SUSCEPTIBILITY OF DIAPREPES ABBREVIATUS (COLEOPTERA: CURCULIONIDAE) LARVAE TO DIFFERENT RATES OF ENTOMOPATHOGENIC NEMATODES IN THE GREENHOUSE

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Abstract

The *Diaprepes* root weevil, *Diaprepes abbreviatus*, is an important field and nursery pest of citrus and ornamentals in Florida and the Caribbean. Entomopathogenic nematodes, *Heterorhabditis indica* Poinar, Kanunakar, and David and *H. bacteriophora* Poinar, and *Steinernema riobrave* (Cabanillas, Poinar, and Raulston) were compared at different rates for their ability to parasitize *D. abbreviatus* under greenhouse conditions. Nematodes (0, 11, 22, 54, and 108 infective juveniles per cm^2) were applied to Candler sandy soil (9% moisture = approximately -0.035 bars), contained in plastic pots with one *D. abbreviatus* larva, and a citrus seedling for food. The experiments were conducted at two temperature ranges (22-25°C and 26-30°C). *Steinernema riobrave* caused higher larval mortality compared with *H. bacteriophora* and *H. indica*, and was more virulent at higher temperatures. At the higher temperature, smaller larvae (ca. 7th instar) were more susceptible to *S. riobrave* and *H. bacteriophora* than later instars (ca. 11th instar). Our results indicate that, under greenhouse conditions, *S. riobrave* has the potential for achieving >90% weevil control at a rate of 22 infective juveniles per cm^2.

Key Words: Biological control, Diaprepes abbreviatus, Steinernema riobrave, Heterorhabditis bacteriophora, H. indica, entomopathogenic nematodes

Resumen

El barrenador de raíz de la caña azúcar (*Diaprepes abbreviatus*) es una plaga importante de los cítricos y cultivos ornamentales en Florida y en el Caribe. Los nemátodos entomopatogénicos, *Heterorhabditis indica* Poinar, Kanunakar y David, *H. bacteriophora* Poinar y *Steinernema riobrave* (Cabanillas, Poinar y Raulston), fueron empleados a diferentes niveles para evaluar su capacidad de parasitar a *D. abbreviatus* bajo condiciones de invernadero. Los nemátodos (a razón de 0, 11, 22, 54 y 108 juveniles infectivos por cm^2) se usaron en suelo arenoso candler (con un contenido de humedad de 9%) empleando macetas de plástico con una larva de *D. addreviatus* y una plántula de de cítrico como fuente de alimento. Los experimentos se llevaron a cabo bajo dos rangos de temperatura, 22-25°C y 26-30°C. *Steinernema riobrave* causó mayor mortalidad de larvas que *H. bacteriophora* y *H. indica*, al mismo tiempo que fue más virulento en el rango alto de temperatura. En el rango alto de temperatura, las larvas más pequeñas (ca. 7° instar) fueron más susceptibles a *S. riobrave* y *H. bacteriophora* que larvas más desarrolladas (ca. 11avo instar). Nuestros resultados indican que *S. riobrave* tiene el potencial de lograr un control del barrenador superior al 90% bajo condiciones de invernadero empleando 22 juveniles infectivos por cm^2.

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The *Diaprepes* root weevil, *Diaprepes abbreviatus* (L.), causes severe damage to citrus, vegetables, sugarcane, and ornamentals in the Caribbean and Florida (McCoy 1995; 1999). This insect is potentially the most damaging weevil in Florida citrus (Schroeder 1994). *Diaprepes abbreviatus* can be found infesting plants both in field and nursery settings (McCoy et al. 1995). Infestation of nursery stock is of particular significance because movement of seedlings and plants is one way *D. abbreviatus* is spread from one area to another (Schroeder 1994; McCoy et al. 1995). Adult weevils feed on foliage and deposit eggs between leaves within the canopy (Schroeder 1992). Upon hatching, neonates fall to the ground and enter the soil where all instars feed on the roots causing damage to the rhizosphere (Schroeder 1992).

Various root weevils, including *D. abbreviatus*, are susceptible to entomopathogenic nematodes (Grewal & Georgis 1998). These nematodes are obligate parasites in the genera *Steinernema* and *Heterorhabditis* that kill insects with the aid of a mutualistic bacterium carried in the nematode's intestine (Poinar 1990). The nematodes complete 2-3 generations within the host resulting in new free-living infective juveniles emerging from the host (Poinar 1990). Numerous biotic and abiotic factors can affect nematode efficacy (Grewal & Georgis 1998).

Field applications of entomopathogenic nematodes can reduce larval populations of *D. abbreviatus* (Schroeder 1990; Downing et al. 1991; Schroeder 1992; Duncan & McCoy 1996; Duncan et al. 1996; Bullock et al. 1999). Four species of entomopathogenic nematodes have been sold commercially to suppress *D. abbreviatus* in citrus: *S. carpocapsae* (Weiser), *S. riobrave* (Cabanillas, Poinar, and Raulston), *H. bacteriophora* Poinar, and H. indica Poinar, Karunakar, and David. Previous studies have indicated clearly that *S. carpocapsae* is relatively inferior for control of *D. abbreviatus* (Schroeder 1994, Duncan et al. 1996).

Previous comparisons between the heterorhabditids and *S. riobrave*, however, may not have portrayed natural differences in species pathogenicity. Duncan et al. (1996) and Duncan & McCoy (1996) reported *S. riobrave* to be superior to *H. bacteriophora* in field suppression of *D. abbreviatus* larvae. It is not known, however, if the observed differences in efficacy were due to innate characteristics of the nematodes or differences in production and formulation. In the laboratory, Shapiro et al. (1999) compared *S. riobrave*, *H. bacteriophora*, and *H. indica* against *D. abbreviatus* and found differences in parasitism among the species that depended on larval age and temperature. The results of these laboratory bioassays were not reflective of natural conditions particularly because nematode searching was limited to a maximum depth of 3.5 cm. In sandy soils *D. abbreviatus* larvae have been found at depths greater than 3 m below the soil surface (McCoy 1999). Additionally the study by Shapiro et al. (1999) was conducted without a natural food source and at a single rate of nematodes. To further elucidate the pathogenicity among candidate nematodes, we compared *H. bacteriophora* and *H. indica* with *S. riobrave* at different rates under greenhouse conditions. The results indicate the potential for the use of entomopathogenic nematodes for larval control in container-grown plants.

Materials and Methods

Larvae of *Diaprepes abbreviatus* (reared on artificial diet) were obtained from the USDA-ARS Horticultural Laboratory (USDA-ARS, Orlando, FL). *Heterorhabditis bacteriophora* (Lewiston strain) and *H. indica* (Homl) were obtained from Integrated BioControl Systems, Inc. (Lawrenceburg, IN). *Steinernema riobrave* (Biovector 355) was obtained from Thermo Triology Corporation (Columbia, MD). Before experimentation, passage of nematodes through live hosts did not exceed five transfers. All nematodes were reared at approximately 25°C in last instar greater wax moth larvae,

Galleria mellonella (L.), according to procedures described in Woodring & Kaya (1988). After harvesting, *S. riobrave* and *H. bacteriophora* were stored in tap water at 10°C (Kaya & Stock, 1997) and *H. indica* at 15°C (unpublished data) for up to 8 d before use. Viability of all nematodes was >95% at the time of application.

Experimental units consisted of plastic pots (4 cm i.d., 20 cm deep). Each pot contained sand at 9% moisture (approximately -0.035 bars), one *D. abbreviatus* larva, and one citrus seedling (Sun Shu Sha, approximately 0.5 cm diameter at the base). For convenience, the seedling was cut off at the base approximately three days prior to nematode application. Four rates of nematodes (11, 22, 54, and 108 infective juveniles per cm^2) were applied in 1 ml of water using a micropipette. One ml of water was added to control pots. After nematode application, the pots were tightly covered with white plastic to inhibit soil moisture loss, and muslin cloth to provide shading. Water was added to the soil every 3-4 d based on observed weight changes in a random selection of eight pots.

Heterorhabditis bacteriophora and *S. riobrave* were compared at two temperature regimes using two stages of larvae. In the first experiment, the nematodes were applied to pots in a greenhouse at a temperature range from 26-30°C. In the second experiment, the temperature ranged from 22-25°C. In both experiments one half of the pots received 7th instars and one half contained 11th instars; the stage of the insect was determined by head capsule size and larval weight (Quintela et al. 1998). There were three replicates of ten pots for each of the nine treatments (4 rates of two nematodes and a control) and each of the two larval stages i.e., 540 pots per experiment. Each experiment was repeated once (resulting in two trials per experiment). Larval mortality was recorded 12 and 15 d post-treatment for the first and second trial, respectively.

A third experiment compared *H. indica* and *S. riobrave* at two temperature ranges simultaneously at the same temperatures as above (i.e., 26-30°C and 22-25°C). The results of the previous experiments indicated that the effects of larval size were minimal compared to the nematode species effect. Therefore, only one stage (7th instar) of *D. abbreviatus* was used in the third experiment. The third experiment was repeated once (i.e., two trials) and larval mortality was determined after 12 and 13 d for the first and second trials, respectively.

The temperatures at which all experiments were conducted are reflective of soil temperatures beneath the tree canopy in a Florida citrus grove during periods appropriate for nematode application (spring to fall). Soil temperatures of 22-25°C are typical 15 cm below the surface under shaded or mature citrus trees (DuCharme 1971). Soil temperatures of 26-30°C are typical 15 cm below the surface in unshaded groves or under young or damaged trees (DuCharme 1971).

Analysis of variance and Student-Newman-Keuls multiple range test (SAS 1985) were used to analyze nematode effect for all rates combined, at each rate separately (nematode × rate). Analysis of variance was also used to test differences due to temperature and susceptibility of larval stages. Regression analysis (SAS 1985) was conducted to determine the relationship between rate of nematode application and larval mortality.

RESULTS

In experiment one (26-30°C), for both larval stages, the nematode effect (for all rates combined) was significant; *S. riobrave* caused greater mortality in *D. abbreviatus* than *H. bacteriophora* ($F = 329.7$; df = 1,37; $P < 0.0001$, and $F = 131.0$; df = 1,40; $P < 0.0001$, for 7th and 11th instars, respectively). Furthermore, *S. riobrave* caused greater mortality than *H. bacteriophora* at each of the rates tested (Figs. 1A and B).
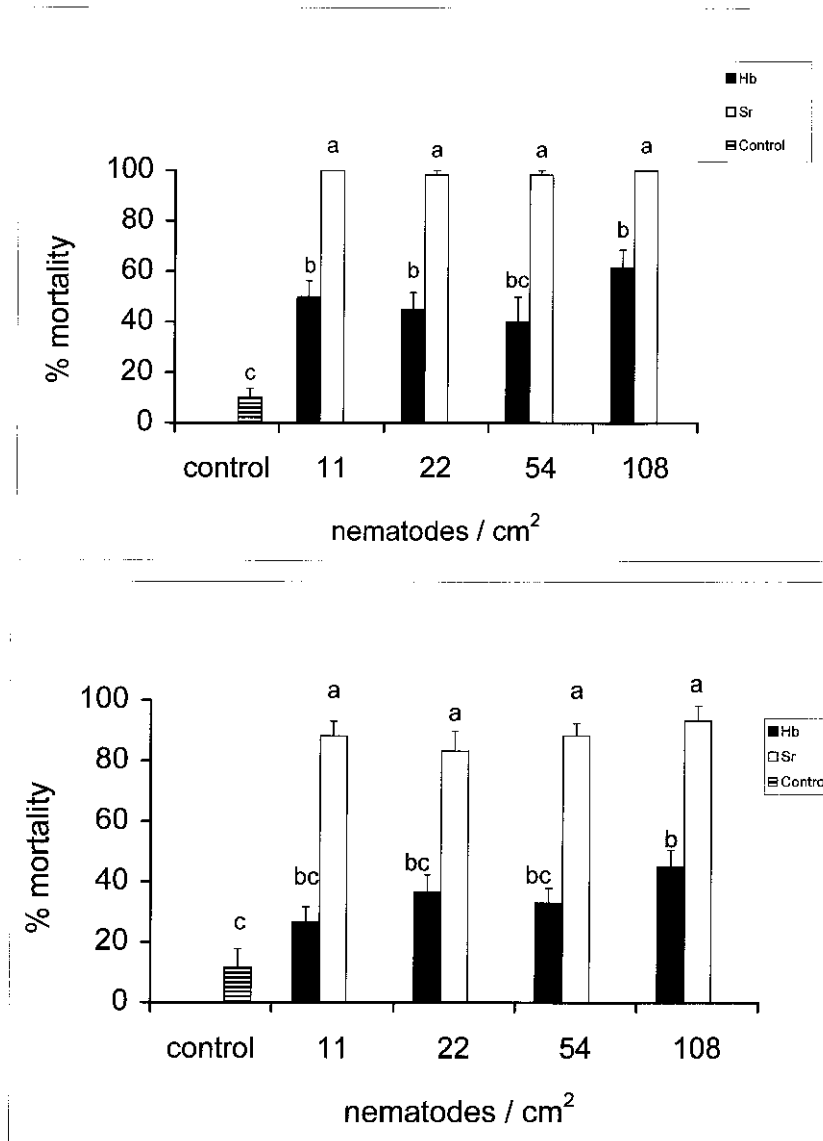
Fig. 1. Nematode induced mortality of 7th (A) and 11th (B) instar *D. abbreviatus* at 26-30°C. Letters above bars indicate statistical significance ($P < 0.05$). Control, water; Hb, *Heterorhabditis bacteriophora*; Sr, *Steinernema riobrave*.

Steinernema riobrave induced mortality (mean ± SE) was greater in 7th instars (99.2 ± 2.8) than 11th instars (88.3 ± 2.5) ($F = 23.4$; df = 1,44; $P < 0.0001$). Heterorhabditis bacteriophora induced mortality (mean ± SE) was also greater in 7th instars (50.5 ±

3.7) than 11th instars (35.4 ± 2.8) ($F = 11.4$; df = 1,41; $P < 0.0016$). Although the slopes were small, a positive linear relationship was observed between rate of *S. riobrave* and *D. abbreviatus* mortality in both stages of larvae ($B_1 = 0.5 \pm 0.16$; $t = 7.6$; $P = 0.005$; $R^2 = 0.22$, and $B_1 = 0.5 \pm 0.15$; $t = 3.3$; $P = 0.0026$; $R^2 = 0.25$ for 7th and 11th instars, respectively). Similar relationships were observed between the rate of *H. bacteriophora* and *D. abbreviatus* mortality ($B_1 = 0.3 \pm 0.1$; $t = 3.3$; $P = 0.003$; $R^2 = 0.28$, and $B_1 = 0.25 \pm 0.07$; $t = 3.5$; $P = 0.0016$; $R^2 = 0.28$ for 7th and 11th instars, respectively).

In experiment two (at 22-25°C), for both larval stages, the nematode effect (for all rates combined) was significant; *S. riobrave* caused higher mortality in *D. abbreviatus* than *H. bacteriophora* ($F = 34.2$; df = 1,40; $P < 0.0001$, and $F = 26.1$; df = 1,40; $P < 0.0001$, for 7th and 11th instars, respectively). Additionally, at most individual rates tested, *S. riobrave* caused higher mortality than *H. bacteriophora* (Figs. 2 A and B). *Steinernema riobrave* induced mortality (mean ± SE) in 7th instars (84.1 ± 2.9) was not significantly different than 11th instars (81.7 ± 3.9) ($F = 0.06$; df = 1,44; $P < 0.81$). Additionally, *H. bacteriophora* induced mortality (mean ± SE) was not significantly different in 7th instars (57.1 ± 4.4) compared with 11th instars (51.7 ± 4.4) ($F = 0.5$; df = 1,44; $P < 0.49$). Although the slopes were small, a positive linear relationship between rate of *S. riobrave* and *D. abbreviatus* mortality was observed in both stages of larvae ($B_1 = 0.06 \pm 0.01$; t = 4.2; $P = 0.0002$; $R^2 = 0.37$, and $B_1 = 0.05 \pm 0.02$; $t = 3.4$; $P = 0.0021$; $R^2 = 0.27$ for 7th and 11th instars, respectively). Similar relationships were observed between the rate of *H. bacteriophora* and *D. abbreviatus* mortality (B1 = 0.05 ± 0.01; $t = 4.3$; $P = 0.0002$; $R^2 = 0.38$, and B1 = 0.03 ± 0.02; $t = 2.7$; $P = 0.011$; $R^2 = 0.18$ for 7th and 11th instars, respectively).

In the third experiment, at both temperatures, the nematode effect (for all rates combined) was significant; *S. riobrave* caused higher mortality than *H. indica* (F = 27.15; df = 2,45; $P < 0.0001$, and $F = 84.91$; df = 2,45; $P < 0.0001$, for the lower and higher temperature regimes, respectively). *Steinernema riobrave* also caused higher mortality than *H. indica* at most rates (Figs. 3 A and B). A positive linear relationship between rate of *Steinernema riobrave* and *D. abbreviatus* mortality was detected at both temperature regimes ($B_1 = 0.4 \pm 0.12$; $t = 3.6$; $P = 0.0014$; $R^2 = 0.29$, and $B_1 = 0.56 \pm 0.12$; $t = 4.5$; P = 0.0001; $R^2 = 0.40$ for the lower and higher temperature regimes, respectively). Similar relationships were observed between the rate of *H. indica* and *D. abbreviatus* mortality ($B_1 = 0.28 \pm 0.10$; $t = 2.8$; $P = 0.0101$; $R^2 = 0.19$, and $B_1 = 0.30 \pm 0.13$; $t = 2.5$; $P = 0.033$; $R^2 = 0.18$ for lower and higher temperature regimes, respectively). Mortality caused by *S. riobrave* was greater at the higher temperature regime compared with the lower temperature regime ($B_1 = 40.61$; df = 5,90; $P < 0.0001$).

DISCUSSION

Our comparisons of pathogenicity were more reflective of the natural or intrinsic abilities of entomopathogenic nematodes to suppress *D. abbreviatus* than previous field and laboratory studies. Previous research (Duncan et al. 1996, Duncan & McCoy 1996) found greater field suppression of *D. abbreviatus* with *S. riobrave* compared with *H. bacteriophora*. In those studies, however, the two nematodes were produced and formulated under different conditions. Therefore, differences in field efficacy involved both nematode production and formulation technology as well as species differences. We demonstrated that *S. riobrave* is innately more virulent to *D. abbreviatus* than *H. bacteriophora* and *H. indica* when the nematodes are cultured and compared in parallel.

Host stage may affect the susceptibility of insect hosts to entomopathogenic nematodes (Fuxa et al. 1988, Shapiro et al. 1999). Shapiro et al. (1999) reported that older
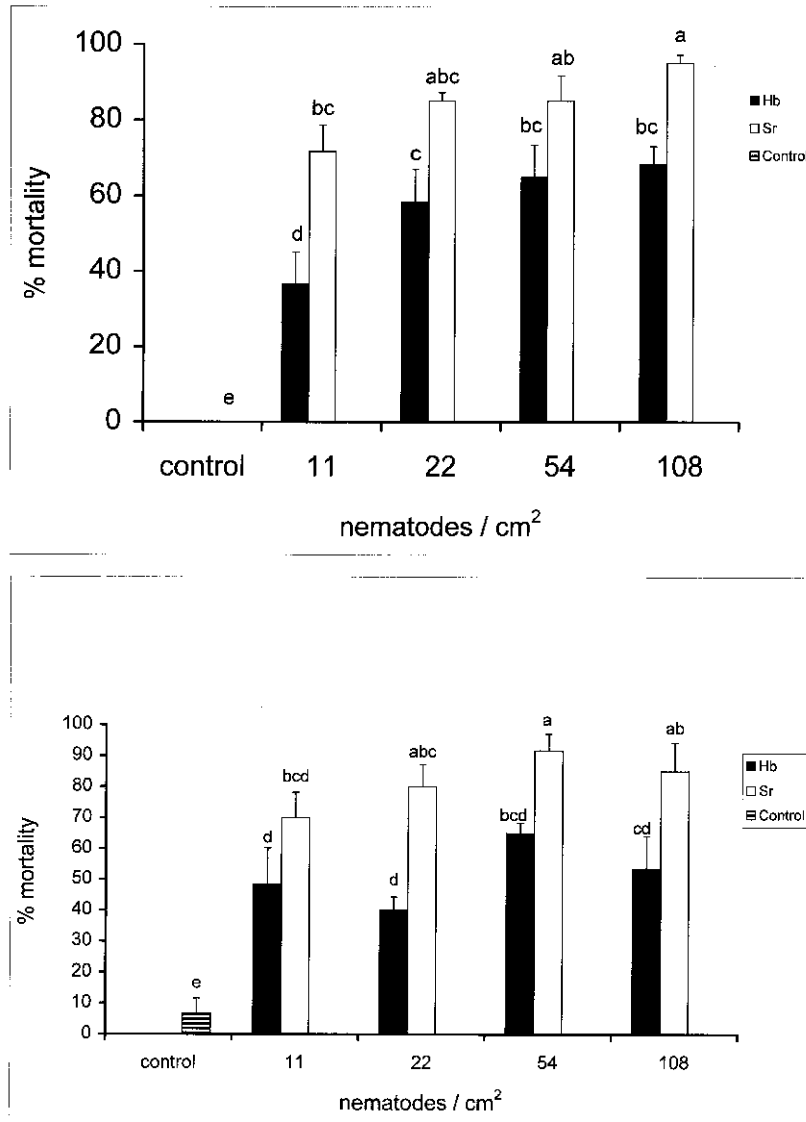
Fig. 2. Nematode induced mortality of 7th (A) and 11th (B) instar *D. abbreviatus* at 22-25°C. Letters above bars indicate statistical significance ($P < 0.05$). Control, water; Hb, *Heterorhabditis bacteriophora*; Sr, *Steinernema riobrave*.

instars of *D. abbreviatus* are more susceptible to entomopathogenic nematodes than younger instars. This study confirmed the above finding at warmer temperatures but not at cooler temperatures. The cause of this discrepancy between temperatures is not
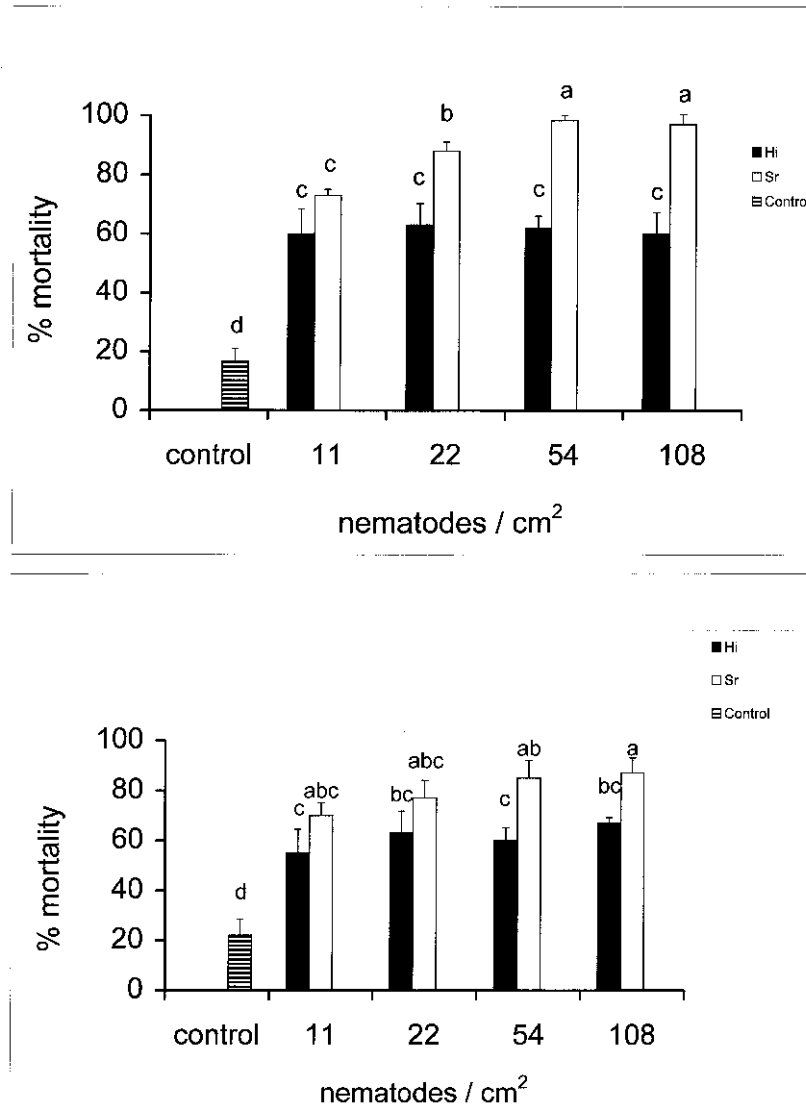
Fig. 3. Nematode induced mortality of 7th instar *D. abbreviatus* at 26-30°C (A) and 22-25°C (B). Letters above bars indicate statistical significance ($P < 0.05$). Control, water; Hi, *Heterorhabditis indica*; Sr, *Steinernema riobrave*.

known but we observed that all variables (species, larval size) were more pronounced at warmer temperatures providing more power to the statistical analyses.

Our results indicate that *S. riobrave* is more virulent to *D. abbreviatus* at warmer temperatures. Similarly, Grewal et al. (1994) reported that *S. riobrave* infectivity

(nematode penetration) in *G. mellonella*, increased continually with temperature up to 35°C. Our results did not detect an effect of temperature on *H. indica* within the ranges tested. Shapiro et al. (1999) reported that both *S. riobrave* and *H. indica* had greater virulence against *D. abbreviatus* at 24°C and 27°C relative to 21°C.

Rate of application is critical to entomopathogenic nematode efficacy (Grewal & Georgis 1998). Field efficacy against *D. abbreviatus* has been achieved using high rates of 108 infective juveniles (Ijs) per cm^2 or more (Downing et al. 1991, Duncan & McCoy 1996, Duncan et al. 1996, Bullock 1999). Field rates currently recommended by commercial companies that sell nematodes for control of *D. abbreviatus* are substantially lower (from 11 to 25 Ijs per cm^2). Successful field application in most other commodities has required rates of 25 Ijs per cm^2 or higher (Georgis & Hague 1991, Grewal & Georgis 1998). In the present study, the heterorhabditid species did not provide >80% suppression of *D. abbreviatus* under any conditions. Contrarily, in the warmer greenhouse, *S. riobrave* provided high levels of mortality (>80%) to *D. abbreviatus* at rates as low as 11 Ijs per cm^2. However, insects are generally less susceptible to nematode parasitism in the field than in the laboratory or greenhouse under controlled conditions (Georgis et al. 1991, Grewal & Georgis 1998). We are currently conducting research to determine optimum rates of *S. riobrave* and *H. indica* under field conditions.

In addition to being severe pests of field crops, *D. abbreviatus* is also an important pest of citrus and woody ornamentals in both nurseries and greenhouses (McCoy et al. 1995). Our data indicate that *S. riobrave* is superior to *H. indica* (Homl) and *H. bacteriophora* (Lewiston) in reducing larval populations of *D. abbreviatus* under greenhouse conditions. In addition, we found that the virulence of several other strains of *H. bacteriophora* (Baine, Hb, HbL, and HP88) and *H. indica* (original strain) are not significantly different from the strains used in this study (unpublished data). The value of nursery stock dictates an extremely low economic threshold. Although *S. riobrave* provided up to 100% larval control with rates as low as 11 Ijs per cm^2, further research is required to determine what rates can achieve adequate root protection for container-grown plants in greenhouses.

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