Journal of Nematology 34(3):246–249. 2002.

Susceptibility of the Plum Curculio, *Conotrachelus nenuphar,* to Entomopathogenic Nematodes

DAVID I. SHAPIRO-ILAN,[1] RUSSELL F. MIZELL, III,[2] AND JAMES F. CAMPBELL[3]

Abstract: The plum curculio, *Conotrachelus nenuphar,* is a major pest of pome and stone fruit. Our objective was to determine virulence and reproductive potential of six commercially available nematode species in *C. nenuphar* larvae and adults. Nematodes tested were *Heterorhabditis bacteriophora* (Hb strain), *H. marelatus* (Point Reyes strains), *H. megidis* (UK211 strain), *Steinernema riobrave* (355 strain), *S. carpocapsae* (All strain), and *S. feltiae* (SN strain). Survival of *C. nenuphar* larvae treated with *S. feltiae* and *S. riobrave,* and survival of adults treated with *S. carpocapsae* and *S. riobrave,* was reduced relative to non-treated insects. Other nematode treatments were not different from the control. *Conotrachelus nenuphar* larvae were more susceptible to *S. feltiae* infection than were adults, but for other nematode species there was no significant insect-stage effect. Reproduction in *C. nenuphar* was greatest for *H. marelatus,* which produced approximately 10,000 nematodes in larvae and 5,500 in adults. Other nematodes produced approximately 1,000 to 3,700 infective juveniles per *C. nenuphar* with no significant differences among nematode species or insect stages. We conclude that *S. carpocapsae* or *S. riobrave* appears to have the most potential for controlling adults, whereas *S. feltiae* or *S. riobrave* appears to have the most potential for larval control.

Key words: biological control, *Conotrachelus nenuphar,* entomopathogenic nematode, *Heterorhabditis,* reproduction, *Steinernema,* virulence.

The plum curculio, *Conotrachelus nenuphar,* (Herbst) is a major pest of pome and stone fruits in North America (Racette et al., 1992). Adult weevils enter orchards from overwintering sites in the spring, feed, and oviposit in fruit. Fruit that is attacked aborts or is deformed, rendering it non-saleable. Larvae continue to develop in fallen fruit, exit as fourth instars, and burrow into the soil (1–8 cm) to pupate (Racette et al., 1992). After emergence, adults feed on fruit and migrate to litter surrounding the orchard to overwinter (Olthof and Hagley, 1993; Racette et al., 1992). In the southern United States, an additional generation occurs in the orchard prior to overwintering.

Current control recommendations for *C. nenuphar* consist solely of above-ground applications of chemical insecticides to suppress adults (Horton et al., 2001; Olthof and Hagley, 1993). Due to environmental and regulatory concerns, research on developing alternative control strategies is warranted. Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* are obligate parasites of insects (Grewal and Georgis, 1998). These nematodes have a mutualistic relationship with a bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively) (Poinar, 1990). Infective juveniles (IJ), the only free-living stage, enter hosts through natural openings (mouth, anus, and spiracles) or, in some cases, through the cuticle. After entering the host's hemocoel, nematodes release their symbiotic bacteria, which are primarily responsible for killing the host and providing the nematodes with nutrition and defense against secondary invaders (Poinar, 1990). The nematodes molt and complete 2 to 3 generations within the host after which IJ exit the cadaver to search out new hosts (Grewal and Georgis, 1998).

Biological control with entomopathogenic nematodes has been particularly successful against certain weevil species that spend a large portion of their life cycle in the soil, e.g., the Diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Duncan et al., 1996; Shapiro-Ilan et al., 2002), and the black vine weevil, *Otiorhynchus sulcatus* (F.) (Bedding and Miller, 1981; Shanks and Agudelo-Silva, 1990; Simons, 1981). Therefore, we may expect *C. nenuphar* to be a good candidate for control with entomopathogenic nematodes. However, research on the potential of entomopathogenic nematodes to suppress *C. nenuphar* has been limited. Only two species have been tested for pathogenicity to *C. nenuphar*, i.e., *S. feltiae* (Filipjev) and *S. carpocapsae* (Weiser), and control resulting from soil or foliar applications was poor or inconsistent unless extremely high rates were applied (Bélair et al., 1998; Olthof and Hagley, 1993; Tedders et al., 1982). *Conotrachelus nenuphar* last instar larvae and adults occur in or on the soil and therefore are potential targets. However, the relative susceptibility of different *C. nenuphar* stages to nematodes has not been determined.

Laboratory testing of additional species and strains may lead to the identification of nematodes with superior virulence toward *C. nenuphar*. Our primary objective was to determine the relative susceptibility of *C. nenuphar* adults and larvae to six commercially available nematode species under laboratory conditions. Additionally, we investigated the reproductive potential of the nematodes in *C. nenuphar* to estimate the potential of entomopathogenic nematode recycling.

MATERIALS AND METHODS

The following nematodes were tested for virulence to, and reproduction in, *C. nenuphar*: *H. bacteriophora*

Received for publication 13 October 2001.
[1] USDA-ARS, SE Fruit and Nut Tree Research Laboratory, Byron, GA 31008.
[2] Department of Entomology, University of Florida, Quincy, FL.
[3] USDA-ARS, GMPRC, Manhattan, KS.
E-mail: dshapiro@saa.ars.usda.gov
The authors thank K. Halat and W. Evans for technical assistance, Ted Cottrell and Ed Lewis for reviewing an earlier draft of this manuscript, R. Gaugler (Rutgers University, New Brunswick), K. Nguyen (University of Florida, Gainesville), P. Stock (University of Arizona), MicroBio Ltd., and Certis USA for providing nematodes.
This paper was edited by Parwinder Grewal.

Poinar (Hb strain), *H. marelatus* Liu and Berry (Point Reyes strain), *H. megidis* Poinar, Jackson, and Klein (UK211 strain), *S. carpocapsae* (All strain), *S. feltiae* (SN strain), and *S. riobrave* Poinar, Karunakar, and David (355 strain). All nematodes for all experiments were reared in parallel on last instar *Galleria mellonella* (L.) at 25 °C according to procedures described in Woodring and Kaya (1988). Nematodes were stored at 13 °C for less than 8 weeks before experimentation. *Galleria mellonella* larvae were obtained from Sunfish Bait (Webster, WI). *Conotrachelus nenuphar* larvae (4th instar) were collected from infested peaches in Monticello, Florida, and stored in sterile potting mix at 15 °C until use. A portion of the larvae was allowed to pupate and reach adulthood, and then kept in glass containers at 25 °C with young peaches for food until use in experiments.

Experiments were conducted in plastic cups (Bioserv Inc., Frenchtown, NJ) based on procedures described by Shapiro et al. (1999). Cups (3–4 cm i.d., 3.5 cm deep) were filled with (oven-dried) autoclaved soil from the USDA-ARS pecan orchard (Byron, GA) and contained one larvae or adult insect each. After autoclaving, the soil (a loamy sand with 84:10:6 percentage sand:silt:clay, pH = 6.1, and organic matter = 2.8% by weight) was kept at 25 °C for at least 2 weeks before use (Kaya and Stock, 1997).

One day after insects were added, nematodes were pipeted onto the soil surface of each cup in 0.5 ml of water so that the final moisture was standardized at field capacity (14% moisture). Approximately 500 IJ were applied to each cup, and the number of insects surviving was recorded after 5 days of incubation at 25 °C. The control received only water. Nematode-infected insects were placed in White traps (Woodring and Kaya, 1988), which were stored at 25 °C, and progeny IJ were collected until they ceased to emerge. The experiment was arranged in randomized block design and contained 3 replicates of 10 cups per treatment (nematode species). Using identical experimental parameters, the experiment was repeated once with a fresh batch of nematodes, except in the second trial three replicates of seven cups were used for the larvae (due to a shortage).

Data recorded as percentages were transformed (arcsin of the square root), and nematode virulence was then analyzed using analysis of variance and Tukey's multiple range test (SAS Institute, Cary, NC). Reproductive capacity in *C. nenuphar* was compared among nematode species using analysis of variance and Tukey's multiple range test (SAS Institute, Cary, NC).

To compare the relative susceptibility of the two insect stages to a nematode species, it was necessary to correct for control mortality (because control mortality differed between insect stages). Abbott's formula (Abbott, 1925) is a standard correction for control mortality, but this formula does not provide an estimate of the variance associated with the ratio of two random variables (Rosenheim and Hoy, 1989). We used bootstrap sampling to generate variance estimates (Buonaccorsi and Liebhold, 1988) for means that were corrected for control mortality using Abbott's formula. Using Resampling Stats software (Resampling Stats Inc., Arlington, VA), 10,000 iterations of Abbott's formula were generated and used to calculate 95% confidence intervals. Overlap of confidence intervals was used to compare the mortalities between larvae and adults within each nematode species.

Results

Survival of treated and non-treated *C. nenuphar* was compared separately for adult and larval stages because control mortality was substantially different between the two stages (Fig. 1A,B). Survival of *C. nenuphar* larvae treated with *S. feltiae* or *S. riobrave* was reduced relative to non-treated larvae ($F = 8.33$; df = 6, 28; $P = 0.0001$) (Fig. 1A), and survival of *C. nenuphar* adults treated with *S. carpocapsae* and *S. riobrave* was reduced relative to the control ($F = 14.81$; df = 6, 28; $P = 0.0015$) (Fig. 1B); survival of *C. nenuphar* was not affected by other nematode treatments (Fig. 1A,B). For most nematode species there was no difference in susceptibility between adults and larvae (95% confidence limits for insect mortality overlapped), but *C. nenuphar* larvae were more susceptible to *S. feltiae* infection than were adults (confidence limits were 0–30% and 52–100% for adult and larval mortality, respectively). *Heteorhabditis marelatus* produced more IJ per *C. nenuphar* larva than all other nematode species ($F = 9.77$; df = 5, 56; $P = 0.0001$)

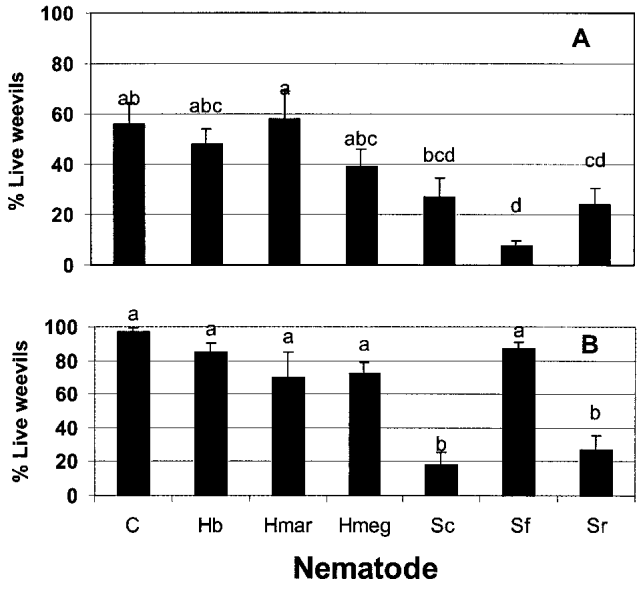

Fig. 1. Survival of *Conotrachelus nenuphar* larvae (A) and adults (B) after exposure to entomopathogenic nematodes. C = control (no nematodes), Hb = *Heterorhabditis bacteriophora,* Hmar = *H. marelatus,* Hmeg = *H. megidis,* Sc = *Steinernema carpocapsae,* Sf = *S. feltiae,* Sr = *S. riobrave.* Different letters above bars indicate statistical significance (Tukey's test, $\propto = 0.05$).

(Fig. 2A) and produced more IJ in adult weevils than *H. megidis* and *S. riobrave* ($F = 2.97$; df = 5, 41; $P = 0.0223$) (Fig. 2B); no other differences were detected in nematode reproduction (Fig. 2A,B).

Discussion

Our results indicate high susceptibility of *C. nenuphar* under laboratory conditions to certain entomopathogenic nematodes but not to others. Previous studies indicated varying levels of potential of entomopathogenic nematodes to suppress *C. nenuphar*. Similar to our research, Olthof and Hagley (1993) observed high susceptibility of *C. nenuphar* larvae to *S. feltiae*. Also similar to our results, Tedders et al. (1982) reported that *S. carpocapsae* was ineffective vs. *C. nenuphar* larvae, but Olthof and Hagley (1993) reported *S. carpocapsae* (All strain) to be highly virulent toward the larvae. The work of Tedders et al. (1992) should be interpreted in the context of a low rate of 15 IJ per insect. The discrepancy between our results and those of Olthof and Hagley (1993) in larval susceptibility to *S. carpocapsae* may have been due to the use of filter paper in some assays in the earlier study, which may increase susceptibility relative to the soil substrate we used (Flanders et al., 1996). Alternatively, the discrepancy may have been due to the conservative analysis we applied; if we had applied a less conservative multiple range test (e.g., SNK test), we would have rejected the null hypothesis that *S. carpocapsae*-treated *C. nenuphar* larvae survival was not different from the control. When Olthof and Hagley (1993) tested *S. carpocapsae* vs. *C. nenuphar* larvae in soil, they also observed significant suppression (73%), but this was using a rate of 10×10^9 per ha, which is substantially higher than a standard rate of $2.5\text{-}5 \times 10^9$ per ha (Georgis and Hague, 1991; Georgis et al., 1995). Using *S. carpocapsae* in field experiments targeted toward *C. nenuphar* adults, Bélair et al. (1998) observed no significant reduction in damage to apples with 5×10^9 IJ per ha, and 30% to 75% damage reduction with 8×10^9 IJ per ha. Certainly some of the inconsistency observed in the study of Bélair et al. (1998) resulted from a loss of nematodes due to desiccation; one half of the nematodes were applied to the canopy and the other half to the soil.

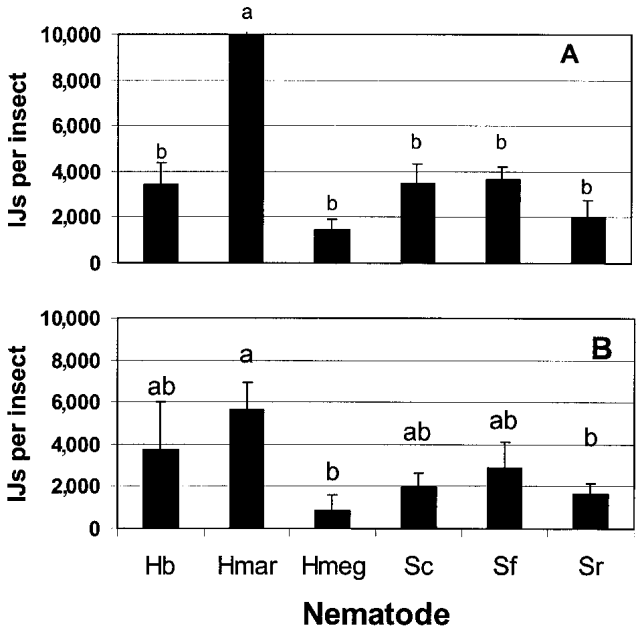

Fig. 2. Reproduction of entomopathogenic nematodes in *Conotrachelus nenuphar* larvae (A) and adults (B). Hb = *Heterorhabditis bacteriophora*, Hmar = *H. marelatus*, Hmeg = *H. megidis*, Sc = *Steinernema carpocapsae*, Sf = *S. feltiae*, Sr = *S. riobrave*. Different letters above bars indicate statistical significance (Tukey's test, $\propto = 0.05$).

Other studies indicate that susceptibility to entomopathogenic nematodes can be affected by insect age or stage (Boivin and Bélair, 1989; Glazer, 1992; Shapiro et al., 1999; Shapiro-Ilan, 2001a). For a number of weevil species, the larval stage is more susceptible to entomopathogenic nematode infection than the adult stage. For example, the Fuller rose beetle, *Asynonychus godmani* Crotch (Morse and Lindegren, 1996), the sweetpotato weevil, *Cylas formicarius,* (F.) (Mannion and Jansson, 1992), and the West Indian sugarcane weevil, *Metamasius hemipterus* (Oliver) (Giblin-Davis et al., 1996) all exhibit stage-specific differential susceptibility with preference for juveniles. This trend also has been observed in various other Coleoptera (Geden et al., 1985; Georgis et al., 1991; Theunis, 1998). Our results also indicated higher virulence of *S. feltiae* to *C. nenuphar* larvae compared with adults. However, recently Shapiro-Ilan (2001a, 2001b) found an exception in pecan weevils *Curculio caryae* (Horn), where adults were more susceptible to entomopathogenic nematodes than larvae. Similarly, we found *S. carpocapsae* to be highly virulent to *C. nenuphar* adults. For some other species, adults are also more susceptible-e.g., mole crickets, *Scapteriscus* spp., exposed to *S. scapterisci* (Parkman and Smart, 1996).

Our results indicate that a management strategy for *C. nenuphar* could entail separate recommendations of one nematode for larval control (e.g., *S. feltiae*) and another for adults (e.g., *S. carpocapsae*). If it would be preferable to recommend only one nematode, *S. riobrave* would likely be the choice because it was the only nematode we found to be virulent to both stages. Potential targets include the emerging first- and second-generation adult weevils (before they reach the trees) and second-stage larvae emerging from fruit. Treating only around the trees or in the border rows would reduce the number of IJ required per acre. We did not test the pupal stage for its susceptibility to entomopathogenic nematodes, but this should be addressed in future studies. Our reproduction data indicate that targeting first-generation *C. nenuphar* may provide some nematodes (through recycling) for suppression of subsequent generations. Further research is required to determine if our results are supported in the field.

Literature Cited

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. Journal of Economic Entomology 18:265–267.

Bedding, R. A., and L. A. Miller. 1981. Use of a nematode, *Heterorhabditis heliothidis,* to control black vine weevil, *Otiorhynchus sulcatus,* in potted plants. Annals of Applied Biology 99:211–216.

Bélair, G., C. Vincent, and G. Chouinard. 1998. Foliar sprays with *Steinernema carpocapsae* against early-season apple pests. Supplement to the Journal of Nematology 30:599–606.

Boivin, G., and G. Bélair. 1989. Infectivity of two strains of *Steinernema feltiae* (Rhabditida: Steinernematidae) in relation to temperature, age, and sex of carrot weevil (Coleoptera: Curculionidae) adults. Journal of Economic Entomology 82:762–765.

Buonaccorsi, J. P., and A. M. Liebhold. 1988. Statistical methods for estimating ratios and products in ecological studies. Environmental Entomology 17:572–580.

Duncan, L. W., C. W. McCoy, and C. Terranova. 1996. Estimating sample size and persistence of entomogenous nematodes in sandy soils and their efficacy against the larvae of *Diaprepes abbreviatus* in Florida. Journal of Nematology 28:56–67.

Flanders, K. L., J. M. Miller, and E. J. Shields. 1996. In vivo production of *Heterorhabditis bacteriophora* 'Oswego' (Rhabditida: Heterorhabditidae), a potential biological control agent for soil-inhabiting insects in temperate regions. Journal of Economic Entomology 89:373–380.

Geden, C. J., R. C. Axtell, and W. M. Brooks. 1985. Susceptibility of the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) to the entomogenous nematodes *Steinernema feltiae, S. glaseri* (Steinernematidae), and *Heterorhabditis heliothidis* (Heterorhabditidae). Journal of Entomological Science 20:331–339.

Georgis R., D. B. Dunlop, and P. S. Grewal. 1995. Formulation of entomopathogenic nematodes. Pp. 197–205 *in* F. R. Hall and J. W. Barry, eds. Biorational pest control agents: Formulation and delivery. Washington, DC: American Chemical Society.

Georgis R., and N. G. M. Hague. 1991. Nematodes as biological insecticides. Pesticide Outlook 2:29–32.

Georgis, R., H. K. Kaya, and R. Gaugler. 1991. Effects of steinernematid and heterorhabditid nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on nontarget arthropods. Environmental Entomology 20:815–822.

Giblin-Davis, R. M., J. E. Pena, and R. E. Duncan. 1996. Evaluation of an entomopathogenic nematode and chemical insecticides for control of *Metamasius hemipterus sericeus* (Coleoptera: Curculionidae). Journal of Entomological Science 31:240–251.

Glazer, I. 1992. Invasion rate as a measure of infectivity of steinernematid and heterorhabditid nematodes to insects. Journal of Invertebrate Pathology 59:90–94.

Grewal P., and R. Georgis. 1998. Entomopathogenic nematodes. Pp 271–299 *in* F. R. Hall and J. J. Menn, eds. Methods in biotechnology, vol. 5. Biopesticides: Use and delivery. Totowa, NJ: Humana Press.

Horton, D., C. Gorsuch, and D. Ritchie. 2001. Southern peach, nectarine, and plum pest management and culture guide, Bulletin 1171. Athens, GA: University of Georgia.

Kaya, H. K., and S. P. Stock. 1997. Techniques in insect nematology. Pp. 281–324 *in* L. A. Lacey, ed. Manual of techniques in insect pathology. San Diego: Academic Press.

Mannion, C. M., and R. K. Jansson. 1992. Comparison of ten entomopathogenic nematodes for control of sweetpotato weevil (Coleoptera: Apionidae). Journal of Economic Entomology 85:1642–1650.

Morse, J. G., and J. E. Lindegren. 1996. Suppression of Fuller rose beetle (Coleoptera: Curculionidae) on citrus with *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Florida Entomologist 79:373–384.

Olthof, T. H., and E. C. Hagley. 1993. Laboratory studies of the efficacy of steinernematid nematodes against the plum curculio (Coleoptera: Curculionidae). Journal of Economic Entomology 86:1078–1082.

Parkman, J. P., and G. C. Smart Jr. 1996. Entomopathogenic nematodes, a case study: Introduction of *Steinernema scapterisci* in Florida. Biocontrol Science and Technology 6:413–419.

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. Boca Raton, FL: CRC Press.

Racette, G., G. Chouinard, C. Vincent, and S. B. Hill. 1992. Ecology and management of plum curculio, *Conotrachelus nenuphar* [Coleoptera: Curculionidae], in apple orchards. Phytoprotection 73:85–100.

Rosenheim, J. A., and M. Hoy. 1989. Confidence intervals for the Abbott's formula correction of bioassay data for control response. Journal of Economic Entomology 82:331–335.

Shanks, C. H., and F. Agudelo-Silva. 1990. Field pathogenicity and persistence of heterorhabditid and steinernematid nematodes (Nematoda) infecting black vine weevil larvae (Coleoptera: Curculionidae) in cranberry bogs. Journal of Economic Entomology 83: 107–110.

Shapiro, D. I., J. R. Cate, J. Pena, A. Hunsberger, and C. W. McCoy. 1999. Effects of temperature and host age on suppression of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) by entomopathogenic nematodes. Journal of Economic Entomology 92:1086–1092.

Shapiro-Ilan, D. I. 2001a. Virulence of entomopathogenic nematodes to pecan weevil larvae *Curculio caryae* (Coleoptera: Curculionidae) in the laboratory. Journal of Economic Entomology 94:7–13.

Shapiro-Ilan, D. I. 2001b. Virulence of entomopathogenic nematodes to pecan weevil adults (Coleoptera: Curculionidae). Journal of Entomological Science 36:325–328.

Shapiro-Ilan, D. I., D. H. Gouge, and A. M. Koppenhofer. 2002. Factors affecting commercial success: Case studies in cotton, turf, and citrus. Pp. 333–355 *in* R. Gaugler, ed. Entomopathogenic nematology. New York, NY: CABI.

Simons, W. R. 1981. Biological control of *Otiorhynchus sulcatus* with heterorhabditid nematodes in the glasshouse. Netherlands Journal of Plant Pathology 87:149–158.

Tedders, W. L., D. J. Weaver, E. J. Wehunt, and C. R. Gentry. 1982. Bioassay of *Metarhizium anisopliae, Beauveria bassiana,* and *Neoaplectana carpocapsae* against larvae of the plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae). Environmental Entomology 11:901–904.

Theunis, W. 1998. Susceptibility of the taro beetle, *Papuana uninodis,* to entomopathogenic nematodes. International Journal of Pest Management 44:139–143.

Woodring, J. L., and H. K. Kaya. 1988. Steinernematid and heterorhabditid nematodes: A handbook of biology and techniques. Southern Cooperative Series Bulletin 331, Fayetteville, AR: Arkansas Agricultural Experiment Station.