INFECTIVITY AND REPRODUCTION OF THREE HETERORHABDITID NEMATODES (RHABDITIDA: HETERORHABDITIDAE) IN TWO INSECT HOSTS

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ABSTRACT

The infectivity, incubation time, and reproduction of three heterorhabditid nematodes, *Heterorhabditis* sp. Bacardis and FL2122 strains and *H. bacteriophora* Poinar HP88 strain were studied in two insect hosts, an apionid weevil, *Cylas formicarius* (F.), and a pyralid moth, *Galleria mellonella* (L.). Two of the nematodes, *Heterorhabditis* sp. Bacardis and FL2122 strains, were of tropical or subtropical origin, whereas the third nematode, *H. bacteriophora* HP88 strain, was of temperate origin. Infectivity did not differ among nematodes within each host; however, it did differ between hosts for the Bacardis strain. *Cylas formicarius* was more susceptible to this nematode than *G. mellonella*. Incubation times also did not differ among nematodes within hosts; however, incubation times were 3.2-4.3 d shorter in *C. formicarius* than in *G. mellonella*. Progeny production differed (although not significantly consistent) among nematodes and was highest for *Heterorhabditis* sp. Bacardis followed by the *Heterorhabditis* sp. FL2122 and *H. bacteriophora* HP88 in both hosts. Percentages of infected cadavers that produced progeny were consistently higher for the tropical and subtropical nematodes, *Heterorhabditis* sp. Bacardis and FL2122, than for the temperate nematode, *H. bacteriophora* HP88, in both hosts. Patterns of emergence from cadavers were consistent in *G. mellonella*; most progeny emerged by 23 d after inoculation and emergence lasted for up to 48 d after inoculation. Conversely, emergence patterns varied markedly in *C. formicarius*. Emergence lasted for up to 29 d after inoculation and peak emergence varied between 12 and 28 d after inoculation. Progeny production in *C. formicarius* was not related to the biomass of the host cadaver.

Key Words: *Heterorhabditis* spp., *Cylas formicarius*, *Galleria mellonella*, infectivity

RESUMEN

Fueron estudiadas la infectividad, el tiempo de incubación y la reproducción de tres nemátodos heterorhabdítidos, las cepas Bacardis y FL2122 de *Heterorhabditis* sp., y la cepa HP88 de *H. bacteriophora* Poinar, en dos insectos hospedantes, un gorgojo apiónido, *Cylas formicarius* (F.), y una polilla pirálida, *Galleria mellonella* (L.). Dos de los nemátodos, las cepas Bacardis y FL2122 de *Heterorhabditis* sp., fueron de origen tropical o subtropical, mientras que el tercer nemátodo, la cepa HP88 de *H. bacteriophora* Poinar, fue de origen templado. La infectividad no difirió entre los nemátodos dentro de cada hospedante; sin embargo, difirió entre los hospedantes para la cepa Bacardis. *Cylas formicarius* fue más susceptible a este nemátodo que *G. mellonella*. Los períodos de incubación tampoco difirieron entre los nemátodos dentro de los hospedantes; sin embargo, los tiempos de incubación fueron 3.2-4.3 días más cortos en *C. formicarius* que en *G. mellonella*. La producción de progenie difirió (aunque no con significación consistente) entre los nemátodos y fue más alta para *Heterorhabditis* sp. Bacardis, seguida por *Heterorhabditis* sp. FL2122 y por *H. bacteriophora* HP88 en ambos hospedantes. Los porcentajes de cadáveres infectados que produjeron progenie fueron consistentemente más altos para los nemátodos tropicales y subtropicales, *Heterorhabditis* sp. Bacardis y *Heterorhabditis* sp. FL2122, que para el templado, *H. bactriophora* HP88, en ambos hospedantes. Los patrones de emergencia de los cadáveres fueron consistentes en *G. mellonella*; la mayoría de la progenie emergió antes de los 23 días posteriores a la inoculación y la emergencia duró al menos 48 días después de la inoculación. Por el contrario, los patrones de emergencia variaron marcadamente entre los 12 y los 28 días después de la inoculación. La producción de progenie en *C. formicarius* no estuvo relacionada con la biomasa del cadáver del hospedante.

Two population parameters of entomopathogenic nematodes that affect their suitability as a biological control agent against specific target insects are their level of infectivity and reproductive capacity. Infectivity refers to the ability of nematodes to cause infection in a target insect (Tanada & Fuxa 1989) and has been shown to vary among nematodes within specific target hosts (Bedding et al. 1983, Molyneux et al. 1983, Morris et al. 1990, Mannion 1992) and among hosts for a given nematode species or strain (Bedding et al. 1983, Morris et al. 1990). The reproductive capacity of nematodes was also shown to differ among nematodes within target insects (Morris et al. 1990, Mannion & Jansson 1992), and among hosts within specific nematode species or strains (Morris et al. 1990). Nematodes with higher levels of infectivity and reproduction within a specific target host may be more effective at controlling a particular insect under field conditions. These two population parameters are also central to long-term persistence. Morris et al. (1990) noted that a high infection rate of soil insects followed by a high rate of reproduction is critical to ensure reinfestation of the habitat by nematode progeny.

Recent studies conducted in the laboratory that used a variety of different bioassay systems showed that heterorhabditid nematodes, especially an undescribed nematode isolated in Florida, *Heterorhabditis* sp. FL2122 strain, were most suitable as biological control agents of the sweetpotato weevil, *Cylas formicarius* (F.) (Mannion 1992). She found that heterorhabditid nematodes had some of the lowest LC_{50} and LC_{90} values, produced more progeny per cadaver, had higher levels of infectivity in sand, soil, and Petri plates, killed more hosts within sweet potato storage roots, and had a greater ability to exit infected weevil cadavers within storage roots and infect new hosts in the soil than steinernematid nematodes. Her data concur with previous

reports (Jansson 1991, Jansson et al. 1990, 1992, 1993) that found heterorhabditid nematodes to be more efficacious against *C. formicarius*. Jansson et al. (1992, 1993) also found that heterorhabditid nematodes persisted longer than steinernematids in the field.

A recent study that determined the potential for applying nematode-infected wax moth, *Galleria mellonella* (L.), cadavers for controlling *C. formicarius* in the field found that an undescribed nematode isolated from Puerto Rico, *Heterorhabditis* sp. Bacardis strain, produced more progeny per *G. mellonella* cadaver than *H. bacteriophora* Poinar HP88 strain (Jansson et al. 1993). The present studies were conducted to more fully compare the suitability of certain nematodes as biological control agents of *C. formicarius* and determined if fitness parameters of three heterorhabditid nematodes differed between two insect hosts, *C. formicarius* and *G. mellonella*.

Materials And Methods

Two insect hosts were used in these experiments: late instar wax moth, *G. mellonella*, and third instar sweetpotato weevil, *C. formicarius*. *Galleria mellonella* larvae were obtained from a commercial supplier (JA-DA Bait, Antigo, Wisconsin). *Cylas formicarius* were reared in the laboratory using methods described previously (Mannion & Jansson 1992).

Three heterorhabditid nematodes were tested: *H. bacteriophora* HP88 strain, and two undescribed nematodes, *Heterorhabditis* sp. FL2122 and Bacardis. The two latter nematodes were of tropical or subtropical origin. The FL2122 isolate was found in central Florida and the Bacardis nematode was isolated near the San Juan harbor in Puerto Rico (R. K. J. et al., unpublished). These two undescribed isolates are presumably local variants of a previously unrecorded species of *Heterorhabditis* (J. Curran, personal communication) reported earlier as *Heterorhabditis* sp. B in Poinar (1990). The HP88 strain was of temperate origin (Poinar 1990). Nematodes were reared *in vivo* (Dutky et al. 1964) in *G. mellonella* larvae as previously described (Mannion & Jansson 1992). Infective juveniles used in these tests were less than two weeks old at the time of the experiment.

Infectivity

The level of infectivity was determined in each host using a single nematode/single host bioassay system (Miller 1989). Single infective juveniles were removed from an aqueous suspension taken from laboratory cultures and pipetted (0.3 ml) onto a double layer of filter paper in individual wells (1.5 cm diam) of a Multiwell™ 24 well, flat bottom tissue culture plate (Falcon®, model 3047, Becton Dickinson and Co., Lincoln Park, New Jersey). One host larva was then placed on the filter paper in each well. Filter paper treated with deionized water served as the control. Microsoap (International Products Corp., Burlington, New Jersey) was added (1.25 ml/liter) to nematode suspensions to improve efficiency of nematode transfer. Tissue culture plates were covered, sealed with parafilm and stored in the dark at 25±2°C. Larvae were checked daily for nematode-induced mortality for four consecutive days. Two trials were conducted against *G. mellonella* larvae and five trials were conducted against *C. formicarius* larvae. A total of four (*G. mellonella*) and twelve (*C. formicarius*) tissue culture plates (96 and 288 larvae, respectively) per nematode treatment were used to determine infectivity of these nematodes.

Efficiency of nematode transfer was determined by removing an aliquot (0.3 ml) with a single nematode from suspensions, dispensing each aliquot onto a Petri plate,

and then counting the number of infective juveniles in each aliquot. A total of 80 aliquots were removed from each suspension on the day of the experiment. The efficiency of transferring single nematodes were 93.7±3.7, 97.5±1.4, and 95.0±2.9 for *H. bacteriophora* HP88, *Heterorhabditis* sp. FL2122, and *Heterorhabditis* sp. Bacardis, respectively. These efficiencies were then used to adjust infectivity data to estimate real infectivity of each nematode.

Incubation Time and Reproduction

All nematode-infected *G. mellonella* larvae and only those *C. formicarius* larvae infected in the first three trials were removed from the tissue culture plates and placed in individual, modified White traps (White 1924) and incubated in the dark at 25±2°C. Cadavers were inspected daily for nematode emergence. The time required for infective juveniles to emerge from each cadaver was recorded. The percentage of cadavers that produced infective juveniles was recorded in both trials with *G. mellonella*, but in only the first and third trials with *C. formicarius*. Once emergence began, infective juveniles were removed from the outer well of each White trap once per week and counted. The time that emergence ceased was also recorded at which time cadavers were dissected and the numbers of infective juveniles within each cadaver were recorded. In the first two trials with *C. formicarius*, the biomass of each larva was recorded before each trial to determine if progeny production was related to host biomass.

Data Analysis

Most data were analyzed by least squares analysis of variance or regression techniques, accordingly (Zar 1984). Percentage infectivity was compared among nematodes within hosts by chi-square analysis (Conover 1980). Mean incubation periods and numbers of progeny produced were compared among nematodes by the Waller-Duncan *K*-ratio *t* test (Waller & Duncan 1984). Numbers of progeny produced per *C. formicarius* cadaver in the first two trials were pooled and regressed on biomass to determine if reproduction was dependent upon host biomass.

RESULTS

Infectivity

Infectivity to *G. mellonella* larvae did not differ ($X^2 = 1.71$, df = 2, $P > 0.05$) among nematodes. Adjusted percentages of infective juveniles that invaded larvae were 15.4, 16.0, and 26.7% for *Heterorhabditis* sp. Bacardis, *Heterorhabditis* sp. FL2122, and *H. bacteriophora* HP88, respectively.

Infectivity to *C. formicarius* also did not differ ($X^2 = 1.9$, df = 2, $P > 0.05$) among nematodes. Adjusted percentages of infective juveniles that invaded *C. formicarius* larvae were 30.3, 25.9, and 20.4% for *Heterorhabditis* sp. Bacardis, *Heterorhabditis* sp. FL2122, and *H. bacteriophora* HP88, respectively.

Infectivity of the *Heterorhabditis* sp. Bacardis nematode was affected by the insect host ($X^2 = 4.9$, df = 1, $P < 0.05$). A higher percentage of infective juveniles invaded *C. formicarius* larvae than *G. mellonella* larvae. Infectivity of *Heterorhabditis* sp. FL2122 and *H. bacteriophora* HP88 did not differ between hosts ($X^2 \leq 2.3$, df = 1, $P > 0.05$).

Incubation Time

The incubation times of nematodes within the two hosts also did not differ ($F \leq 1.1$, df = 2,4, $P > 0.05$) among nematodes. In *G. mellonella* larvae, *Heterorhabditis* sp. Bacardis, *Heterorhabditis* sp. FL2122, and *H. bacteriophora* HP88 required 11.9±0.1, 12.1±0.1, and 12.0±0.1 d, respectively, to emerge from cadavers. In *C. formicarius* larvae, these nematodes required only 8.7±0.2, 7.8±0.2, and 8.1±0.2 d, respectively, to emerge from hosts. Incubation periods for each nematode were significantly shorter in *C. formicarius* than in *G. mellonella*, which is probably related to differences in host size.

Reproduction

In the first trial, percentages of *G. mellonella* cadavers that produced progeny differed among nematodes ($X^2 = 10.7$, df = 2, $P < 0.01$). Higher percentages of *G. mellonella* infected with *Heterorhabditis* sp. Bacardis (66.7%; $n = 6$) and FL2122 (71.4%; $n = 7$) produced progeny than those infected with *H. bacteriophora* HP88 (38.5%; $n = 13$). In the second trial, percentages of cadavers that produced progeny did not differ among nematodes ($X^2 = 3.6$, df = 2, $P > 0.05$). All nematode-infected cadavers produced progeny in high percentages and were 75% ($n = 8$), 100% ($n = 8$), and 91% ($n = 11$) for Bacardis, FL2122, and HP88, respectively.

Patterns of progeny production were consistent for each nematode in *G. mellonella* larvae (Fig. 1). Most progeny emerged from cadavers infected with *Heterorhabditis* sp. Bacardis (67.7-79.3%), *Heterorhabditis* sp. FL2122 (69.0-81.3%) and *H. bacteriophora* HP88 (85.6-96.2%) within 23 d after inoculation. Emergence of progeny declined 23 d after inoculation for all nematodes and lasted for up to 48 d after inoculation.

Total progeny production in *G. mellonella* cadavers did not differ ($F = 2.3$, df = 2,12, $P > 0.05$) among nematodes in the first trial, but did differ ($F = 4.4$, df = 2,12, $P < 0.05$) among nematodes in the second trial (Table 1). Trends in the data were consistent between the two trials. More progeny were consistently produced by *Heterorhabditis* sp. Bacardis followed in decreasing order by *Heterorhabditis* sp. FL2122 and *H. bacteriophora* HP88. Considerably more progeny were produced in the second trial than in the first trial, and the reasons for this are unclear.

Percentages of *C. formicarius* cadavers that produced progeny differed among nematodes in the two trials ($X^2 \geq 15.3$, df = 2, $P < 0.001$). In the first trial, the highest percentages of *C. formicarius* cadavers produced progeny when infected with *Heterorhabditis* sp. Bacardis (90%; $n = 20$) followed in decreasing order by those infected with *Heterorhabditis* sp. FL2122 (41.7%; $n = 12$) and *H. bacteriophora* HP88 (35.3%; $n = 17$). In the third trial, cadavers infected with *Heterorhabditis* sp. FL2122 had the highest percentage of nematode production (90%, $n = 20$) followed by *Heterorhabditis* sp. Bacardis (58.8%; $n = 17$) and *H. bacteriophora* HP88 (46.7%; $n = 15$).

No consistent patterns of emergence of infective juveniles from *C. formicarius* cadavers were found in the three trials (Fig. 2). In the first trial, emergence of *Heterorhabditis* sp. Bacardis peaked 19 d after inoculation, whereas those of *H. bacteriophora* HP88 and *Heterorhabditis* sp. FL2122 peaked 28 d after inoculation. In trial 2, emergence of *H. bacteriophora* HP88 peaked 12 d after inoculation, whereas those of *Heterorhabditis* sp. FL2122 and Bacardis peaked 19 d after inoculation. Few progeny emerged after 19 d. In the third trial, emergence of all three nematodes peaked 16 d after inoculation and few progeny emerged after 16 d.

Total progeny production in *C. formicarius* cadavers did not differ ($F \leq 1.3$, df = 2,24, $P > 0.05$) among nematodes in the first two trials, but did differ ($F = 11.2$, df = 2,32, $P < 0.001$) in the third trial (Table 1). As found in *G. mellonella*, more progeny

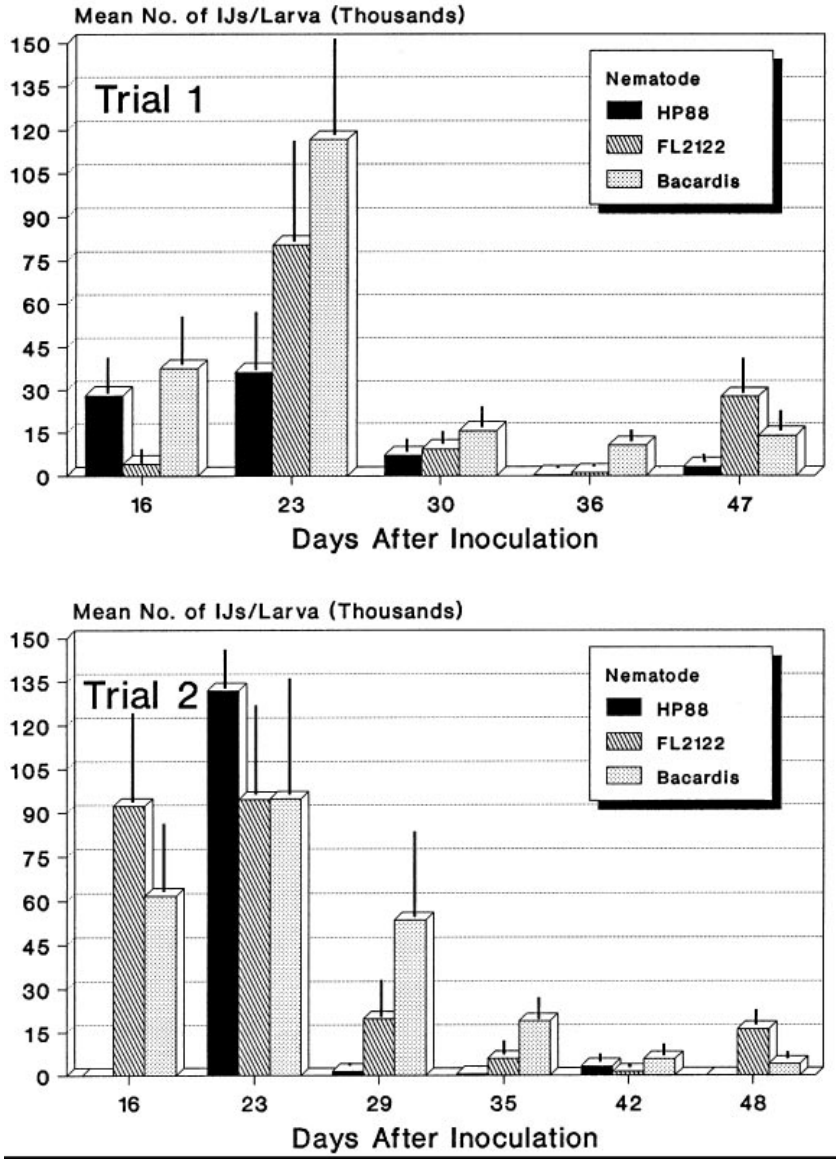

Fig. 1. Emergence patterns of infective juveniles from *G. mellonella* cadavers infected with three different heterorhabditid nematodes: *Heterorhabditis* sp. Bacardis strain, *Heterorhabditis* sp. FL2122 strain, and *H. bacteriophora* HP88 strain. Numbers counted on the last sample date of each trial are the sum of those that emerged plus any remaining infective juveniles within cadavers.

TABLE 1. PROGENY PRODUCTION OF THREE HETERORHABDITID NEMATODES IN TWO INSECT HOSTS, *CYLAS FORMICARIUS* AND *GALLERIA MELONELLA*.

	Trial		
Host × Nematode	1	2	3
C. formicarius			
Heterorhabditis sp. Bacardis	6,522.7(1,844.0)a[1]	7,919.5(1,061.0)a	8,625.6(1,162.6)a
Heterorhabditis sp. FL2122	5,610.6(1,086.5)a	6,896.8(1,167.1)a	4,834.6(676.7)b
H. bacteriophora HP88	1,880.8(469.0)a	3,387.0(259.0)a	2,071.6(388.7)c
G. mellonella			
Heterorhabditis sp. Bacardis	194,222.5(54,228.0)a	238,692.2(45,442.2)a	
Heterorhabditis sp. FL2122	122,580.2(34,579.4)a	229,903.4(30,825.3)a	
H. bacteriophora HP88	74,992.5(30,940.0)a	137,228.8(13,072.1)b	

[1]Data are means ± sem. Means within a column followed by the same letter do not differ by the Waller-Duncan *K*-ratio *t* test (Waller & Duncan,1969).

were consistently produced (although not consistently significant) by *Heterorhabditis* sp. Bacardis followed in decreasing order by *Heterorhabditis* sp. FL2122 and *H. bacteriophora* HP88.

The nematode host influenced progeny production. Approximately 28.1-, 30.5-, and 43.4-fold more progeny were produced in *G. mellonella* than in *C. formicarius* for *Heterorhabditis* sp. Bacardis, *Heterorhabditis* sp. FL2122, and *H. bacteriophora* HP88, respectively.

Progeny production in *C. formicarius* was not related to host biomass for any of the three nematodes (Bacardis: Y = 1054 + 646713X, $F = 1.3$, df = 1,31, $P > 0.05$, $r^2 = 0.04$; FL2122: Y = 91 + 687518X, $F = 1.2$, df = 1,13, $P > 0.05$, $r^2 = 0.09$; HP88: Y = -1901 + 510346X, $F = 2.4$, df = 1,6, $P > 0.05$, $r^2 = 0.29$).

DISCUSSION

Few differences in infectivity were found among nematodes within insect hosts; however, infectivity differed between hosts for *Heterorhabditis* sp. Bacardis strain. This nematode was more infective against the weevil, *C. formicarius*, than against the lepidopteran, *G. mellonella*, despite the fact that these nematodes were all reared *in vivo* in *G. mellonella* before experiments. Bedding et al. (1983) showed that *H. bacteriophora* was least infective against the host from which it was isolated, *Heliothis punctigera* (Wallengren). They also found that *Steinernema feltiae* (Filipjev) (= *Neoaplectana bibionis*) isolated from *Lucilia cuprina* (Wiedemann) and *Otiorhynchus sulcatus* (F.) was least infective to these insects. Kaya (1987) suggested that the host from which a nematode is isolated in soil probably has little, if any, affect on the suitability of the nematode/host encounter. Entomopathogenic nematodes attack a broad

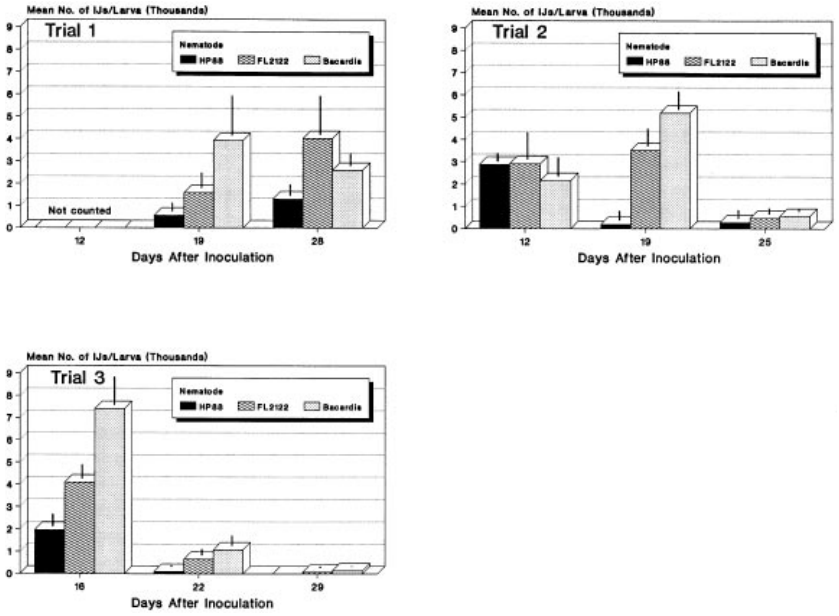

Fig. 2. Emergence patterns of infective juveniles from *C. formicarius* cadavers infected with three different heterorhabditid nematodes: *Heterorhabditis* sp. Bacardis strain, *Heterorhabditis* sp. FL2122 strain, and *H. bacteriophora* HP88 strain. Numbers counted on the last sample date of each trial are the sum of those that emerged plus any remaining infective juveniles within cadavers.

spectrum of insects; thus, isolation from soil is, in part, due to a chance encounter between the isolation host and the nematode. Kaya (1987) also suggested that, in certain cases, continued association with the same insect species may reduce virulence rather than enhance it.

Incubation times also did not differ among nematodes within each species; however, incubation times were considerably shorter for all nematodes in the weevil, *C. formicarius*, than in the lepidopteran, *G. mellonella*. It is well known that emergence of infective juveniles is related to depletion of food reserves and crowding within the host cadaver (Kaya 1985, 1987).

Patterns in total reproduction of nematodes differed among the three nematodes in both hosts. *Heterorhabditis* sp. Bacardis consistently produced more (although not consistently significant) progeny than the other two nematodes in both hosts. These data concur with previous studies (Jansson & Lecrone 1994, Jansson et al. 1993) which showed that *Heterorhabditis* sp. Bacardis produced more progeny per cadaver than *H. bacteriophora* HP88 in *G. mellonella* larvae. Progeny production of Bacardis was higher in the previous study (range, 272,576-396,598 per cadaver) than in the present study (range, 137,229-238,692 per cadaver). However, a dose of 20 infective juveniles per larva was used in the previous study compared with only 1 per larva in the present study. Progeny production of *H. bacteriophora* HP88 was comparable between the two studies despite large differences in dose (range of previous study, 76,260-219,181 per cadaver; range of present study, 74,992-194,222 per cadaver).

Patterns of emergence from cadavers were consistent in *G. mellonella*, but not in *C. formicarius*. In *G. mellonella*, most infective juveniles emerged within 23 d after inoculation. In *C. formicarius*, emergence patterns varied among trials. As noted earlier, emergence of infective juveniles is related to depletion of food reserves and crowding (Kaya 1985, 1987). These factors may have been less apparent to emerging infective juveniles from *C. formicarius* due to the smaller size of this host compared with *G. mellonella*.

Total progeny production of heterorhabditid nematodes in *C. formicarius* concurred with a previous report (Mannion & Jansson 1992), although progeny production was higher for FL2122 and lower for HP88 in the present study. The dose used might have affected these data. Mannion & Jansson (1992) used a dose of 25 infective juveniles per larva compared with 1 per larva in the present study.

It is recognized that a laboratory bioassay that predicts performance of entomopathogenic nematodes in the field is needed to facilitate selection of nematodes in biological control programs (Hominick 1990, Mannion 1992). Mannion (1992) conducted Petri dish, sand, soil, and simulated field bioassays to select suitable entomopathogenic nematodes for the biological control of *C. formicarius* and consistently found that heterorhabditids were superior to steinernematids in all bioassay systems tested. The present bioassay system may also have potential for selecting suitable entomopathogenic nematodes, especially heterorhabditid nematodes, for *C. formicarius*.

Morris et al. (1990) noted that both infectivity and reproduction within hosts were important attributes of nematodes capable of reinfesting new hosts in the field. The present study demonstrated that *Heterorhabditis* sp. Bacardis was most infective and reproductive against *C. formicarius*, and, for this reason, it should be a suitable nematode for use in biological control of this weevil. Recent results from field studies confirm this belief. *Heterorhabditis* sp. Bacardis was shown to be very efficacious at controlling weevil damage to storage roots and persisted at levels higher than those of all other nematodes tested, including *H. bacteriophora* HP88, *S. carpocapsae* (Weiser) All and S20, and *S. feltiae* N27 (Jansson et al. 1993). Collectively, these data suggest that the use of this single nematode/single host bioassay may be an important tool for identifying potential candidate heterorhabditid nematodes in biological control programs of target insect pests. More work is needed, however, to confirm this belief.

These data also suggest that certain Neotropical and subtropical nematodes, *Heterorhabditis* sp. Bacardis and FL2122, may be more suitable as biological control agents against the pantropical sweetpotato weevil, *C. formicarius*, than the temperate nematode, *H. bacteriophora* HP88 strain. Recent studies by Lawrence (1994) also suggested that certain Neotropical nematodes may be more suitable as biological control agents of this weevil; however, not all tropical and subtropical isolates were superior to all temperate isolates.

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nematodes. These nematodes are currently being held at the University of Florida, I.F.A.S., Entomology and Nematology Department, Gainesville, FL and at the Department of Entomology, Rutgers University. The present research was supported by the U.S. Department of Agriculture under CSRS Special Grant No. 91-34135-6134 (to R.K.J.) managed by the Caribbean Basin Administrative Group (CBAG). Research was conducted at the University of Florida, I.F.A.S., T.R.E.C., Homestead. The manuscript was prepared, in part, at Merck & Co., Inc.

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