

Differential susceptibility of subterranean termite castes to entomopathogenic nematodes

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(Received 28 May 2004; returned 9 July 2004; accepted 15 September 2004)

Abstract

We examined the attachment and infectivity of two entomopathogenic nematode species, *Steinernema carpocapsae* and *Heterorhabditis indica*, on soldiers and workers in two subterranean termite species, *Coptotermes formosanus* and *C. vastator*. In attachment tests with *S. carpocapsae*, more nematodes attached to soldiers of *C. formosanus* and *C. vastator* in the absence of workers compared to soldiers that were in the presence of workers. In tests with soldiers alone, workers alone, and mixture of equal numbers of workers and soldiers, soldiers in the mixed groups had lower mortality than soldiers alone after 1 and 4 days for both termite species. Exposure of small groups of either termite species to *S. carpocapsae* resulted in higher mortality of soldiers after 1 and 4 days post exposure. Mortality in soldiers alone exposed to *H. indica* was not significant compared to mortality in mixed groups at 1 day exposure, but was significant after 4 days. In concentration–mortality tests, a significant two-way interaction existed between nematode concentration and termite caste for *C. formosanus* exposed to *S. carpocapsae* for 1 and 4 days. A significant effect of nematode concentration was found for *C. vastator* exposed to *H. indica* for 1 day. This termite species had lower mortality when exposed to *H. indica* after 1 and 4 days compared to *C. formosanus*. At 4 days post exposure to *H. indica*, the effects of nematode concentration and termite caste were significant. *Steinernema carpocapsae* caused higher mortality than *H. indica*, but mortality of workers was higher when exposed to *H. indica*. Soldiers of both species experienced rapid mortality when exposed to *S. carpocapsae*, whereas soldiers of *C. vastator* experienced lower mortality when exposed to *H. indica*. Thus, our results show that when soldiers alone or workers alone are exposed to the nematodes, there is a differential susceptibility of soldiers and workers to nematode infection with soldiers being more susceptible than workers. In a mixed group of soldiers and workers or workers alone, it appears that grooming behavior of the workers serves as a mechanism to reduce nematode infection of soldiers and workers. The reason for this differential response to nematode infection is that soldiers do not exhibit grooming behavior.

Keywords: Behavioral ecology, biological control, *Coptotermes formosanus*, *Coptotermes vastator*, *Formosan subterranean termite*, *Philippine milk termite*, entomopathogenic nematodes, *Heterorhabditis indica*, *Steinernema carpocapsae*

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ISSN 0958-3157 print/ISSN 1360-0478 online © 2005 Taylor & Francis Group Ltd
DOI: 10.1080/09583150400016951

Introduction

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is a severe pest in wooden structures throughout many parts of the world. Its cryptobiotic lifestyle and aggressiveness make it difficult to manage and control (Culliney & Grace 2000). This and other subterranean termite species have been estimated to cause 2 billion US dollars in damage and repairs annually (Culliney & Grace 2000). The predominant control strategies for subterranean termites consist of chemical treatment either pre-construction or post-infestation, and baiting systems using a matrix containing a slow acting insecticide or insect growth regulator that is consumed by foraging termites and disseminated throughout the colony via trophallaxis. However, current social perceptions of chemical insecticides have lowered public opinion of this form of treatments and have increased interest in biological control approaches for these types of destructive termites.

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae have been the focus of biological control of soil insect pests (Kaya & Gaugler 1993). These nematodes have attributes of both parasitoids and insect pathogens (Kaya & Gaugler 1993) and are exempt from registration by the Environmental Protection Agency in the USA (Gorsuch 1982). They are associated with mutualistic bacteria in the genus *Xenorhabdus* for *Steinernema* and *Photorhabdus* for *Heterorhabditis* (Burnell & Stock 2000). It is this nematode–bacterium complex that works together to kill their insect host. The bacterium is housed in the intestine of the non-feeding, free-living, third-stage infective juvenile nematode. This infective juvenile searches for hosts in the soil environment (Kaya & Gaugler 1993; Campbell & Lewis 2002). Upon finding a host, the infective juvenile enters through natural openings (mouth, anus or spiracles) and then penetrates into the hemocoel where it releases the mutualistic bacterium. With *Heterorhabditis* species, the infective juvenile can also penetrate through soft cuticle. The bacterium kills the host within 2 days and the nematode develops by feeding on the bacterial cells and degraded host tissues. There are one to three nematode generations, and when food resources become limiting, infective juveniles are produced and exit the cadaver to start the life cycle over again.

Subterranean termites occupy the same soil habitat as the nematode species, but they are not particularly susceptible to nematode infection (Fujii 1975). Although nematodes can kill termites in the laboratory, they have had little effect on subterranean termites in field tests (Epsky & Capinera 1988; Mauldin & Beal 1989). Wang et al. (2002) found that under laboratory conditions continued exposure of termites to *Heterorhabditis indica* Poinar, Karunakar, and David at 400 infective juveniles per termite resulted in 58 and 73% mortality of *Reticulitermes flavipes* (Kollar) and *C. formosanus*, respectively. These authors also found that *Steinernema carpocapsae* (Weiser) at the same rate incurred mortalities of 42 and 44% in *R. flavipes* and *C. formosanus*, respectively. In spite of this low mortality at high nematode concentrations, Wang et al. (2002) stated that there might be a nematode species that could be used for termite control, particularly if termites are first stressed with low concentrations of imidacloprid as suggested by Boucias et al. (1996).

The reason for the poor efficacy of nematodes against termites has been attributed to termite social behavior. Fujii (1975) demonstrated that large numbers of *S. carpocapsae* are required for infection of *C. formosanus* workers and suggested

that colony behavior of removing or walling off nematode-killed termites was a contributing mechanism to low nematode infection. With social hymenopterans (Formicidae), Oi and Pereira (1993) suggested that rapid movement of ants and behaviors like nest mate grooming contributed to low mortality of workers exposed to nematodes. Drees et al. (1992) observed fire ant workers fastidiously removing infective juveniles of *S. carpocapsae* from brood, alates and other workers resulting in negligible worker mortality.

The few studies of entomopathogenic nematodes with subterranean termites have primarily examined the concentration–mortality of various nematode species/strains and concentrations on groups of termite workers (Fujii 1975; Wang et al. 2002). These studies have shown that uneconomic numbers of infective juveniles per termite are needed to obtain some degree of termite worker mortality. However, we are not aware of any studies that have examined nematode attachment to different termite castes. Since termite soldiers cannot groom themselves of debris or pathogens (Rath 2000), including nematodes, they are most likely at higher risk to nematode infection than worker termites. We hypothesize that termites are resistant to nematode infection because nematodes have difficulty in attaching to the termite cuticle due to grooming behavior. Continuous exposure may overcome this phenomenon such that eventually some termites are infected. We examined nematode attachment to workers and soldiers, mortality of soldiers in the presence and absence of workers, and concentration–mortality of soldiers of two subterranean termite species, *C. formosanus* and *C. vastator* Light, to elucidate the behaviors that limit termite susceptibility to nematodes.

Materials and methods

Termites

We tested our hypotheses on two subterranean termite species, *C. formosanus* and *C. vastator*, found in Hawaii. Although much is known about *C. formosanus*, little is known about the biology and habits of the latter species. *Coptotermes vastator* is a relatively new introduction to Hawaii from the Philippines (Woodrow et al. 2001) and occurs in similar habitats as *C. formosanus* (Uchima & Grace 2003).

Coptotermes formosanus was collected from wooden bait traps (Tamashiro et al. 1973) on the Manoa campus of the University of Hawaii. Termites were separated from the trap material 1–2 days before testing and kept in metal trays (30 × 26 × 6 cm) with moistened filter paper at 27 ± 1°C. *Coptotermes vastator* was collected from Barbers Point, Oahu, Hawaii and processed as above. Termites were gathered and counted with an aspirator and transferred to test containers.

Nematodes

Steinernema carpocapsae All strain and *H. indica* MG-14 strain, originally obtained from stock cultures at the University of California, Davis, were propagated in greater wax moth larvae, *Galleria mellonella* (L.). Infective juveniles were harvested from White traps (Kaya & Stock 1997). The harvested infective juveniles were collected and kept in tissue culture flasks at 15°C and used within 30 days of emergence.

Attachment tests

We hypothesized that grooming behavior of workers affects nematode attachment. Therefore, soldiers alone, workers alone, and a mix of soldiers and workers together were conducted as follows using *S. carpocapsae* and *H. indica*. Treatment 1 consisted of groups of 20 workers alone. Treatment 2 consisted of groups of 20 soldiers alone. Treatment 3 was composed of a combination of 10 workers and 10 soldiers together. Each treatment was performed in one petri dish (100 × 15 mm) with a piece of Whatman No. 2 filter paper moistened with either 1 ml of water for the control replicates or 1 ml suspension of 6000 infective juveniles (=300 nematodes/termite). The dishes were maintained at room temperature ($23 \pm 2^\circ\text{C}$) in a dark box for 3 h. After this time period, 10 individual workers or soldiers from the different groups were removed, placed individually in a 60-mm petri dish, washed with ca. 10 ml of distilled water from a squirt bottle, and removed. The water in the dish was examined with a dissecting microscope at $\times 60$ to count the number of nematodes that had been washed off each termite. Accordingly, nematode counts were made from 10 worker termites from treatment 1, 10 soldiers from treatment 2, and 10 soldiers and 10 workers from treatment 3. This procedure was conducted three times with *C. formosanus* and *C. vastator*.

Mortality tests: Differential response of workers and soldiers

The objective of this study was to determine if workers and soldiers of *C. formosanus* or *C. vastator* responded differently to infection by *S. carpocapsae* or *H. indica*. Groups of six workers alone, six soldiers alone, or mixed groups of three soldiers and three workers were placed into 60 × 15-mm petri dishes lined with filter paper. Then, 0.5 ml of water (control) or 1800 infective juveniles (300/termite) in a 0.5-ml suspension of water was added to each dish. All dishes were kept in a large plastic tub lined with wet paper towels and maintained at room temperature ($23 \pm 2^\circ\text{C}$) in a dark box. The dishes were checked daily for mortality and the number of dead termites was recorded. The dead termites were dissected to confirm mortality by nematodes. Each treatment was replicated three times and the experiment was repeated three times.

Concentration mortality studies of workers and soldiers

The response of workers and soldiers of *C. formosanus* and *C. vastator* to different concentrations of *S. carpocapsae* or *H. indica* was determined. Twenty workers or 20 soldiers were placed separately into 100 × 15-mm petri dishes containing a piece of filter paper. The paper was moistened with 1 ml of distilled water (control) or with 1 ml suspension of nematodes at five different concentrations (20, 40, 80, 160, or 320 infective juveniles/termite). Each rate was replicated three times. The replicates were kept in a randomized design in plastic tubs (48 × 30 × 15 cm) lined with damp paper toweling. The experiment was repeated three times and mortality of termites was checked after 1 and 4 days exposure. Since high control mortality occurred after 4 days post treatment, we analysed the data at the 1- and 4-day checkpoints.

Statistical analysis

The mean number of nematodes removed by washing in the attachment test was analysed by ANOVA (SAS Institute 2002). Means were compared using the Ryan–Einot–Gabriel–Welsh multiple range test. For the differential response and the concentration–mortality tests, the mortality data were normalized using an arcsine transformation and analysed as above. In the concentration–mortality test, transformed mortality data were analysed as a randomized complete block design using PROC Mixed, modeling the interaction of nematode concentration and termite caste. We used least square means to identify differences associated with termite caste and nematode rate.

Results*Attachment tests*

In the attachment tests with *S. carpocapsae*, for both termite species, soldiers from the mixed group treatments had a lower (*C. formosanus*: $F = 13.95$, $df = 3, 76$, $P < 0.0001$; *C. vastator*: $F = 31.31$, $df = 3, 116$, $P < 0.0001$) number of nematodes washed off after 3 h of exposure than soldiers placed in groups without workers. *Coptotermes formosanus* soldiers placed in groups without workers had an average of 30.0 ± 8.0 infective juveniles washed off after 3 h, whereas soldiers of this species from mixed worker–soldier groups had an average of 1.0 ± 0.4 infective juveniles washed off after the same exposure period. Groups of *C. formosanus* workers alone and workers from mixed groups had 0.1 ± 0.1 and 0.2 ± 0.2 infective juveniles washed off, respectively. Similar results were obtained for *C. vastator* where soldiers alone had an average of 23.0 ± 4.0 infective juveniles washed off, and soldiers from the mixed groups had an average of 7.0 ± 1.0 infective juveniles washed off. Worker termites alone and from mixed groups had 0.5 ± 0.1 and 2.0 ± 0.3 nematodes washed off, respectively.

In the attachment tests with *H. indica*, no nematodes were washed off either termite species during the 3-h period. Therefore, individual termite tests were not conducted with this nematode species.

Mortality tests: Differential response of workers and soldiers

General observations: In the mortality tests, rapid soldier mortality was observed after 24 h of exposure to *S. carpocapsae*, whereas soldier mortality generally took longer with *H. indica*. Dissection of dead termites in the nematode treatments confirmed the presence of nematodes. Interestingly, most of the developing termites were found in the head capsules of the termites. None of the dead control termites died from nematode infection. Their cause of death is unknown. In nematode treatments with worker termites, dead termites were isolated by live termites by surrounding the dead individuals with chewed filter paper fibers forming a small ‘cocoon’ around them. However, this behavior was also observed with dead termites in the control.

Differential response of workers and soldiers: After 1 day exposure, *C. formosanus* soldier alone groups exposed to *S. carpocapsae* had $57 \pm 17\%$ mortality, whereas soldiers in mixed groups experienced lower ($4 \pm 4\%$; $F = 10.83$, $df = 7, 64$, $P < 0.0001$) mortality. Exposure of *C. formosanus* to *H. indica*, however, showed no differences between treatments after 1 day ($P > 0.54$). Low mortality occurred in the

soldiers alone group and soldiers from the mixed worker–soldier group, but the means were not significantly different. Similarly for *C. vastator* at 1 day exposure, soldier termites alone experienced higher mortality ($76 \pm 10\%$) than soldiers with workers ($26 \pm 12\%$, $F=11.3$, $df=7, 64$, $P<0.0001$) when exposed to *S. carpocapsae*. Exposure to *H. indica* also resulted in much lower mortality of both soldiers alone ($11 \pm 5\%$) and soldiers from mixed groups ($4 \pm 4\%$), but this difference was not significant. Worker termites from the mixed groups experienced higher mortality ($26 \pm 7\%$, $F=5.82$, $df=7, 64$, $P<0.0001$) than soldiers in the mixed groups ($4 \pm 4\%$) when exposed to *H. indica* (Table I).

At 4-days exposure, *C. formosanus* soldiers alone exposed to *S. carpocapsae* had significantly higher mortality (100%) than soldiers in the mixed worker–soldier groups ($44 \pm 16\%$, $F=20.11$, $df=7, 64$, $P<0.0001$) (Table I). Workers in mixed groups experienced a higher mortality ($41 \pm 15\%$) than workers alone ($9 \pm 7\%$). Exposure to *H. indica* after 4 days also showed similar mortality trends but these were not as great as with *S. carpocapsae*. Soldiers alone experienced significantly higher mortality ($61 \pm 15\%$, $F=8.96$, $df=7, 64$, $P<0.0001$) than soldiers from worker–soldier groups ($15 \pm 8\%$) when exposed to *H. indica*. Workers alone experienced much lower mortality ($6 \pm 3\%$) than soldiers alone, but workers from mixed groups had higher mortality ($22 \pm 8\%$) than soldiers from mixed groups ($15 \pm 8\%$).

At 4 days exposure, *C. vastator* soldiers without workers exposed to *S. carpocapsae* had higher mortality ($96 \pm 4\%$) than soldiers in the mixed worker–soldier groups ($67 \pm 11\%$, $F=24.33$, $df=7, 64$, $P<0.0001$) (Table I). Workers in mixed groups experienced higher mortality ($90 \pm 8\%$) than workers alone ($52 \pm 9\%$). Exposure to *H. indica* showed similar mortality trends but these were not as great as with *S. carpocapsae*. Soldiers alone experienced significantly higher mortality ($74 \pm 9\%$, $F=11.46$, $df=7, 64$, $P<0.0001$) than soldiers from worker–soldier groups ($22 \pm 6\%$) when exposed to *H. indica*. Workers alone experienced lower mortality ($52 \pm 3\%$) than

Table I. Percent mortality (\pm SE) of worker, soldier and mixed worker–soldier groups of *Coptotermes formosanus* and *C. vastator* 1 or 4 days after exposure to each of two nematode species (*Steinernema carpocapsae* or *Heterorhabditis indica*) at 300 nematodes/termite.

Exposure/ Nematode	Workers control	Soldiers control	Workers Nematodes	Soldiers Nematodes	Workers mixed control	Soldiers mixed control	Workers mixed nematodes	Soldiers mixed nematodes
Mortality - 1 day exposure								
<i>C. formosanus</i>								
<i>S. carpocapsae</i>	0 ^b	0 ^b	0 ^b	57 ± 17^a	0 ^b	0 ^b	0 ^b	4 ± 4^b
<i>H. indica</i>	0	0	0	6 ± 6	0	0	0	4 ± 4
<i>C. vastator</i>								
<i>S. carpocapsae</i>	4 ± 3^{bc}	4 ± 3^{bc}	22 ± 6^{bc}	76 ± 10^a	0 ^c	7 ± 5^{bc}	33 ± 11^b	26 ± 12^{bc}
<i>H. indica</i>	0 ^b	2 ± 2^b	15 ± 5^{ba}	11 ± 5^{ba}	0 ^b	0 ^b	26 ± 7^a	4 ± 4^b
Mortality - 4 day exposure								
<i>C. formosanus</i>								
<i>S. carpocapsae</i>	0 ^c	0 ^c	9 ± 7^c	100 ^a	0 ^c	0 ^c	41 ± 15^b	44 ± 16^b
<i>H. indica</i>	4 ± 3^b	0 ^b	6 ± 3^b	61 ± 15^a	0 ^b	4 ± 4^b	22 ± 4^b	15 ± 8^b
<i>C. vastator</i>								
<i>S. carpocapsae</i>	7 ± 4^d	57 ± 7^c	52 ± 9^c	96 ± 4^a	4 ± 4^d	15 ± 8^d	90 ± 8^{ab}	67 ± 11^{bc}
<i>H. indica</i>	4 ± 4^c	50 ± 4^{ba}	52 ± 8^{ba}	74 ± 9^a	22 ± 8^{bc}	7 ± 5^c	63 ± 12^a	22 ± 6^{bc}

*Means with same letter are not significantly different within rows between columns.

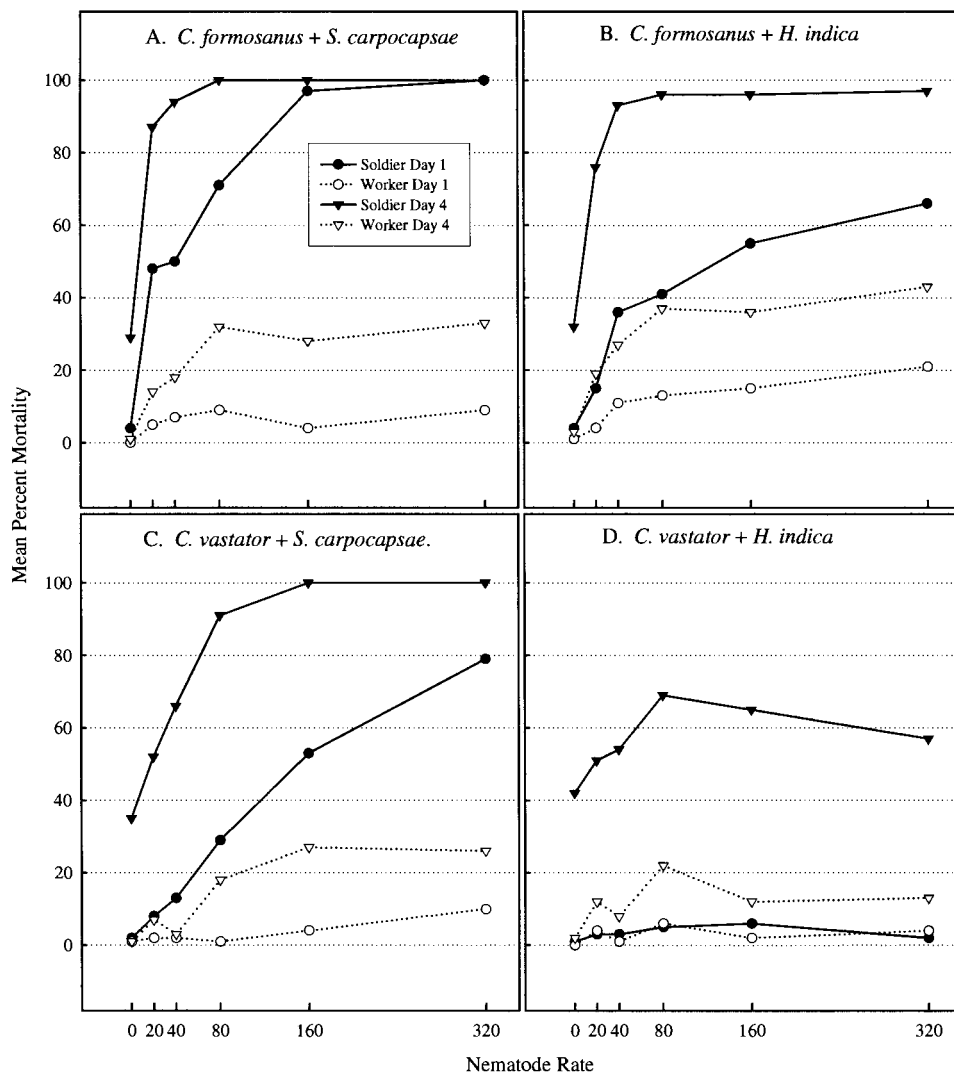


Figure 1. Percent mortality of soldiers and workers of *Coptotermes formosanus* and *Coptotermes vastator* 1 and 4 days after exposure to six rates of either *Steinernema carpocapsae* or *Heterorhabditis indica*.

soldiers alone, but workers from mixed groups had higher mortality ($63 \pm 12\%$) than soldiers from mixed groups ($22 \pm 6\%$) (Table I). Although these results with *C. vastator* at 4 days exposure are similar to the other results, we noted that the average control mortality for both soldiers and workers was rather high.

Concentration mortality studies of workers and soldiers

After 1 day exposure, *C. formosanus* exposed to *S. carpocapsae* showed high soldier mortality in all treatments except the control. Soldier mortality was highest in the treatments of 160 ($97 \pm 2\%$) and 320 (100%) nematodes per termite. A significant two-way interaction existed between nematode rate and termite caste ($F=26.8$,

df=5, $P < 0.0001$). Worker mortality after 1 day was low at $9 \pm 3\%$ in the 320 nematodes/termite treatment. At 4 days exposure, soldier mortality was 100% at 80, 160 and 320 nematodes/termite. Soldier mortality at other rates was also high ($0 = 29 \pm 2$, $20 = 87 \pm 5$, $40 = 94 \pm 4\%$) (Figure 1A). Worker mortality increased compared to 1 day exposure but remained much lower than soldier mortality. At 4 days, worker mortality was $33 \pm 4\%$ in the 320 nematodes per termite treatment. The same two-way interaction between nematode rate and termite caste was significant at 4 days exposure ($F = 16.8$, df=5, $P < 0.0001$).

Coptotermes formosanus exposure to *H. indica* also resulted in a significant two-way interaction between nematode rate and termite caste at both 1 day exposure ($F = 4.83$, df=5, $P < 0.0006$) and 4 days exposure ($F = 7.49$, df=5, $P < 0.0001$). One day soldier mortality from *H. indica* exposure was not as high as that of *C. formosanus* soldiers exposed to *S. carpocapsae* (Figure 1B). Soldier mortality reached $66 \pm 9\%$ in the 320 nematodes/termite treatment at 1 day exposure. At 4 days, soldier mortality was slightly lower than with soldiers exposed to *S. carpocapsae* and did not reach 100% in any of the treatments. The highest soldier mortality was $97 \pm 2\%$ in the 320 nematodes/termite treatment. Workers exposed to *H. indica* incurred higher mortality than workers exposed to *S. carpocapsae*. Mortality at 1 day reached $21 \pm 3\%$ in the 320 treatment and $43 \pm 4\%$ after 4 days in the same treatment (Figure 1A,B).

Exposure of *C. vastator* to *S. carpocapsae* yielded results similar to *C. formosanus* exposed to this nematode. A significant two-way interaction existed between nematode rate and termite caste at 1 day exposure ($F = 18.3$, df=5, $P < 0.0001$) and 4 days exposure ($F = 7.09$, df=5, $P < 0.0001$). Soldiers of this termite species incurred lower mortality at 1 day exposure than *C. formosanus* soldiers (Figure 1C). Mortality in the 320 nematodes/termite treatment reached $79 \pm 6\%$ at 1 day post exposure. After 4 days exposure, however, soldier mortality was 100% in the 160 and 320 nematodes/termite treatments. Compared to soldier mortality, *C. vastator* workers experienced a lower mortality at 1 and 4 days exposure. At 1 day exposure, worker mortality reached $10 \pm 3\%$ at the highest nematode rate. At 4 days exposure, worker mortality was $26 \pm 5\%$ at the same rate.

Results for *C. vastator* soldiers exposed to *H. indica* differed from other tests. At 1 day exposure, a significant effect was found for nematode rate only ($F = 2.74$, df=5, $P < 0.02$) indicating that termite caste did not affect mortality at 1 day. This is evident in Figure 1D where worker and soldier mortalities at 1 day show a similar trend. Soldier mortality was highest in the 160 nematodes/termite treatment ($6 \pm 1\%$) and lower in the 320 nematodes/termite treatment ($2 \pm 1\%$). Worker mortality at 1 day was highest in the 80 nematodes/termite treatment ($6 \pm 2\%$) and lower in the 320 nematodes/termite treatment ($4 \pm 2\%$). At 4 days exposure to *H. indica*, the main effects of nematode rate ($F = 5.17$, df=5, $P < 0.0003$) and termite caste ($F = 245.3$, df=5, $P < 0.0001$) were significant. The interaction of nematode rate and termite caste was not significant. Mortality of *C. vastator* soldiers was much lower at 4 days compared to soldier mortality of *C. vastator* exposed to *S. carpocapsae* (Figure 1C) and *C. formosanus* exposed to *S. carpocapsae* (Figure 1A) or *H. indica* (Figure 1B). Soldier mortality at 4 days was highest in the 80 nematodes/termite treatment ($69 \pm 6\%$) and lower in the 160 ($65 \pm 6\%$) and 320 ($57 \pm 6\%$) nematodes/termite treatment. Worker mortality remained low for *C. vastator* exposed to *H. indica* 4 days exposure reaching $22 \pm 4\%$ in the 80 nematodes/termite treatment. Mortality at the higher nematode rates was lower (Figure 1D).

Discussion

Our results provide strong evidence that the presence of worker termites with soldiers greatly affects nematode attachment and reduces soldier mortality. Since soldiers cannot effectively groom themselves, grooming behavior by workers reduces termite susceptibility to nematode infection. Our data support those presented by Rath (2000) who showed that soldiers of *Nasutitermes exitiosus* (Hill) were more susceptible to an entomopathogenic fungus than workers when they were alone. When soldiers and workers were mixed, this difference disappeared. Rath (2000) speculated that the grooming and feeding behavior of the workers probably extended the life of soldiers in the mixed group. Other studies have shown that the self and social grooming behavior of the termites, *Zootermopsis angusticollis* Hagen (Rosengaus et al. 2000) and *R. flavipes* (Boucias et al. 1996), can reduce entomopathogenic fungal infections. Self-grooming by white grubs (Gaugler et al. 1994; Koppenhöfer et al. 2000) can reduce entomopathogenic nematode infection. In our study, the attachment and the differential response tests showed that soldiers in the presence of workers had lower mortality than lone groups of soldiers exposed to nematodes, indicating that workers were cleaning nematodes from the soldiers and thus reducing infection. Worker mortality in mixed groups was also higher than with workers alone, indicating that workers may become more susceptible to the nematodes when they have to clean both themselves and soldiers. In fact, we observed workers grooming other workers and soldiers in the control and nematode treatments. However, because the termites were held in the dark during most experiments, these observations were made when the mortality data were recorded.

In other results with the attachment and the differential response tests, we found that *S. carpocapsae* was effective against soldiers of both *C. formosanus* and *C. vastator*. *Heterorhabditis indica* was more effective against *C. formosanus* than *C. vastator*. Little is known about the biology of *C. vastator*, and this is the first study to record its response to nematode infection. *Coptotermes vastator* responded at a slower rate to nematode infection than *C. formosanus*, but at some of the higher rates of exposure, it survived better than when exposed to lower rates of nematodes. At present, we cannot explain this and further studies examining the effects of *H. indica* on *C. vastator* are needed. *Steinernema carpocapsae* caused high mortality to soldiers of *C. formosanus*.

In the concentration–mortality tests, *S. carpocapsae* appeared to be more infectious to soldiers than *H. indica*. This was especially true with *C. vastator*. Wang et al. (2002) found that *H. indica* was more infectious than *S. carpocapsae* to *C. formosanus* workers. In our study, *C. formosanus* workers exposed to *H. indica* also sustained higher mortality than *C. formosanus* workers exposed to *S. carpocapsae*. As our experiment continued, mortality of soldier termites exposed to *H. indica* increased. In replicates containing worker termites in these tests, we observed live termites surrounding nematode-killed termites with filter paper particles. This ‘walling-off’ behavior of dead termites by living workers may serve as a mechanism that prevents nematodes from spreading to termites in a colony (Fujii 1975). However, as the filter paper particles would be porous and allow infective juveniles through, this behavior by itself does not explain the need for high nematode concentrations to kill the termites within the petri dish test. The walling-off behavior may be more important in a colony situation where the dead termites may be sealed off from the rest of the colony by frass, soil and other debris.

The differences in foraging behavior of *S. carpocapsae*, a sit and wait strategist (ambusher), versus *H. indica*, a widely foraging strategist (cruiser) (Campbell & Lewis 2002), do not adequately explain our results with the subterranean termite workers. All things being equal, it would seem logical that *S. carpocapsae* would be the more effective nematode against actively moving termites, compared with *H. indica*, which would be expected to be more effective against sedentary insect hosts. Yet, our data and those of Wang et al. (2002) show that *H. indica* is more effective against workers than *S. carpocapsae*. These data suggest that foraging behavior, per se, is only one of several factors that can affect host susceptibility to nematodes. The actual mechanism by which a cruising nematode would be more efficacious against actively moving termites needs to be elucidated.

Kaya and Gaugler (1993) and Gaugler et al. (2000) have stated that it is important to match entomopathogenic nematode species with the host they are adapted to infect. Wang et al. (2002) suggested that the right nematode species has not yet been found for termite biological control. Because of the many state restrictions in the importation of exotic organisms into Hawaii, we were not able to test a number of other nematode species against the subterranean termites. However, our data show that workers do remove nematodes from soldiers and each other during grooming and that there is a need for unrealistically high nematode concentrations to obtain efficacious results. At least, this is the case with the two nematode species tested in our study. In addition, because nematodes are slow moving, termite workers are fast moving, and subterranean termites exist in large colonies with expansive foraging ranges, nematodes do not seem to be the best biological control agents against these social insects. Some laboratory tests have shown marginal efficacy (Epsky & Capinera 1988; Mauldin & Beal 1989), but it must be kept in mind that entirely new complications arise when nematodes are applied to the soil around termite infestations. Even though both organisms share a subterranean habitat, the physical, chemical, and biological properties of the soil environment greatly affect nematode efficacy. Adding termite grooming behavior to this list only increases the chances of failure with nematode treatments against termites. Since encounters with nematodes in the soil would most likely occur at a lower rate than in a petri dish, the effects of grooming would be amplified. Nevertheless, much can be learned about behavioral aspects of resistance mechanisms to nematode infection with these social insects, and continued research may find ways to overcome this type of resistance among this and other insect species.

Acknowledgements

Partial funding was provided by USDA-ARS Specific Cooperative Agreement 58-6615-9-018 (J.K. Grace, PI). This is Journal Series No. 4688 of the College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa.

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