Effects of Soil Type on Virulence and Persistence of Entomopathogenic Nematodes in Relation to Control of *Diaprepes abbreviatus* (Coleoptera: Curculionidae)

D. I. SHAPIRO,[1] C. W. MCCOY,[2] A. FARES,[2] T. OBREZA,[3] AND H. DOU[4]

University of Florida, Citrus Research and Education Center, Lake Alfred, FL

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ABSTRACT The Diaprepes root weevil *Diaprepes abbreviatus* (L.) is a major pest of citrus, ornamentals, and vegetables in Florida and the Caribbean. Entomopathogenic nematodes can provide substantial control of the root feeding larvae, but their efficacy can be affected by soil type. Our objective was to determine the effects of three soil types on the control of *D. abbreviatus* with *Steinernema riobrave* (Cabanillas Poinar & Raulston) and *Heterorhabditis bacteriophora* Poinar. In the laboratory we measured nematode virulence and persistence in a Marl, Ridge (entisol), and Flatwoods (spodosol) soil. The Marl soil contains a high silt and clay content (80 and 15%, respectively), whereas the other soils are >93% sand and typical soils of citrus production in Florida. The virulence of *S. riobrave* was greater than *H. bacteriophora* in all soils. Both nematode species exhibited greater virulence and persistence in Marl soil compared with sandy soils. Nematode virulence was greater in the spodosol than in the entisol soil. Oxygen levels (in the cups) were not significantly different among the soils. Further research is required to determine the cause of these trends and the applicability of these findings under different water tensions and under field conditions.

KEY WORDS *Steinernema riobrave*, *Heterorhabditis bacteriophora*, *Diaprepes abbreviatus*, soil type, biological control

THE DIAPREPES ROOT weevil *Diaprepes abbreviatus* (L.) causes severe damage to ornamentals, vegetables, sugarcane, and citrus in the Carribean and Florida (McCoy 1995, 1999). This insect is the most damaging weevil in Florida citrus (McCoy 1999). In citrus, adults feed on foliage (causing a characteristic notching) and deposit eggs between leaves within the canopy (Schroeder 1992). After hatching, neonates fall to the ground and enter soil, where all instars feed on the roots (Schroeder 1992). Entomopathogenic nematodes are an important biological component of an integrated pest management (IPM) program for *D. abbreviatus*. These nematodes can be effective control agents of *D. abbreviatus* (Schroeder 1990, 1992; Downing et al. 1991; Duncan and McCoy 1996; Duncan et al. 1996; Bullock et al. 1999a), and are the only recommended control method for larvae that have established themselves in the citrus grove (Bullock et al. 1999b).

Entomopathogenic nematodes are obligate parasites in the genera *Steinernema* and *Heterorhabditis* that kill insects with the aid of a mutualistic bacterium carried in their intestine (Poinar 1990). The nematodes complete two to three generations within the host, after which free-living infective juveniles emerge to seek new hosts (Poinar 1990).

Reported levels of *D. abbreviatus* control with entomopathogenic nematodes have varied. *Steinernema riobrave* (Cabanillas, Poinar & Raulston) suppressed a *D. abbreviatus* population by 77 to >98% (Duncan and McCoy 1996, Duncan et al. 1996, Bullock et al. 1999a). Downing et al. (1991) reported 56–83% *D. abbreviatus* suppression with *H. bacteriophora* Poinar, whereas other researchers have reported no effect with this nematode (Adair 1994, Duncan and McCoy 1996). Numerous biotic and abiotic factors can affect nematode efficacy (Kaya and Gaugler 1993, Grewal and Georgis 1998).

Soil characteristics can contribute to variations in nematode efficacy (Kaya 1990, Barbercheck 1992). Soil moisture is a critical factor influencing nematode survival, movement, and infectivity (Molyneux and Bedding 1984, Kung et al. 1991, Koppenhofer et al. 1995). Increased levels of various soil nutrients can be beneficial to entomopathogenic nematodes (Bednarek and Gaugler 1997, Jaworska et al. 1999). Soil texture can also influence nematode efficacy. As clay content increases, nematode dispersal (Georgis and Poinar 1983, Barbercheck and Kaya 1991) and survival (Kung et al. 1990a) has been reported to decrease.

[1] Current address: SE Fruit and Tree Nut Research Laboratory, USDA-ARS, Byron, GA.

[2] Department of Entomology and Department of Soil and Water Science, University of Florida, IFAS, CREC, Lake Alfred, FL.

[3] Department of Soil and Water Science, University of Florida, IFAS, SWREC, Immokalee, FL.

[4] Florida Department of Citrus, Lake Alfred, FL.

Table 1. Soil characteristics of Ridge, Flatwoods, and Marl soil

Soil	pH	Ca	Mg	K	P	Om	N	Sand	Silt	Clay
Marl	8.0	6,450.0	61.7	2.4	2.4	4.1	2,140.0	4.4	80.3	15.3
Flatwood	6.9	538.0	74.6	88.3	25.5	1.1	490.0	94.0	3.0	3.0
Ridge	6.3	214.0	39.1	36.0	83.0	0.3	172.0	96.6	2.0	1.4

All qualities are expressed as mg/kg except organic matter (OM) and soil texture parameters, which are in percentages, and pH. Ca, K, Mg, and P were measured using Mehlich I extractable elements procedure, N represents total Kjeldahl nitrogen, and OM was measured using Walkley–Black dichromate methodology.

Although some general conclusions have been drawn concerning effects of different soils on entomopathogenic nematode efficacy, various nematode species may be affected differently by soil types (Molyneux and Bedding 1984, Kung et al. 1990a). Furthermore, each soil type contains a variety of unique characteristics that may have different effects on soil biota (Barbercheck 1992). Therefore, studying the effects of specific soil types within a particular biocontrol program is warranted.

Our objective was to determine the persistence and virulence of *S. riobrave* and *H. bacteriophora* in three soil types. Two of the soils are representative of two distinct regions for citrus propagation in Florida: Ridge (Entisol) and Flatwoods (Ft. Pierce, Spodsol) (Obreza et al. 1997). The third soil, Marl, is common in south Florida where it is used for growth of ornamental plants. *D. abbreviatus* is prevalent in all of these soils.

Materials and Methods

Larvae of *Diaprepes abbreviatus* (reared on artificial diet) were obtained from the USDA-ARS Horticultural Laboratory (USDA-ARS, Orlando, FL). Voucher specimens were deposited in the Florida State Collection of Arthropods (Division of Plant Industry, Gainesville, FL). *Heterorhabditis bacteriophora* (Lewiston strain) was obtained from Integrated BioControl Systems (Lawrenceburg, IN). *Steinernema riobrave* (Biovector 355) was obtained from Thermo Triology Corporation (Columbia, MD). All nematodes were reared at ≈25°C in last instar greater waxmoth larvae, *Galleria mellonella* (L.), according to procedures described in Woodring and Kaya (1988). After harvesting, *S. riobrave* and *H. bacteriophora* were stored in tap water at 10°C (Kaya and Stock 1997). All nematodes were stored <4 d before they were used in experiments.

Characteristics of each soil type are listed in Table 1. The Ridge and Flatwoods soils both have a sandy texture and differ in their organic matter content (Table 1). In contrast, the Marl soil is a heavy soil (Table 1). Water release curves are represented in Fig. 1.

Experimental units consisted of plastic cups (2.7–4.0 cm i.d., 4 cm deep) filled to a depth of ≈3 cm with oven dried (≈43°C) soil at a final water tension of −0.04 bars. A water tension of −0.04 bars is frequently encountered in Florida soils during times of nematode application (late spring or early fall) and corresponds to 6.8, 9.8, and 57.0% moisture (by weight) for the Ridge, Flatwoods, and Marls soils, respectively.

Nematode virulence and persistence were determined by measuring mortality of *D. abbreviatus* larvae over time. Approximately 500 *S. riobrave* or *H. bacteriophora* infective juveniles were pipetted onto the soil surface of each cup in 0.5 ml tap water. A single *D. abbreviatus* (seventh to eighth instar) was added to each cup (which was then sealed). To estimate nematode persistence, the larvae were added at three different intervals: on the day of nematode application, 10 d after nematode application, or 25 d after application. Larval mortality was recorded 7 d after the insects were added.

The experiment was a factorial in a completely randomized design. The factorial consisted of all nine treatments: including three soil levels (Ridge, Flatwoods, and Marl) × three nematode levels (*S. riobrave*, *H. bacteriophora*, and a water-control). Each treatment contained three replicates of 10 cups. Thus, there were 30 cups per treatment × nine treatments × three time intervals resulting in 810 cups per experiment. The entire experiment was repeated once. Experiments were contained in a greenhouse at ≈23–25°C.

Oxygen levels inside cups were measured using a flow-through system (model 261112, Orbisphere, Geneva, Switzerland). Soil cups were constructed in a manner identical to those described above. Oxygen levels were measured 2 and 14 d after adding water and sealing the cups. Oxygen was sampled by puncturing the top of each cup with a 4-cm needle attached to a 1-ml syringe (Becton-Dickinson, NJ). Approximately 0.4 ml of gas was removed from each of four replicates of each soil.

A repeated measure analysis (PROC mixed) was used to determine statistical significance of the main

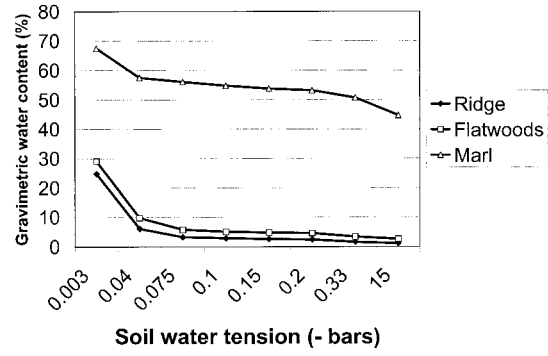

Fig. 1. Soil water release curves for Ridge, Flatwoods, and Marl soils.

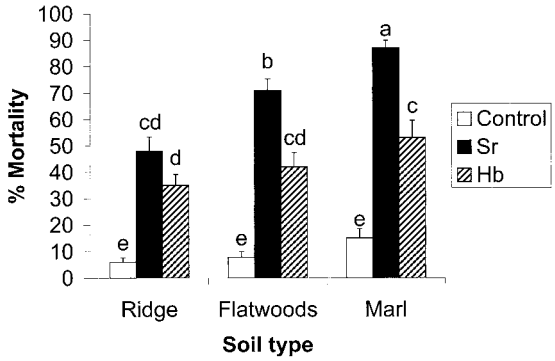

Fig. 2. Mortality of *D. abbreviatus* (averaged over three exposure periods) in three soils after exposure to entomopathogenic nematodes. Insects were added 0, 10, or 25 d after entomopathogenic nematode inoculation. Different letters above bars indicate statistical significance (Student–Newman–Keuls test). Hb, *H. bacteriophora;* Sr, *S. riobrave.*

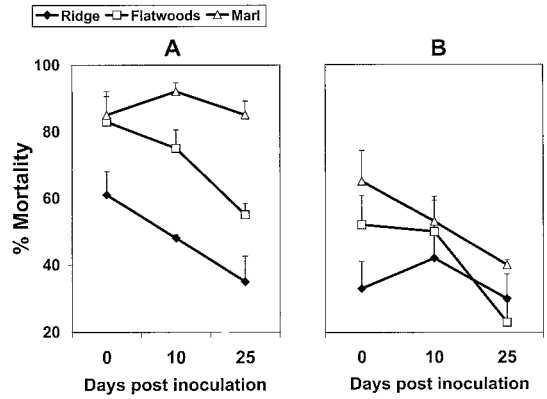

Fig. 3. Mortality of *D. abbreviatus* over time after application of *S. riobrave* (A) or *H. bacteriophora* (B) in three soils. Error bars represent a single standard error from the mean.

effects (soil type and nematodes) over the entire experimental period (SAS Institute 1985). Treatment effects within each of the three time intervals were analyzed separately using analysis of variance (ANOVA) (PROC GLM). If interactions between main effects were detected, the effects of combination treatments (nematode × soil type) were separated using ANOVA (PROC GLM) and the Student–Newman–Keuls test (SAS Institute 1985). Changes in virulence over time were analyzed using linear regression (PROC Reg) (SAS Institute 1985). Oxygen levels were analyzed with ANOVA (PROC GLM) (SAS Institute 1985).

Results

When averaged over the entire experimental period, statistical significance was detected for the main effects and the interaction between them (F = 17.1, 151.3, and 3.44; df = 2,143, 2, 143, and 4, 143; P = 0.0001, 0.0001, and 0.01 for the soil, nematode, and interaction effects, respectively). Mortality caused by *S. riobrave* was greatest in the Marl soil followed by the Flatwoods soil (Fig. 2). The virulence of *H. bacteriophora* was less than that of *S. riobrave* in the Marl and Flatwoods soil and was greater in the Marl soil compared with Ridge (Fig. 2). Mortality was greater in all nematode treatments relative to the controls, which did not differ from each other (Fig. 2).

When each time interval was analyzed separately, significant main effects were detected. For the soil effects, F = 5.6, 10.4, and 3.7; P = 0.007, 0.0002, and 0.033 in the 0-, 10-, and 25-d intervals, respectively (df = 2, 53). For the nematode effects, F = 70.4, 48.3, and 40.2 in the 0-, 10-, and 25-d intervals, respectively (df = 2, 53; P = 0.0001). A significant interaction (soil type × nematode) was only detected in the 25-d interval (F = 2.6; df = 4, 53; P = 0.048). The combination treatments (soil type × nematode) in the 25-d interval were significantly different (F = 23.3; df = 8, 53; P = 0.0001). The Student–Newman–Keuls test ($P \leq 0.05$) detected differences among treatments. In the first two time intervals, nematode virulence in the Marl was not significantly different from virulence in the Flatwoods soils but was greater than the Ridge (Fig. 3). In the third time interval (25 d), *S. riobrave* virulence was greater in the Marl soil than the Flatwoods and the Ridge, which were not significantly different from each other (Fig. 3A). The virulence of *H. bacteriophora* was not significantly affected by soil type in the 25-d interval (Fig. 3B). The virulence of *S. riobrave* was greater than *H. bacteriophora* in all soils and time intervals except in Ridge soil 25 d after application, in which case no difference between species was detected. Larval mortality was greater in all nematode treatments relative to the controls, which did not differ from each other.

The changes in nematode virulence over time are depicted in Fig. 3. Linear regression indicated a significant decline in *S. riobrave* virulence in the Ridge ($y = 60.6 - 0.1x$; $P = 0.04$; $R^2 = 0.2$) and Flatwoods soils ($y = 84.5 - 0.1x$; $P = 0.002$; $R^2 = 0.4$), yet virulence in the Marl soil remained relatively stable ($P = 0.97$). A significant decline in *H. bacteriophora* virulence over time was detected in the Flatwoods soil ($y = 55.4 - 0.1x$; $P = 0.02$; $R^2 = 0.25$) but not in the Ridge or Marl (P = 0.67 and 0.13 for the Ridge and Marl soils, respectively).

No significant differences in percentage oxygen content were detected among soils 2 or 14 d after the experimental units were constructed (F = 0.19; df = 2, 11; P = 0.83 and F = 0.9; df = 2, 11; P = 0.44, respectively). After 2 d, mean ± SD oxygen levels were 5.9 ± 2.6, 6.7 ± 2.0, and 5.8 ± 1.0%, for the Ridge, Flatwoods, and Marl soils, respectively. After 14 d, mean ± SD oxygen levels were 9.1 ± 3.2, 10.1 ± 0.6, and 7.2 ± 4.3% for the Ridge, Flatwoods, and Marl soils, respectively.

Discussion

We found entomopathogenic nematodes to persist and be more virulent against *D. abbreviatus* in a heavy

soil relative to lighter soils. This finding is not consistent with prior research that indicated an opposite trend (Molyneux and Bedding 1984, Geden et al. 1985, Kung et al. 1990a). The discrepancy may be the result of differences in the unique characteristics of the soils tested, which may have varying effects on soil fauna (Barbercheck 1992). Additionally, effects of soil type can vary among entomopathogenic nematode species (Molyneux and Bedding 1984, Kung et al. 1990a), and perhaps insect species (Villani and Wright 1990).

Experimental methods may also have contributed to the divergence in observations on effects of soil type. Molyneux and Bedding (1984) did not compare soil types simultaneously, and therefore their comparisons among soils may be questionable. Geden et al. (1985) did not report the moisture levels in their soils, and hence we cannot ascertain if the observed effects were caused by soil type or moisture level. Kung et al. (1990a) attempted to standardize soil moisture at 50% field capacity. However, the relationship between water content and water tension is not linear (Obreza et al. 1997). Therefore, using 50% field capacity to standardize moisture content among soil types is not appropriate because resulting water tensions are likely to differ. Standardizing water tension has been accepted as an appropriate method of normalizing moisture among soil types (Barberchek 1991).

Low oxygen levels can be detrimental to entomopathogenic nematode survival (Kung et al. 1990b). In our study, however, oxygen levels were not a factor in determining nematode virulence within the soil types. Kung et al. (1990b) reported that *S. carpocapsae* and *S. glaseri* were not able to kill *G. mellonella* after 2 wk exposure to soil with an oxygen level of 10%. Our results, however, indicate that *S. riobrave* and *H. bacteriophora* were able to survive and cause high levels of mortality to *D. abbreviatus* at oxygen levels below 10%. Oxygen levels below 10% are not commonplace, at least within the first 30 cm of soil (Baver et al. 1972). In deeper soil, or in water-saturated soil, oxygen may be an important factor, but we contend that, in general, other factors are likely to be more important in determining efficacy of entomopathogenic nematode in different soil types.

Entomopathogenic nematode mobility has been demonstrated to decrease as soil particle size decreases (Georgis and Poinar 1983, Barbercheck and Kaya 1991). Smaller pore sizes in heavy soils are often not conducive to nematode movement relative to soils with larger particles (Wallace 1958). In our study, dispersal was not a limiting factor because the highest virulence was observed in the heaviest soil. However, in circumstances where nematodes must traverse greater distances than in our experiments, movement (and thus efficacy) might be hindered in heavy soils.

It is unclear what physical or chemical characteristics caused the Marl soil to support the greatest nematode virulence and the Flatwoods to be second. Based on research conducted by Kung et al. (1990b) the differences in pH among the soil types we tested could not account for any differences in virulence or persistence. The trends we observed in virulence are consistent with the trends in amounts of nitrogen, calcium, and organic matter in the soils (i.e., Marl soil has the highest level followed by Flatwoods soil) and are opposite to the amounts of phosphorous in the soil. Perhaps one or all of these soil characteristics affected nematode virulence. Depending on the nematode species, length of exposure, and concentration, various soil nutrients may enhance or hinder entomopathogenic nematode infectivity and virulence (Shapiro et al. 1996, Bednarek and Gaugler 1997, Jaworska et al. 1999). For example, Jaworska et al. (1999) reported an increase in virulence of *S. carpocapsae* and *H. bacteriophora* upon treatment with Mg or Mn ions. Addition of organic matter to soil (i.e., compost) has also been suggested to improve entomopathogenic nematode survival and efficacy (Ishibashi and Kondo 1986). Further research is required to determine if the soil nutrients and organic matter content in the Marl and Flatwoods soil may have been factors improving nematode virulence.

We hypothesize that the amount of moisture in the soil may have contributed to the observed trend. Although the water tensions were equal among soil types, the amount of water that was held in the Marl soils was greatest followed by the Flatwoods soil and least in the Ridge. Indeed, the approximate available pore space filled with water was 85, 33, and 24% for the Marl, Flatwoods, and Ridge, respectively. Thus, our data indicate that higher water content in the soil may be favorable to nematode virulence and survival.

Different water tensions may have variable effects on different nematode species (Molyneux and Bedding 1984, Koppenhofer et al. 1995). We only tested the effects of soil type at a single water tension (-0.04 bars); it is conceivable that the trends we observed may have been different at other water tensions. One might argue that, rather than water tension, field capacity could be used to standardize moisture among soils types. Field capacity is the moisture level a soil will hold against gravity (Obreza et al. 1997). Field capacity, however, also represents only a single point in time during the fluctuating water content of a field soil. To obtain a more complete view of the system, the effects of Marl, Flatwoods, and Ridge soils on nematode virulence and survival should be investigated at various water tensions.

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