate was conducted in 2009 and contained 5 insects per treatment. Treatment effects were determined through repeated measures analysis (Proc Mixed, averaging mortality over the experimental period); replicate variation was accounted for as a random block effect, and (if F was significant at $P \leq 0.05$) mean separation was elucidated with LSMEANS (SAS 2002, SAS Software: Version 9.1. SAS Institute, Cary, NC; Shapiro-Ilan et al. 2008, Environ. Entomol. 37: 162 - 171).

Results indicated that *S. carpocapsae* and *S. riobrave* are pathogenic to *C. hicoriae*, i.e., higher *C. hicoriae* mortality was observed in these nematode treatments relative to the control (Fig. 1; $F = 7.37$; df = 3, 6; $P = 0.0195$). No difference in virulence was detected between the 2 steinernematids (Fig. 1). In contrast, pathogenicity was not detected with *H. indica* (Fig. 1). The lack of pathogenicity in *H. indica* is somewhat surprising given that this nematode was shown to be pathogenic to other weevil species, such as the citrus root weevil, *Diaprepes abbreviatus* (L.) (Shapiro et al. 1999, J. Econ. Entomol. 92:1086 - 1092) and a curculionid in the same genus as the weevil in this study, the guava weevil, *Conotrachelus psidii* Marshall (Dolinski et al. 2006, Biol. Control 38: 422 - 427).

Although *S. carpocapsae* and *S. riobrave* were pathogenic to *C. hicoriae*, virulence appears to be relatively low. Mortality of *C. hicoriae* did not exceed 60% for either steinernematid species (Fig. 1). Furthermore, if Abbott's formula (Abbott 1925. J. Econ. Entomol. 18: 265 - 267) is applied to the data to correct for natural mortality, the level

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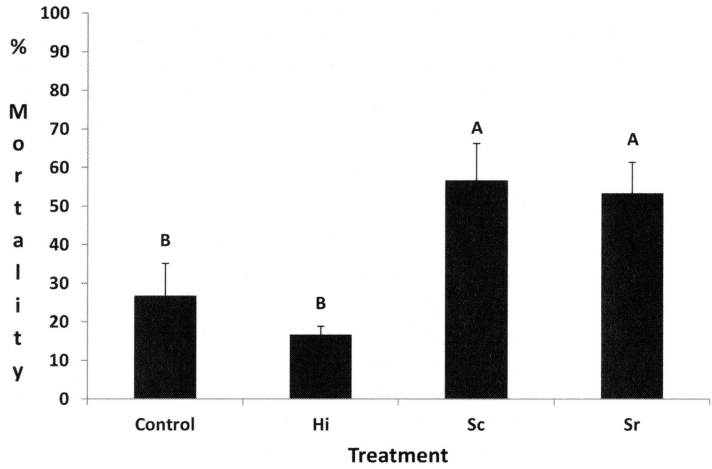

Fig. 1. Percentage mortality of *Conotrachelus hicoriae* adults in the laboratory following exposure to entomopathogenic nematodes *Heterorhabditis indica* (Hi), *Steinernema carpocapsae* (Sc), *S. riobrave* (Sr), or an untreated (water-only) control. Bars with different letters indicate a statistical difference (LSMEANS, $P \leq 0.05$).

of control due to the nematode treatments does not exceed 45%. This level of mortality must be considered low given that the rate of application (approx.. 40 IJs per cm^2) was within the range of a standard field rate, and commercially successful nematodes applications for control of other target pests tend to show higher mortality in the laboratory when applied at similar rates (Shapiro-Ilan et al. 2002. Pp 333 - 355, *In* R. Gaugler, (ed.) Entomopathogenic nematology. CABI, NY; Shapiro-Ilan et al. 2012. Pp. 29 - 72, *in*: F. Vega and H. K. Kaya (eds.) Insect pathology. Elsevier, San Diego). Whereas it is difficult to compare across studies, in a prior study conducted under very similar conditions (soil, moisture, experimental arena) and at the same application rate, *S. carpocapsae* and *S. riobrave* caused substantially higher mortality in the closely-related (same genus) plum curculio, *Conotrachelus nenuphar* (Herbst), than in *C. hicoriae* as observed in this study; adult *C. nenuphar* corrected mortality caused by *S. carpocapsae* and *S. riobrave* after 5 d was approx. 80 and 70%, respectively (Shapiro-Ilan et al. 2002, J. Nematol. 34:346 - 349). Substantially higher mortality was also observed after only 4 d in adult pecan weevil, *Curculio caryae* (Horn), when *S. carpocapsae* and *S. riobrave* were applied at the same rates and assay conditions used in the present study (99% and 67% corrected mortality was observed, respectively) (Shapiro-Ilan 2001, J. Entomol. Sci. 36: 325 - 328).

Despite the relatively low virulence, the use of entomopathogenic nematodes may have merit in contributing to *C. hicoriae* suppression, particularly if the approach utilizes *S. carpocapsae* in conjunction with *C. caryae* control. *Steinernema carpocapsae* is highly virulent to *C. caryae* adults (Shapiro-Ilan 2001, J. Entomol. Sci. 36: 325 - 328) and high levels of field efficacy have been demonstrated when targeting ground-dwelling stages (Shapiro-Ilan and Gardner 2012, J. Entomol. Sci. 47: 1 - 16). *Curculio caryae* can be effectively targeted with entomopathogenic nematodes in the spring (once soil

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temperatures are conducive) (Shapiro-Ilan and Gardner 2012, J. Entomol. Sci. 47: 1 - 16), which coincides with a feasible period to apply nematodes to overwintering *C. hicoriae*. Therefore, it is conceivable that a combined benefit could be gained if targeting *C. hicoriae* and *C. caryae* simultaneously.

Additional research to determine the potential of entomopathogenic nematodes to control *C. hicoriae* is warranted. The susceptibility of other life stages (larvae and pupae) should be determined, and additional nematode species can be tested for virulence. Furthermore, studies are required to determine efficacy under field conditions. Also, additional information on the insect's biology and life-history may facilitate improved control with entomopathogenic nematodes or other control agents. For example, it would be useful to determine whether *C. hicoriae* overwinters exclusively within the orchard or if a proportion overwinters on the perimeter (similar to *C. nenuphar*). The bulk of our insect captures were made in cone traps (data not shown) indicating an overwintering population within the orchard, but that does not rule out the possibility that some insects may overwinter outside of the orchard.

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