INTERACTIONS OF A *RHABDITIS* SP. ON THE VIRULENCE OF *HETERORHABDITIS* AND *STEINERNEMA* IN PUERTO RICO

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Entomopathogenic nematodes (EPN) have been widely used to control pests in the soil, but the efficacy of EPNs can be affected by a variety of factors, among these, competitive interactions with other nematode species, e.g., free-living bactivorous nematodes (Duncan et al. 2003). Surveys conducted separately by D. A. J. and J. M. G. indicated that a free-living nematode, *Rhabditis* sp., was commonly found in agricultural soils of Puerto Rico and emerged from cadavers of insects, indicating it might be an entomopathogen or an opportunistic invader (Jenkins, unpub.). Therefore, we tested the hypothesis that the native nematode would impact the efficacy of EPN's used to control soil pests in Puerto Rico, either by augmenting the efficacy of the EPNs or by reducing their efficacy through competition. In addition to affecting the mortality caused by the EPNs, the *Rhabditis* sp. could potentially affect the rate at which target organisms were killed by the EPNs.

The bioassay methods used were based on those described by Shapiro & McCoy (2000a, b) and Jenkins et al. (2007). Nematodes and insects were obtained through soil surveys or laboratory cultures. Soil samples were taken from 5 citrus orchards in the University of Puerto Rico Agricultural Experiment Stations at Isabela and Adjuntas with soil probes sterilized with 10% bleach solution. Each sample was divided into five containers, 400 grams of soil (wet weight) per container and five larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) were placed into each container. Soil was moistened to field capacity. After 7 days the larvae were extracted and evaluated for mortality. Dead larvae were placed in White traps (Kaya & Stock 1997) to collect, quantify and identify any nematodes coming from the cadavers. These nematodes were then placed on *G. mellonella* larvae to demonstrate infection and thereby satisfy Koch's postulates. EPNs were not found in any of the soil samples collected, but most of the soil samples did yield an unidentified *Rhabditis* sp. Nematodes were stored in tap water at 13°C. Cultures of *Heterorhabditis bacteriophora* Oswego strain, and *Steinernema glaseri* NJ93 strain were obtained from the laboratory of D. S. I. and subsequently reared in *G. mellonella* in Puerto Rico. *Diaprepes abbreviatus* larvae were obtained from Florida Division of Plant Industry.

To assess whether nematode interactions were additive, antagonistic or synergistic we used the analyses outlined in Nishimatsu and Jackson (1998). The nature of the interaction was determined by comparing expected percentage mortality of *D. abbreviatus* to observed mortality, adjusted using Abbott's formula (Abbott 1925), and subjecting these comparisons to χ^2 tests. Expected mortality was derived from the formula $P_E = P_O + (1 - P_O)(P_1) + (1 - P_O)(1 - P_1)(P_2)$, where P_E is the expected mortality, P_O is the control mortality, P_1 is the mortality from 1 nematode sp. treatment alone, and P_2 is the mortality from the other nematode sp. applied alone. The χ^2 value was derived from the formula $\chi^2 = (L_O - L_E)^2/L_E + (D_O - D_E)^2/D_E$, where L_O is the number of living larvae observed, D_O is the number of dead larvae observed, and D_E is the number of dead larvae expected (Nishimatsu and Jackson 1998; SAS 2003). Interactions were deemed additive if the χ^2 value was less than 3.84, antagonistic if the χ^2 value was greater than 3.84 and the observed mortality from the combination was less than the expected mortality from the combination, and synergistic if the χ^2 value was greater than 3.84 and the observed mortality from the combination was greater than the expected mortality from the combination.

There was no difference between the expected and observed mortalities when the nematodes were combined. No interaction was observed between *Rhabditis* sp. and the EPNs at the doses we assayed, indicating that the native and common *Rhabditis* sp. is not likely to interfere with applications of the EPNs we assayed (Table 1). *Heterorhabditis bacteriophora* and *S. glaseri* caused higher mortality of *D. abbreviatus* than either the control or the *Rhabditis* sp. treatment. At the doses tested there was no difference between the mortality caused by *Rhabditis* sp. and the control treatment. However, in the second trial, 10 days after treatment the combination of *S. glaseri* and *Rhabditis* sp. caused higher mortality than the control; whereas neither the *S. glaseri* treatment nor the *Rhabditis* treatment applied alone was different from the control. This may indicate

TABLE 1. MEAN (±SEM) PROPORTION OF *DIAPREPES ABBREVIATUS* LARVAE SURVIVING AFTER INOCULATION WITH VARIOUS NEMATODE SPECIES AND COMBINATIONS. MEANS FOLLOWED BY THE SAME LETTER WITHIN A COLUMN WERE NOT DETERMINED TO BE SIGNIFICANTLY DIFFERENT.

	Mean proportion surviving ±SEM Days post inoculation		
	2	5	10
Trial 1			
Control	1.00 ± 0.00 a	0.98 ± 0.02 a	0.78 ± 0.08 a
Rhabditis sp. (10 IJs/cm^2)	0.92 ± 0.02 a	0.88 ± 0.02 ab	0.76 ± 0.06 a
H. bacteriophora (10 IJs/cm^2)	0.82 ± 0.02 a	0.40 ± 0.04 d	0.16 ± 0.01 c
H. bacteriophora ± *Rhabditis* sp. (5 IJs/cm^2)	0.82 ± 0.05 a	0.42 ± 0.05 d	0.16 ± 0.07 c
S. glaseri (10 IJs/cm^2)	0.84 ± 0.08 a	0.70 ± 0.11 bc	0.38 ± 0.10 b
S. glaseri ± *Rhabditis* sp. (5 IJs/cm^2)	0.90 ± 0.05 a	0.64 ± 0.12 c	0.38 ± 0.04 b
Trial 2			
Control	0.88 ± 0.05 a	0.72 ± 0.04 a	0.62 ± 0.04 a
Rhabditis sp. (10 IJs/cm^2)	0.96 ± 0.03 a	0.84 ± 0.03 a	0.68 ± 0.07 a
H. bacteriophora (10 IJs/ cm^2)	0.94 ± 0.03 a	0.30 ± 0.04 b	0.24 ± 0.04 c
H. bacteriophora ± *Rhabditis* sp. (5 IJs/cm^2)	0.94 ± 0.04 a	0.34 ± 0.03 b	0.30 ± 0.05 bc
S. glaseri (10 IJs/cm^2)	0.94 ± 0.04 a	0.68 ± 0.05 a	0.48 ± 0.07 ab
S. glaseri ± *Rhabditis* sp. (5 IJs/cm^2)	0.96 ± 0.03 a	0.66 ± 0.03 a	0.34 ± 0.08 bc

some potential for enhanced suppression of the target pest when the 2 nematodes are in the soil simultaneously. On the other hand, when applied in conjunction with *S. glaseri*, which is visibly different in size from the *Rhabditis* sp., infective juveniles of *Rhabditis* sp. were observed emerging from cadavers, indicating that the 2 species may compete for resources within the host.

SUMMARY

A *Rhabditis* sp. of nematode was collected from soil and then assayed for virulence to last instar larvae of *Diaprepes abbreviatus*, both alone and in conjunction with the entomopathogenic nematode species, *Steinernema glaseri* and *Heterorhabditis bacteriophora*. When *Rhabditis* sp. was applied at low doses (10 infective juveniles per cm^2), we did not detect a significant difference between the mortality in the *Rhabditis* sp. treatment and the control treatment. However, cadavers from soil that had been treated with the *Rhabditis* sp. yielded *Rhabditis* sp. nematodes, indicating that it is an opportunistic invader of cadavers. When applied with either *S. glaseri* or *H. bacteriophora* at low doses, *Rhabditis* sp. nematodes had no detectable impact on the virulence of the other nematodes, either in total mortality caused or in the speed of the mortality. Because *S. glaseri* is so much larger than the *Rhabditis* sp., it was easy to distinguish which nematode emerged from cadavers exposed to both, and in several cases *Rhabditis* sp. nematodes were detected emerging from cadavers, demonstrating its opportunistic nature.

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