Laboratory Evaluations of Four Entomopathogenic Nematodes for Control of Subterranean Termites (Isoptera: Rhinotermitidae)

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ABSTRACT The infectivity of four species of entomopathogenic nematodes, *Steinernema carpocapsae* (Weiser) [Breton strain], *Steinernema riobrave* Cabanillas, Poinar & Raulston [Weslaco strain], *Heterorhabditis bacteriophora* Poinar [HP88 strain], and *Heterorhabditis indica* Poinar, Karunakar & David [Coimbatore strain] was examined in the laboratory against two subterranean termites: *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki. In petri dish tests, they were all effective against *C. formosanus* at ≥400 nematodes per termite. *Steinernema riobrave* had no detectable effect against *R. flavipes* even at a rate of 2,000 nematodes per termite. The virulence of the nematodes for *R. flavipes* was *H. indica* > *H. bacteriophora* > *S. carpocapsae* > *S. riobrave.* The virulence of the nematodes for *C. formosanus* was *H. indica* and *H. bacteriophora* > *S. carpocapsae* and *S. riobrave* at $\alpha = 0.10$ level. The LD_{50} of *H. indica* against *R. flavipes* in petri dishes and in containers with vermiculite/sand medium were 296 (95% FL: 231–353) and 264 (95% FL: 176–344) nematodes per termite, respectively. The LD_{50} of *H. bacteriophora* against *R. flavipes* in petri dishes was 494 (95% FL: 357–625) nematodes per termite. *Heterorhabditis indica* repelled termites at high concentrations in sand and vermiculite medium. The length of repellency varied with the nematode concentration. Nematodes were able to reproduce from *R. flavipes* and *C. formosanus.* The possibility of using nematodes to control termites is discussed.

KEY WORDS *Reticulitermes flavipes*, *Coptotermes formosanus*, entomopathogenic nematodes, biological control

SUBTERRANEAN TERMITES ARE serious pests of wooden structures in the United States, and cause tremendous amounts of damage to buildings and loss to homeowners (Beal et al. 1994, Su and Scheffrahn 1998). *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki are especially important because of their wide distribution and large colonies (Snyder 1954, Su and Scheffrahn 1988). Currently, chemical control is still playing a vital role in controlling these pests (Su and Scheffrahn 1998), but use of chemicals around homes and gardens poses direct danger to humans and the environment. Due to increasing concerns about these side effects, there has been great interest in finding other methods, especially biological agents, of controlling termites and reducing the use of chemicals (Grace 1997).

Entomopathogenic nematodes, steinernematid and heterorhabditid, as a group of biological control agents, received great attention in the 1990s (Gaugler and Kaya 1990, Bedding et al. 1993, Kaya and Gaugler 1993). Hundreds of different species from most orders of insects were susceptible to various entomopathogenic nematodes in laboratory tests. Nematodes have the advantages of being easy to apply, compatible with many pesticides, and finding their hosts either actively or passively (Smart 1995). Termites live and forage in habitats that are moist, cool, and without direct sunlight such as soil or wood materials. These environmental conditions are ideal for the survival and movement of steinernematid and heterorhabditid nematodes, and, therefore, provide the basis for the interest in their role in control of subterranean termites. Five species of nematodes have been shown to cause mortality of termites: *Steinernema carpocapsae* (Weiser) (Reese 1971, Epsky and Capinera 1988, Mauldin and Beal 1989, Guangdong Entomological Research Institute 1982), *Heterorhabditis bacteriophora* (=*heliothidis*) (Khan, Brooks & Hirschmann) (Mauldin and Beal 1989), *Steinernema feltiae* (=*bibionis*) (Bovien) (Guangdong Entomological Research Institute 1982, Mauldin and Beal 1989), *Steinernema glaseri* (Steiner) (Guangdong Entomological Research Institute 1982), and *Heterorhabditis* sp. (Danthanarayana and Vitarana 1987). Nematodes showed effectiveness against subterranean termites in the lab-

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oratory, but did not cause colony elimination in the field with *R. flavipes* (Mauldin and Beal 1989), *R. tibialis* (Banks) (Epsky and Capinera 1988), or *C. formosanus* (Reese 1971, Fujii 1975, Tamashiro 1976). The reasons suggested were termite social behavior like walling off dead termites and avoiding foraging in nematode infested areas (Reese 1971, Fujii 1975, National Pest Control Association 1985). The past failures posed great doubt of the feasibility of using nematodes to control termites (Mix 1986).

The rapid development in the study and commercial application of entomopathogenic nematodes in pest control during the last 20 yr instigated further interest in finding useful nematodes and better application methods to control subterranean termites. Currently, our knowledge about the infectivity of entomopathogenic nematodes to termites has been limited. There is little or no information on comparative infectivity of different nematodes species, median lethal doses, propagation of nematodes in termites, repellence of nematodes to termites, and so on. In this study, we evaluated four species of entomopathogenic nematodes against *R. flavipes* and *C. formosanus* in the laboratory. Our specific objectives were to compare the infectivity of four nematodes against *R. flavipes* and *C. formosanus*, to determine the LD_{50} and LD_{90} of the best performing nematodes, to determine the repellence of nematodes to termites, and to determine whether nematodes can successfully reproduce in termites.

Materials and Methods

Collection and Preparation of Termites. *Reticulitermes flavipes* was collected from cardboard bait buried near trees in the field in Stoneville, MS. *Coptotermes formosanus* was collected in cardboard bait buried in the field in New Orleans, LA. The termite colonies used in the experiment were kept in large plastic containers in the laboratory at room temperature (25–28°C). One day before testing, termite workers (at least third instar) were taken from rearing containers, counted, and transferred to 9.0 by 1.5-cm plastic petri dishes lined with a wet filter paper. Dead and weak termites were replaced with healthy termites immediately before the test. For tests in containers with sand and vermiculite medium, termites were counted 2 d before the test, then recounted 1 d before the test and transferred to test containers. For the two-container choice test, termite numbers (including soldiers, larvae, and nymphs) were estimated by weight and put in test devices 7 d before the test.

Nematode Preparation. Four nematode species from the live nematode culture of the Department of Entomology and Nematology, University of Florida, USA, were used in this study. They were *S. carpocapsae* Breton strain, *S. riobrave* Weslaco strain, *H. bacteriophora* HP88 strain, and *H. indica* Coimbatore strain. Before tests were started, nematodes were propagated in greater wax moth, *Galleria mellonella* (L.) larvae (Kaya and Stock 1997). Nematode infective juveniles emerging from the *G. mellonella* larvae within 7 d from the first day of emergence were collected and were kept in a tissue culture flask at 13.7°C. They were used within 5 d of collection.

Experiment Design. Experiments 1, 3, and 5 had a randomized complete block design (3–5 blocks). The experiments were blocked by different colonies. In experiments 2 and 4, a completely random design on the same colony was used for all replicates. Each experiment had a factorial treatment structure with one to four nematode species and different rate combination. The experiment unit was a plastic container or a two-container choice device with a known number of termite workers. All experiment units were kept under dark at 25°C in an incubator.

Experiment 1. Comparative Infectivity of Four Nematodes Against R. flavipes. The test was conducted in 9.0 by 1.5-cm plastic petri dishes with one 7.5-cm-diameter Reeve Angel filter paper (Whatman, Clifton, NJ) per dish. The area of the bottom half of the dish was 58.1 cm^2. Forty termites were added to each petri dish. Nematode infective juveniles were applied to filter paper at 0, 400, 1,200, and 2,000 per termite. More water was added to each dish so that the final water content was ≈250% (wt:wt) of the filter paper. Each rate was applied to five replicates, with each replicate representing a different colony of termites. Termites survival was checked at 2, 4, 8, and 15 d. One colony was eliminated in data analysis because of high mortality in the control.

Experiment 2. Comparative Infectivity of Four Nematodes Against C. formosanus. Methods were similar to experiment 1 except that three filter paper discs and 30 termites were placed in each dish. The numbers of infective juveniles applied were 0, 400, 2,000, and 5,000 per termite. Each rate was applied to five replicates. The termites were all from the same colony.

Experiment 3. Dose–Response Relationship for H. indica *and* H. bacteriophora *Against* R. flavipes. Methods were similar to experiment 1 with one filter paper and 40 termites per petri dish. Nematode infective juveniles were added to the filter paper at eight rates: 0, 200, 400, 600, 900, 1,200, 1,500, and 1,800 nematodes per termite. Each rate was replicated three times with each replicate representing a different termite colony. Termite survival was checked at 4, 8, 12, and 15 d.

Experiment 4. Dose–Response Relationship for H. indica *Against* R. flavipes. Nematodes may act differently with or without the presence of soil. To examine the effect of nematodes against termites with the presence of medium, 40 termites were put in each container (5.1 cm diameter by 3.5 cm high) with rearing medium in it. The medium was a mixture of vermiculite, sand, and water (16:14:9 by volume). A southern pine (*Pinus* sp.) block (1.9 by 1.9 by 1.9 cm) was buried at the center of the container. The final depth of the rearing medium was 2.5 cm. The volume of the medium excluding the pine block in each container was 44.2 cm^3. Nematodes were applied at 10 rates: 0, 100, 200, 300, 400, 500, 600, 700, 800, and 900 nematodes per termite. Each rate was replicated three times with termites all from the same colony. The number of live termites were checked at 22 d. The mean number of

live termites from each rate was used for calculating LD_{50} and LD_{90} of *H. indica* against *R. flavipes.*

Experiment 5. Infectivity of H. indica *Against* R. flavipes *in Two-Container Choice Device.* This experiment was designed to test the effect of nematodes against termites in a near natural condition. Termites were able to move between nematode treated and nonetreated containers. The two-container choice test device described by Mauldin and Beal (1989) was used to examine the infectivity of *H. indica* against *R. flavipes.* The device was composed of two round plastic containers connected by a plastic tube. The large container was 15.5 cm diameter (15.1 cm i.d.) by 4 cm high. The small container was 5.1 cm diameter by 3.5 cm high. The plastic tube (15 cm long, 1.3 cm outside diameter, 0.9 cm i.d.) was inserted into a 1.3 cm hole near the base of the large container and connected to a similar hole near the base of the small container. The containers were half-filled (2 cm deep) with the rearing media described above. One thousand *R. flavipes* workers were added to the large container 7 d before inoculation with nematodes. Three replicates, each representing a different colony, were applied. *Heterorhabditis indica* was added to the large container or small container at the rate of 400,000 nematodes/device. At 30 d, live termites were counted and the presence of nematodes in the rearing media was examined by repeated baiting with *Galleria mellonella* L. larvae (Koppenhöfer et al. 1998). Medium from treatment devices (both the large and small containers) was mixed thoroughly. Half of the medium was transferred to a 23 by 32 by 5-cm container. Sixty *G. mellonella* larvae were added to the medium at two different times. Dead larvae killed by nematodes were counted and removed.

Repellence of *H. indica* to *R. flavipes*. One day before the test, 200 *R. flavipes* workers were put in a two-container choice device similar to experiment 5, except the two containers were the same size (5.1 cm diameter by 3.5 cm high). Termites were evenly distributed between the two containers on the test day. *Heterorhabditis indica* was added to one of the two containers of each device at rate of 1,000, 2,000, 4,000, 8,000, and 16,000 nematodes/device. Each rate was replicated three times with termites all from the same colony. The location of the termites was checked every day for 14 d.

Reproduction of *H. indica* in *R. flavipes* and *C. formosanus*. Three dishes of *R. flavipes* and *C. formosanus* (100–120 workers per dish) were prepared as in experiment 1. All *R. flavipes* and *C. formosanus* were from one colony. The mean worker weight of *R. flavipes* and *C. formosanus* at the day of the treatment were 31.1 ± 0.6 and 40.4 ± 0.1 mg (mean ± SE), respectively. Each dish was inoculated with 48,000 *H. indica* infective juveniles. *Reticulitermes flavipes* and *C. formosanus* killed by *H. indica* were transferred to wet filter paper disks (2 cm diameter) and maintained individually in 4.0 by 1.2-cm plastic dishes to ascertain nematode reproduction. The dishes were kept in a moist container at 25°C for up to 21 d. Nematodes that emerged from the dead termites were rinsed into a petri dish and counted using a dissecting microscope.

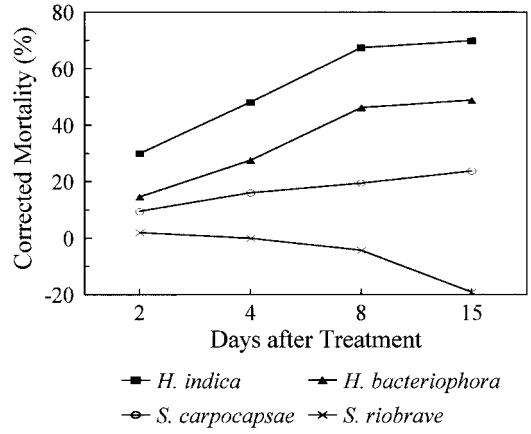

Fig. 1. Temporal changes in mortality of *Reticulitermes flavipes* exposed to entomopathogenic nematodes at 1,200 nematodes per termite in petri dishes.

Statistical Analysis. Mortality data were transformed (arcsine of the square root) and analyzed using analysis of variance (ANOVA) for comparison among species and between different rates and the control. PROC MIXED in the SAS software (SAS Institute 1999) is used where "species, rate, and species × rate" are the fixed effects and "block (or colony)" is the random effect. For experiment 2, there was no random effect because all termites were from the same colony. Mean mortalities of different nematode species and different rates were compared by least significant difference (LSD) after ANOVA (SAS Institute 1999). One-tailed tests were used for comparisons between rates of the same nematode species. Two-tailed tests were used for comparisons between nematode species at the same rate. Comparisons between different nematode species also were performed by fitting a linear regression between mortality (arcsine of the square root transformed) and rate (log10 transformed) for each species, then comparing their slopes using *F*-tests for homogeneity of slopes. Corrected mortality was calculated based on Abbott (1925). Median lethal dose causing 50 and 90% mortality (LD_{50} and LD_{90}) and their 95% fiducial limits (FL) were determined by probit regression analysis (PROC PROBIT) (SAS Institute 1999).

Results

Comparative Infectivity of Nematodes Against *R. flavipes* and *C. formosanus* in Petri Dish Tests. In experiment 1, *R. flavipes* exposed to the four entomopathogenic nematodes exhibited increased mortality until 8 d after exposure (Fig. 1). Termite mortality at 8 d varied significantly among nematode species ($F = 32.9$; df = 3, 45; $P < 0.01$). *Steinernema riobrave* had no detectable effect against *R. flavipes* ($P > 0.10$, Table 1). *Steinernema carpocapsae* was effective against *R. flavipes* at ≥1,200 nematodes per termite

Table 1. Percent mortality of *R. flavipes* induced by nematodes 8 d after exposure in petri dish test (means ± SE)

Nematode species	Rate (nematodes per termite)			
	Control	400	1,200	2,000
S. riobrave	14.4 ± 5.5a	10.6 ± 2.1aA	10.6 ± 2.1aA	19.4 ± 3.7aA
S. carpocapsae	13.1 ± 5.0a	17.5 ± 4.7aA	30.0 ± 12.0bB	45.0 ± 12.5bB
H. bacteriophora	10.6 ± 5.4a	42.5 ± 5.1bB	51.9 ± 3.3bC	65.6 ± 9.5cC
H. indica	9.9 ± 5.0a	58.1 ± 12.1bB	70.6 ± 10.2bD	91.9 ± 4.0cD

Means within a row followed by different lowercase letters are significantly different ($P \leq 0.10$); means within a column followed by different uppercase letters are significantly different ($P \leq 0.05$; LSD; arcsine of the square root transformed). Mean of four replicates with 40 termites per replicate.

level. *Heterorhabditis bacteriophora* and *H. indica* were effective against *R. flavipes* at all rate levels ($P \leq 0.10$, Table 1). *Heterorhabditis indica* caused the highest mortality to *R. flavipes* among the four nematodes. *Heterorhabditis indica* was significantly more effective against *R. flavipes* than *H. bacteriophora* at rates of 1,200 and 2,000 nematodes per termite ($P \leq 0.05$, Table 1). The virulence of the nematode species was *H. indica* > *H. bacteriophora* > *S. carpocapsae* > *S. riobrave* at $\alpha = 0.10$ level based on slope of the regression between termite mortality and nematode concentration (Table 2). *Heterorhabditis indica* and *H. bacteriophora* were significantly more virulent against *R. flavipes* than *S. carpocapsae* and *S. riobrave* at $\alpha = 0.05$ level (Table 2).

Similar to experiment 1, mortality of *C. formosanus* caused by nematodes ceased to increase 8 d after inoculation with nematodes. All four of the nematodes caused significant mortality to *C. formosanus* after 8 d exposure to nematodes at 400 nematodes per termite ($P \leq 0.10$, Table 3). *Heterorhabditis indica* caused the highest mortality to *C. formosanus. Heterorhabditis indica* was significantly more effective than *H. bacteriophora* at rates of 400 and 2,000 nematodes per termite ($P \leq 0.05$, Table 3), but was not significantly different from *H. bacteriophora* based on the slopes of the linear regressions ($P = 0.83$, Table 4). *Heterorhabditis indica* and *H. bacteriophora* were significantly more effective than *S. carpocapsae* and *S. riobrave* against *C. formosanus* ($P \leq 0.05$, Table 4).

Dose–Response Relationship Among *H. indica*, *H. bacteriophora*, and *R. flavipes*. In petri dish tests (experiment 3), *R. flavipes* exposed to *H. indica* and *H. bacteriophora* suffered increased mortality as nematode number increased. The response patterns varied among colonies. In petri dish tests, the dose–response of two colonies fitted the probit regression model at $\alpha = 0.05$ level for both *H. indica* and *H. bacteriophora* (Table 5). But one of these two colonies only marginally fitted the model ($P = 0.09$). For *H. indica*, the LD_{50} of the colony (colony 1) that had good fit to the probit regression model was 296 (95% FL: 231–353) nematodes per termite or five (95% FL: 4–6) nematodes/cm^2 expressed by concentration. For *H. bacteriophora*, the LD_{50} of the colony (colony 3) that had good fit to the probit regression model was 494 (95% FL: 357–625) nematodes per termite or nine (95% FL: 6–11) nematodes/cm^2 expressed by concentration. The LD_{50} and LD_{90} values of these two colonies show that *H. indica* was more virulent than *H. bacteriophora.* Comparing the LD_{50} and LD_{90} values obtained from petri dish tests based on the same termite colony (colony 2) with a marginal good fit between the two nematode species also shows that *H. indica* was more virulent than *H. bacteriophora* against *R. flavipes* (Table 5).

Reticulitermes flavipes exposed to *H. indica* in small containers with vermiculite and sand (experiment 4) also showed increased mortality as the application rate increased. The LD_{50} at 22 d was 264 (95% FL: 176–334) nematodes per termite, or six (95% FL: 4–8) nematodes/cm^3 expressed by concentration. The LD_{50} was very similar to that of petri dish assays, but the LD_{90} was much larger than that of the test in petri dishes (Table 5).

Infectivity of *H. indica* to *R. flavipes* in Two-Container Choice Test. Before nematodes were added to the containers, termites foraged freely between the two containers. After addition of nematodes to one of the two containers, most of the termites from the treated container retreated and moved to the untreated container or to the tube connecting the two containers. *Heterorhabditis indica* was observed migrating to the connecting tube and the untreated container (≥13 cm distance) 24 h after inoculation. Dead termites killed by nematodes (recognized by brick red color) were observed at the bottom and the surface of the rearing medium 3 d after treatment. At 30 d, *R.*

Table 2. Comparisons of the slopes of linear regressions between *R. flavipes* mortality (arcsine of the square root) and treatment rate (log10) of the four nematode species

Nematode species	Comparison between regression slopes of different species (df = 1, 53)		
	S. riobrave	*S. carpocapsae*	*H. bacteriophora*
H. indica	$F = 35.6$, $P < 0.01$	$F = 18.1$, $P < 0.01$	$F = 5.5$, $P = 0.06$
H. bacteriophora	$F = 16.5$, $P < 0.01$	$F = 5.5$, $P = 0.02$	
S. carpocapsae	$F = 2.9$, $P = 0.09$		

Table 3. Percent mortality of *C. formosanus* induced by nematodes 8 d after exposure in petri dish test (means ± SE)

Nematode species	Rate (nematodes per termite)			
	Control	400	2,000	5,000
S. riobrave	2.7 ± 1.9a	42.0 ± 9.8bA	50.3 ± 3.3bA	62.7 ± 12.1bA
S. carpocapsae	1.9 ± 1.3a	43.7 ± 6.2bA	50.7 ± 7.8bA	58.0 ± 2.5bA
H. bacteriophora	6.6 ± 2.7a	53.3 ± 9.0bA	86.7 ± 6.9cB	97.3 ± 1.9cB
H. indica	16.0 ± 7.1a	73.3 ± 10.1bB	98.7 ± 1.3cC	96.0 ± 4.0cB

Means within a row followed by different lowercase letters are significantly different ($P \leq 0.10$); means within a column followed by different uppercase letters are significantly different ($P \leq 0.05$; LSD; arcsine of the square root transformed); Mean of five replicates with 30 termites per replicate.

flavipes suffered significantly higher mortality than controls after nematodes were applied to the large container ($F = 42.4$; df = 1, 2; $P = 0.03$). Nematodes killed 37% of the termites (corrected by Abbott 1925). *Reticulitermes flavipes* exhibited only slightly higher mortality than control after nematodes were applied to the small container than that of the control. This difference was insignificant ($F = 0.7$; df = 1, 2; $P = 0.50$). Baiting with *Galleria* larvae in vermiculite/sand at the end of the test found 27 (out of 60) *Galleria* larvae being killed by *H. indica.* Therefore, the rearing medium still had live *H. indica* infective juveniles at the end of the test. However, no nematode-induced termite mortality was observed from 22 d after treatment.

Repellence of Nematodes to *R. flavipes.* In two-container choice devices, nematodes repelled *R. flavipes* (defined as no termites entering the treated container) at ≥4,000 infective juveniles per container (or 90 nematodes/cm^3). The repellence varied not only between nematode concentration, but also between replicates of the same nematode concentration. Repellence of nematodes at the highest concentration (16,000 per container, or 362 nematodes/cm^3) to termites lasted for 3–11 d. In experiment 5, repellence of nematodes lasted for up to 17 d after nematodes were applied to the small container.

Reproduction of *H. indica* in *R. flavipes* and *C. formosanus.* Dead *R. flavipes* and *C. formosanus* showed brick red color. *Heterorhabditis indica* was seen through the cuticle of dead *R. flavipes* and *C. formosanus* 4–5 d after inoculation. Nematodes began to emerge at 5 d after infestation. Most of the nematode-killed termites were consumed by healthy termites or by a saprophagous mite, *Australhypopus* sp. (Acari: Acaridae). Average numbers of infective juveniles produced from *R. flavipes* and *C. formosanus* (based on 11 and eight workers, respectively) were 289 ± 50 and 642 ± 93 per worker, respectively. Thus, nematodes have the potential to continue their infestation to termites after an initial treatment.

Discussion

The nematode infectivity test in petri dishes or containers with medium showed that different nematodes species had very different levels of infectivity against termites. *Heterorhabditis* spp. were more effective than *Steinernema* spp. *Heterorhabditis indica* was the best of the four species tested in terms of virulence. Its LD_{50} value is very low compared with that of *S. carpocapsae* against *Reticulitermes tibialis* (Banks) (1.5×10^4 nematodes per termite, Epsky and Capinera 1988), *S. carpocapsae* against *C. formosanus* (average of 3,069 nematodes per termite; termites were anesthetized before treatment, Fujii 1975), and *Heterorhabditis* sp. against *Glyptotermes dilatatus* (Bugnion & Popoff) (3,670 nematodes per termite, Danthanarayana and Vitarana 1987). In a field study on *Heterorhabditis* sp., effective control was achieved against a dry-wood termite, *G. dilatatus*, to protect tea bushes in Sri Lanka (Danthanarayana and Vitarana 1987). However, *H. indica* did not eliminate termites in two-container choice tests. This was expected, because the termites avoided nematode-treated containers. In a similar test, Mauldin and Beal (1989) did not find significant differences in *R. flavipes* survival rates after *S. carpocapsae* was applied at 80,000 nematodes in the smaller container of the two-container choice test device. Repellence of nematodes to termites might be the main reason for the ineffectiveness of nematodes to termites in choice test device.

Although nematodes can reproduce in termites, they were rarely seen emerging from dead termites because mites usually consumed the nematode-killed termites. The mite *Australhypopus* sp. (Acari: Acaridae) is very common on the *R. flavipes* body, especially on the heads (unpublished data). Once a termite

Table 4. Comparisons of the slopes of linear regressions between *C. formosanus* mortality (arcsine of the square root) and treatment rate (log10) of the four nematode species

Nematode species	Comparison between regression slopes of different species (df = 1, 72)		
	S. riobrave	*S. carpocapsae*	*H. bacteriophora*
H. indica	$F = 4.7$, $P = 0.03$	$F = 5.2$, $P = 0.03$	$F = 0.1$, $P = 0.83$
H. bacteriophora	$F = 5.7$, $P = 0.02$	$F = 6.2$, $P = 0.01$	
S. carpocapsae	$F = 0.01$, $P = 0.92$		

Table 5. Dose-mortality relationship between nematodes and *R. flavipes*

Nematode species	Contents in containers	Colony no.	Lethal dose (mean and 95% fiducial limit) (nematodes per termite)		Model fitting information
			LD_{50}	LD_{90}	
H. bacteriophora	Filter paper	1	865 (155–1,164)	1,640 (1,217–11,545)	$\chi^2 = 15.3$, df = 5, $P = 0.01$
		2	1,074 (492–6,827)	13,938 (3,590–2.4×10^{10})	$\chi^2 = 9.6$, df = 5, $P = 0.09$
		3	494 (357–625)	2,625 (1,842–4,854)	$\chi^2 = 5.8$, df = 5, $P = 0.32$
H. indica	Filter paper	1	296 (231–353)	660 (557–829)	$\chi^2 = 5.9$, df = 5, $P = 0.32$
		2	332 (107–439)	583 (441–1,135)	$\chi^2 = 9.6$, df = 5, $P = 0.09$
		4	363 (174–526)	760 (525–1,823)	$\chi^2 = 22.7$, df = 5, $P < 0.01$
H. indica	Sand/vermiculite	1[a]	264 (176–344)	1,364 (975–2,447)	$\chi^2 = 5.8$, df = 7, $P = 0.55$

[a] This test was done 38 d later than the previous two tests using the same colony kept in the laboratory.

dies, mites quickly reproduce in large numbers and feed on the dead termites. Another cause for the failure of nematode recycling in termites was that healthy termites ate dead termites. Fujii (1975) suggested that the walling-off behavior of termites prevented the nematodes from reinfesting. Although the walling-off behavior may impede the movement of nematodes because of the coverings, it might not prevent the dispersal of nematodes from inside the occlusion. At least, nematodes that were produced from partially or loosely buried termites were observed outside the occlusion during our experiment.

The high numbers of termites in a colony and the wide foraging range are obstacles for nematodes to eliminate termite colonies. The limited mobility of nematodes and low rate of reproduction in dead termites make it unlikely that nematodes will reach and maintain a large enough density to eliminate a termite colony in the field. The repellence of nematodes to termites, as seen in two-container choice tests, will render field control with nematodes ineffective unless measures are taken to eliminate this impediment. Therefore, it may not be feasible to use these entomopathogenic nematodes in classical biological control for subterranean termites. Inundative release of these nematodes will only be useful for short-term protection and local control until means are developed to enhance survival and pathogenicity in systems such as bait matrices.

Should we eliminate nematodes as candidates for termite biological control? It may be too early to say so. Pathogens such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin have been continuously studied even though they have not been successful in the field for termite control, except for one preliminary test by Hänel (1983). There is a lot to learn about nematode biology, ecology, and relationships with their hosts. Termites stressed by sublethal doses of chemical or pathogens probably are more susceptible to entomopathogenic nematodes. A combination of nematodes with other biocontrol agents or chemicals may improve their control over termites. Additive or synergistic interactions between entomopathogenic nematodes and *Bacillus thuringiensis* Berliner were observed for scarab species (Koppenhöfer et al. 1999). It is known that imidacloprid improves the effect of nematodes against scarab species (Koppenhöfer et al. 2000). Imidacloprid also showed ability to increase termite susceptibility to *Metarhizium anisopliae* and *Beauveria bassiana* (Boucias et al. 1996, Ramakrishnan et al. 1999). More study on nematode biology, screening for more infective nematode species, strains, or application techniques will provide new valuable information on possible use of nematodes for termite control.

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