Effects of Temperature and Host Age on Suppression of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) by Entomopathogenic Nematodes

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ABSTRACT Effects of temperature and host age on the biocontrol potential of entomopathogenic nematodes against the sugarcane rootstalk borer weevil *Diaprepes abbreviatus* (L.) were tested under laboratory conditions. Virulence and reproductive potential were compared among 3 nematode species: *Steinernema riobrave* (Cabanillas, Poinar & Raulston), *Heterorhabditis bacteriophora* Poinar, and *H. indica* Poinar, Karunakar & David. Assays were conducted in plastic cups filled with moist sand. Three soil temperature regimes (21, 24, and 27°C) and 5 larval ages (20, 30, 40, 50, and 100 d old) were combined in various treatments. The larval ages were estimated to represent 4th–10th instar. Older larvae (i.e., 100 d old) were less susceptible to nematode infection than younger larvae. Nematodes were less virulent at 21°C than at 24 or 27°C. The virulence of *H. indica* was greater than *H. bacteriophora* in 50-d-old *D. abbreviatus* larvae at all temperatures, and greater than the other 2 nematode species in 20-d-old larvae at 24°C. *Heterorhabditis bacteriophora* was more virulent than *S. riobrave* in 20-d-old larvae (at 24°C), whereas *S. riobrave* was more virulent than *H. bacteriophora* at 21°C (in 50-d-old larvae). Reproductive potential was greatest in *H. indica* followed by *H. bacteriophora*. The high level of reproduction in heterorhabditid species indicates a potential for nematode recycling in field applications. We conclude that temperature and host age should be considered critical factors in determining the time of nematode application.

KEY WORDS *Diaprepes abbreviatus*, *Steinernema*, *Heterorhabditis*

THE SUGARCANE ROOTSTALK borer weevil *Diaprepes abbreviatus* (L.) causes severe damage to citrus, sugarcane, ornamentals, and vegetables in the West Indies and Florida (Woodruff 1964, McCoy 1995). It is potentially the most destructive weevil in Florida citrus (Schroeder 1994). Adults feed on foliage (causing a characteristic notching of leaves) and lay eggs between webbed-together leaves within the canopy (Schroeder 1992). Upon hatching, neonates fall to the ground and enter soil. All instars will feed on the roots, progressing from small to very large roots as the larvae mature (Schroeder 1992). Larval feeding can kill trees and reduce production beyond a level of profitability (McCoy 1995). There are no chemical insecticides available that can provide substantial control of *D. abbreviatus* larvae (Bullock et al. 1988, McCoy 1995).

Entomopathogenic nematodes represent an attractive solution for the suppression of *D. abbreviatus* in Florida citrus. Various studies have indicated that field applications with entomopathogenic nematodes can produce substantial control of *D. abbreviatus* populations (Schroeder 1990, 1992; Downing et al. 1991; Duncan and McCoy 1996; Duncan et al. 1996). Levels of suppression, however, have varied greatly among different nematode species and among different applications or formulations of the same species (Downing et al. 1991, Duncan and McCoy 1996, Duncan et al. 1996). For example, reports of *D. abbreviatus* control with the nematode *Heterorhabditis bacteriophora* Poinar vary from no significant control (Duncan and McCoy 1996) to >80% suppression (Downing et al. 1991). The causes of this variation must be explored to maximize the potential of using entomopathogenic nematodes against *D. abbreviatus*.

Efficacy of entomopathogenic nematode applications can be strongly affected by environmental conditions and by suitability of the nematode species or strain for the target host (Georgis and Gaugler 1991, Georgis and Manweiler 1994). For example, soil temperature can have a drastic impact on the success or failure of entomopathogenic nematode applications (Kaya 1990). Efficacy may also depend on the age of the host (Fuxa et al. 1988, Jackson and Brooks 1995). The objective of this study was to determine the effects of temperature and host age on the ability of entomopathogenic nematodes to suppress *D. abbreviatus*. The virulence and reproductive potential of 3

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nematodes were compared: *Steinernema riobrave* (Cabanillas, Poinar & Raulston), *H. bacteriophora*, and *Heterorhabditis indica* Poinar, Karunakar & David. Two of the species, *S. riobrave* and *H. bacteriophora*, have previously been shown to be effective against *D. abbreviatus* (Schroeder 1992, Duncan and McCoy 1996), whereas *H. indica* has not been reported previously as a pathogen of *D. abbreviatus*.

Materials and Methods

Nematodes and Insects. Larvae of *D. abbreviatus* (reared on artificial diet) were obtained from the Horticulture Laboratory (USDA-ARS, Orlando, FL). The Hbl strain of *H. bacteriophora* was used in this study (originally obtained from Dr. R. Gaugler, Rutgers University, New Brunswick, NJ). *Heterorhabditis indica* (Hom1) was obtained from the laboratory of J. Pena (University of Florida, Homestead) and was identified by P. Stock (University of California, Davis). *S. riobrave* was obtained from J. Raulston (USDA-ARS, Weslaco, TX). All nematodes were reared at ≈24°C in last instar greater waxmoth larvae, *Galleria mellonella* (L.), according to the procedures described in Woodring and Kaya (1988). After harvesting nematodes, *S. riobrave* and *H. bacteriophora* were stored in tap water at 10°C. *H. indica* was stored at 15°C because of an increased shelf-life at this temperature relative to 10 or 25°C (D.I.S., unpublished data). In all experiments, nematodes were used within 1 mo of harvest.

Virulence. The virulence of entomopathogenic nematodes was tested using an assay method similar to the one described by Berry et al. (1997). Experimental units consisted of (30 ml) plastic Dixie cups (James River County, Norwalk, CT) (3–4 cm i.d., 3.5 cm deep) filled with sand at 10% gravimetric moisture. A single *D. abbreviatus* larva was placed on the bottom of each cup and a slice of carrot was inserted vertically into the sand until it's upper edge was level with the soil surface where ≈500 infective juveniles were applied the next day. The cups were then wrapped in aluminum foil to maintain soil moisture and to prevent the larvae from chewing out of the plastic. The cups were incubated at various test temperatures for 14 d at which time percentage mortality of the larvae was recorded.

Virulence of the 3 nematode species (*S. riobrave*, and *H. bacteriophora*, and *H. indica*) was determined in 4 different aged hosts and at 3 temperatures. To test the effect of host age, experiments were conducted on 20-, 40-, 50-, and 100-d-old larvae. Based on average weight and development time, these 20-, 40-, 50-, and 100-d-old larvae correspond approximately to 4th-5th, 6th-7th, 7th-8th, and 9th-10th, instars, respectively (Quintela et al. 1998). The experiments contained 3 nematode treatments (*S. riobrave*, *H. bacteriophora*, and *H. indica*) and a control (water). To test the effects of temperature, a single experiment was conducted simultaneously at 21, 24, and 27°C. This experiment contained 9 treatments (a factorial of 3 temperatures and 3 nematodes) and water as a control. The experiment at varying temperatures was conducted on 50-d-old larvae. All experiments consisted of 3 replicates per treatment with 10 cups per replicate. Each experiment was repeated once.

Reproductive Potential. Experiments to determine the reproductive potential of *S. riobrave*, *H. bacteriophora*, and *H. indica* in *D. abbreviatus* larvae of different ages were conducted in cups containing sand using methods similar to those described above. Larvae were checked for mortality 7 and 14 d after nematode application. Each dead larva was placed on a White trap (White 1927, Woodring and Kaya 1988). The number of infective juveniles exiting the host was determined at 5- to 8-d intervals until no further emergence was observed (30–37 d after inoculation). At the end of the experiment, the average number of infective juveniles that emerged was compared among treatments. Cadavers in which nematodes did not emerge were dissected to determine if nematodes had invaded host tissue. If nematodes were not observed in a cadaver then that cadaver was not included in calculating the number of nematodes emerging.

The reproductive potential of 50-d-old larvae was tested simultaneously at 21, 24, and 27°C resulting in 9 treatments (3 nematode species and 3 temperatures). The experiment was repeated to obtain at least 12 replicates per treatment. Smaller experiments were conducted at 24°C to determine the reproductive potential in 40 and 100-d-old larvae. The experiment on 40-d-old larvae contained a minimum of 7 replicates. The experiment on 100-d-old larvae contained 5–8 replicates.

Data Analyses. The effects of host age and temperature on virulence and reproduction of entomopathogenic nematodes were analyzed by analysis of variance (ANOVA) (SAS Institute 1985). The experiments with host age were not combined for all nematode species and larval age groups because experiments on different age groups were not all done simultaneously. Therefore, effects of larval age were analyzed separately for each nematode species. Additionally, differences among nematode species (in virulence and reproductive potential) were analyzed separately for each larval age group. Treatment differences were detected with Duncan multiple range test (SAS Institute 1985).

Results

The susceptibility of *D. abbreviatus* to each nematode species was significantly affected by host age (F = 31.5, 5.2, and 8.7, P = 0.0001, 0.008, and 0.0007 for *H. indica*, *S. riobrave*, and *H. bacteriophora*, respectively; df = 3, 20). For all nematode species, susceptibility of *D. abbreviatus* was markedly less in older larvae (100 d) relative to younger larvae (50 d or younger) (Fig. 1). Twenty-day-old larvae were more susceptible to *H. indica* than 40- or 50-d-old larvae, but no significant difference among 20-d and 40- or 50-d-old larvae was detected when exposed to the other nematode species (Fig. 1). Characteristic signs of nematode infection (Woodring and Kaya 1988) were observed for each

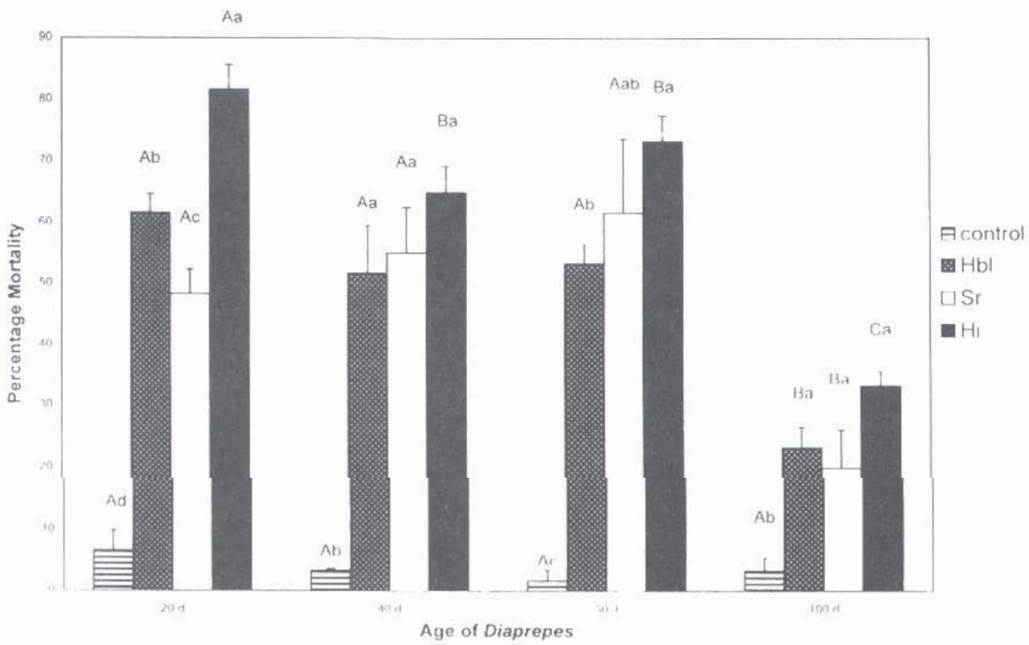


Fig. 1. Nematode-induced mortality of different aged *D. abbreviatus* larvae. Within each age group, different lowercase letters above bars indicate statistical significance ($P < 0.05$) among nematode species and the control. For each nematode species, different uppercase letters represent statistical significance ($P < 0.05$) among age groups. Control, water; Hbl, *H. bacteriophora*; Sr, *S. riobrave*; Hi, *H. indica*

genus; cadavers infected with *Heterorhabditis* spp. were flaccid and colored brownish red to brick red whereas cadavers infected with *S. riobrave* were flaccid and colored brown.

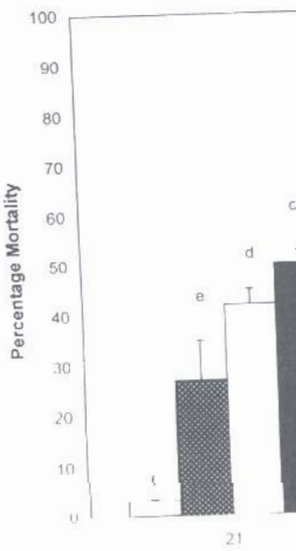
Some differences in virulence were detected among nematode species in different larval age groups (tested at 24°C) (Fig. 1). In 20-d-old larvae, *H. indica* had the greatest virulence followed by *H. bacteriophora* and then *S. riobrave* ($F = 72.5$; df = 3,16; $P = 0.0001$). In 50-d-old larvae, the virulence of *H. indica* was greater than *H. bacteriophora* (the virulence *S. riobrave* was not significantly different from either of the other nematode species) ($F = 40.0$; df = 3, 16; $P = 0.0001$).

Susceptibility of *D. abbreviatus* larvae to nematode infection was significantly affected by temperature ($F = 29.2$; df = 11, 48; $P = 0.0001$) (Fig. 2). The virulence of all nematode species was reduced at 21°C relative to 24°C and 27°C (which were not different from each other). The virulence of *H. indica* was greater than that of *H. bacteriophora* (but not different from *S. riobrave*) at all temperatures that were tested. The virulence of *H. bacteriophora* and *S. riobrave* did not differ significantly at 24°C or 27°C; but at 21°C, greater virulence was detected in *S. riobrave* relative to *H. bacteriophora*.

Nematode reproductive capacity was also affected by host age and temperature. Reproduction was generally greatest in *H. indica* followed by *H. bacteriophora* (Figs. 3 and 4). Reproduction in *S. riobrave* was poor in all age groups and temperatures (Figs. 3 and 4). The reproductive capacity of *H. indica* and *H. bacteriophora* were greater in 100-d-old larvae compared with younger larvae (i.e., 40 d old) ($F = 13.4$, 12.4; $P = 0.0011$, 0.0038; for *H. bacteriophora* and *H. indica*, respectively; df = 1, 26). The reproductive capacity of *S. riobrave* was not significantly affected by age of larvae ($F = 0.45$; df = 1, 13; $P = 0.51$). Temperature did not significantly affect the reproductive capacity of *H. indica* or *S. riobrave* but reproduction was reduced at 27°C relative to 21 and 24°C for *H. bacteriophora* ($F = 19.27$; df = 8, 153; $P = 0.0001$) (Fig. 4).

Discussion

Our results indicate a decreased susceptibility of older *D. abbreviatus* larvae to entomopathogenic nematodes. This relationship has been observed with entomopathogenic nematodes and other insects such as *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Fuxa et al. 1988) and *S. littoralis* Boisduval (Lepidoptera: Noctuidae) (Glazer 1992). Two (of many) factors that can affect host susceptibility to entomopathogenic nematodes are size and immune response (Kaya 1990). Insects or insect stages that are too small may physically inhibit nematode infection or development (Jackson and Brooks 1995). Yet as insect



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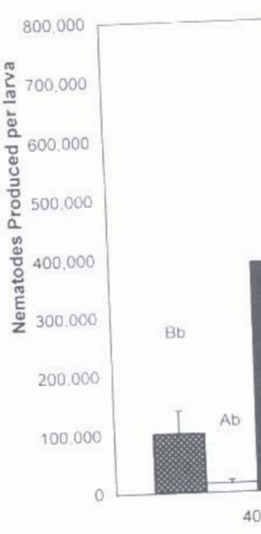

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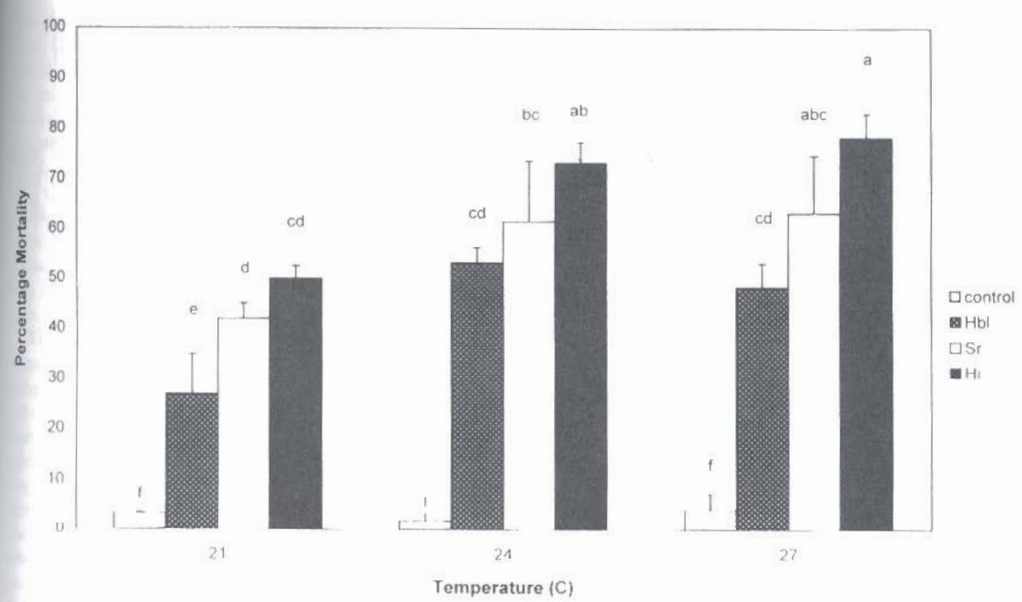

Fig. 2. Nematode-induced mortality of 50-d-old *D. abbreviatus* larvae at different temperatures. Different letters above bars indicate statistical significance ($P < 0.05$). Control, water; Hbl, *H. bacteriophora*; Sr, *S. riobrave*; Hi, *H. indica*.

larvae grow and age, their immune systems generally become stronger and they become less susceptible to pathogens (Watanabe 1987). Therefore, once an insect grows beyond a certain threshold size that is inhibitory to nematode infection, susceptibility begins to decrease with age. This seems to be the case with

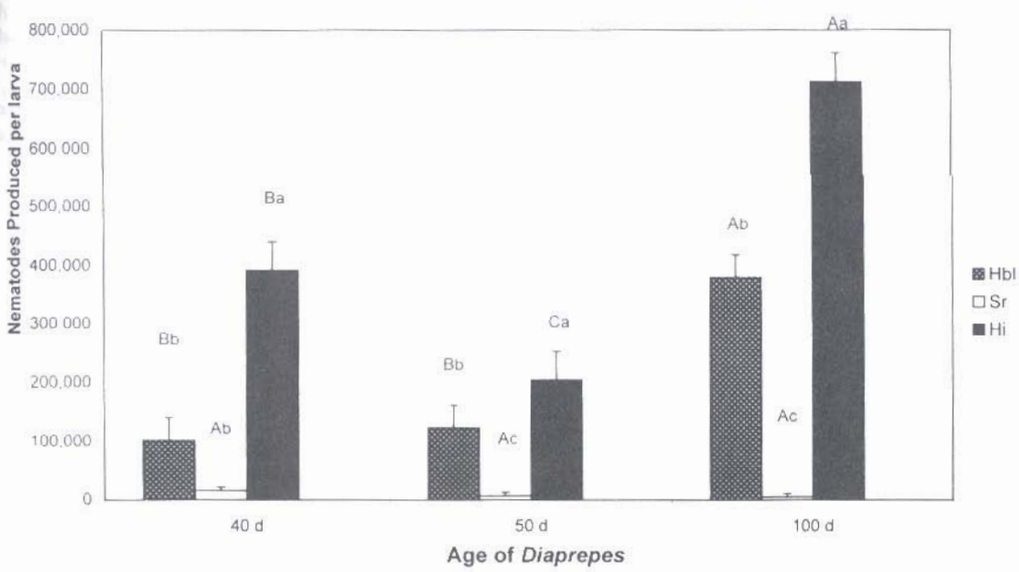

Fig. 3. Reproduction of nematodes in different aged *D. abbreviatus* larvae. Within each age group, different lowercase letters above bars indicate statistical significance ($P < 0.05$) among nematode species. For each nematode species, different uppercase letters represent statistical significance ($P < 0.05$) among age groups. Hbl, *H. bacteriophora*; Sr, *S. riobrave*; Hi, *H. indica*.

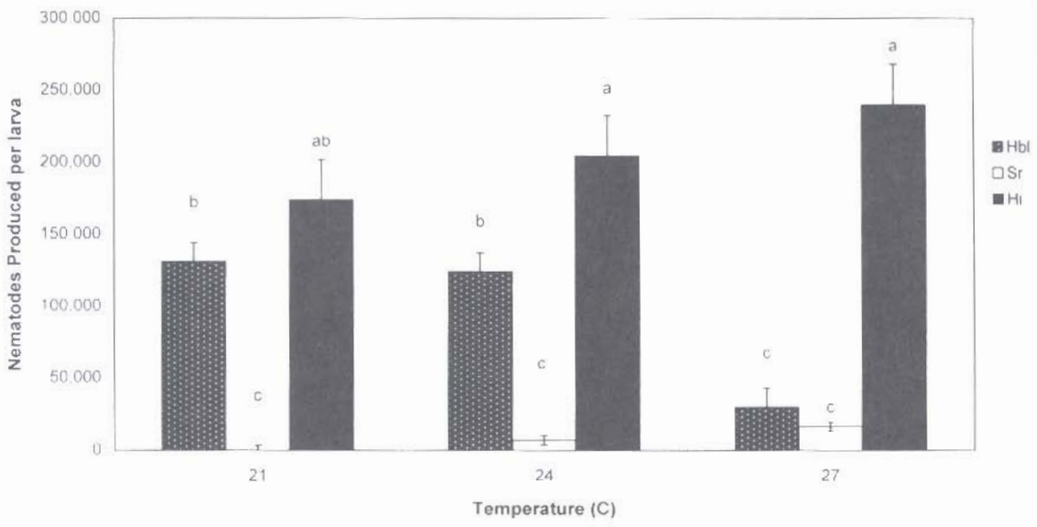

Fig. 4. Reproduction of nematodes in 50-d-old *D. abbreviatus* larvae at different temperatures. Different letters above bars indicate statistical significance ($P < 0.05$). Hbl, *H. bacteriophora*; Sr, *S. riobrave*; Hi, *H. indica*.

D. abbreviatus because neonates were found to be not susceptible (Schroeder 1987) and our data show that later instars become less susceptible with age (at least within the range of ages we tested). This trend has also been observed in the susceptibility of the leafminer *Liromyza trifolii* (Diptera: Agromyzidae) to *Steinernema carpocapsae* (Weiser) (LeBeck et al. 1993). Second-instar leafminers were the most susceptible stage and neonates could not support development of nematodes.

The decreased susceptibility to nematodes in older *D. abbreviatus* larvae may have been the result of reduced feeding in later instars. Quintela et al. (1998) reported a substantial drop in weight gain after the 8th instar for root-fed or artificial diet-fed larvae. Therefore, it is likely that little or no feeding occurs in the later instars (9th–11th). The insect mouth is one of the entry points for entomopathogenic nematodes (Woodring and Kaya 1988). In this study, it is conceivable that by feeding on carrot, the younger instars became more susceptible to nematodes than older larvae because the former opened their mouths more frequently. Schroeder (1994) reported no difference in nematode susceptibility between *D. abbreviatus* larvae with carrot and without. However, Schroeder (1994) used 3-mo-old larvae, which were probably already past the point of intensive feeding.

Greater reproductive potential of entomopathogenic nematodes was observed in older *D. abbreviatus*. As expected, larger larvae yield more infective juveniles. This tendency has been observed in *G. mellonella* in which large last instar larvae produced twice as many infective juveniles as smaller last instar larvae (Flanders et al. 1996).

Our study indicates that applications of the entomopathogenic nematodes tested at warmer soil temperatures (24–27°C) will likely provide better control of *D. abbreviatus* than applications at lower temperatures (e.g., 21°C). Our results are consistent with previous studies in which infectivity of *H. bacteriophora* and *S. riobrave* to *G. mellonella* decreased as temperatures dropped from 25 to 20°C (Grewal et al. 1994). The temperature range chosen in this study (21–27°C) is representative of the soil temperatures range that might be encountered under the canopy of a citrus grove in central Florida during the months that nematodes are applied (March–November) (DuCharme 1971).

The effects of temperature on entomopathogenic activity (e.g., virulence, reproduction, infection) has been related to the nematode's place of origin (Molyneux 1986, Grewal et al. 1994). *S. riobrave* and *H. indica* have only been isolated from warm climates (Poinar et al. 1992, Cabanillas et al. 1994, Berry et al. 1997), whereas *H. bacteriophora* has been isolated from sources diverse in temperature (e.g., Florida, South Dakota, Indiana) (Poinar 1990; J. J. Jackson, USDA-ARS, Brookings, SD, unpublished data; D.I.S., unpublished data). Therefore, it is surprising that the virulence of *H. bacteriophora* was particularly reduced at 21°C relative to the other nematode species. Different strains of *H. bacteriophora* have different temperature tolerances (Shapiro et al. 1996); thus, another strain may have performed differently at 21°C.

Steinernema riobrave did not reproduce well in *D. abbreviatus* regardless of the temperature or host's age. In other insects, the reproductive capacity of *S. riobrave* has been observed to be high. Under laboratory conditions similar to those in this study, *S. riobrave* produced an average of >300,000 infective juveniles per *G. mellonella* larva at 25°C (Grewal et al. 1994). Production of >300,000 *S. riobrave* per insect

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has also been documented in *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (the nematode from which the nematode was originally isolated) (Cabanillas and Raulston 1994). Relative to other nematodes *S. riobrave* can be highly effective in killing certain coleopteran pests such as *D. abbreviatus* (Schroeder 1994, Duncan and McCoy 1996, Duncan et al. 1996) and the dried fruit beetle, *Carpophilus hemipterus* L. (Coleoptera: Nitidulidae) (Vega et al. 1994). However, the low reproduction of *S. riobrave* in *D. abbreviatus* relative to lepidopteran hosts may suggest that the nematode is more naturally adapted to Lepidoptera than to Coleoptera.

The results of this study have important implications for the control of *D. abbreviatus* with entomopathogenic nematodes. Foremost is that our laboratory data indicate that the *H. indica* (Homl strain) has great potential as a biological control agent of *D. abbreviatus*. The overall performance of *H. indica* was superior to *H. bacteriophora* and *S. riobrave*. Other studies have shown good biocontrol potential for *H. indica* in other Coleopteran pests. For example, Berry et al. (1997) observed that *H. indica*, along with *H. marelatus*, provided the best control for Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), from among 7 nematode species or strains. Secondly, the high reproductive capacity observed in the heterorhabditids warrant further investigation. High levels of reproduction in infected cadavers after field applications may result in prolonged control of *D. abbreviatus* through several seasons assuming susceptible hosts are available. In general, entomopathogenic nematodes are applied in an inundative approach (i.e., no recycling is expected). Under certain conditions, however, continued suppression of the target pest has been observed (Klein and Georgis 1992, Parkman et al. 1994) Finally, our research illustrates that timing of entomopathogenic nematode applications for control of *D. abbreviatus* should be made with consideration of host age and temperature. Investigations are underway to determine if predictions from this laboratory study will hold true in the field.

Acknowledgments

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Infectivity Studies
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ABSTRACT This s
diamondback moth,
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KEY WORDS di

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proprietary product does not co
mendation by USDA for its use.

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