Bull. Inst. Zool. Academia Sinica **30**(1): 31-39 (1991)

SUSCEPTIBILITY OF TWO FORMOSAN TERMITES TO THE ENTOMOGENOUS NEMATODE, *STEINERNEMA FELTIAE* FILIPJEV[1]

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(Accepted June 12, 1990)

Huei-Jung Wu, Zu-Nan Wang, Chia-Fei Ou, Ru-Shiow Tsai and Yien-Shing Chow (1991) Susceptibility of two Formosan termites to the entomogenous nematode, *Steinernema feltiae* Filipjev. *Bull. Inst. Zool., Academia Sinica* **30**(1): 31-39. The effect of nematode, *Steinernema feltiae* Filipjev, on constrained Formosan termites, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe) was studied. The relationship between the nematode and host termites is a case of parasitism. Nematode invasion is via the mouth of the hosts. All castes of the hosts were very susceptible to the nematode. But the host eggs were resistant to *S. feltiae* under conditions in the experiments.

The susceptibility of the host termites to *S. feltiae* was evaluated under an imitative artificial nest environment. Hosts were exposed to six concentrations of *S. feltiae* ranging from 5,000 to 1,500,000 per nest. Mean nematode-associated host mortality 7 and 20 days after exposure was dose-related ranging from 4.8% at the lowest nematode dosage level to 97.9% at the highest dose. Although host termites could detect the nematodes and seek ways to avoid nematode attacks by extending their earthern tunnels to the outside of the nest, a considerable number of the termites had been parasitized before running away from the nematodes.

Key words: Nematode, Termites, Susceptibility, Parasitism, Biocontrol.

Two Formosan termites, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe), have distributed widely and become major structural pests in Taiwan. These insects crossed the Pacific to the United States by ship 20 years ago and have a serious propensity to infest and destroy condominiums (Hardy, 1988). Hardy notes that extensive trials with highly stable and toxic organochlorines such as chlordane and heptachlor in North America have not proven efficient in the prevention and control of the Formosan termites. One reason for this inefficiency may be that the termites' nests are

1. Paper No. 346 of the Journal Series of the Institute of Zoology, Academia Sinica.
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extensively burrowed and inaccessible, causing the efficacious application of chemical insecticides difficult.

The entomogenous nematode, *Steinernema feltiae* Filipjev, along with its associated bacterium, *Xenorhabdus nematophilus* (Poinar & Thomas), has great potential to biological control certain insect pests (Lindegren and Vail, 1986; Kaya *et al.*, 1982; Poiner and Deschamps, 1981). Our research focuses on determing the effect of *S. feltiae* on Formosan termites and to test the ability and efficiency of this nematode in infecting and killing the termites in an environment of designed imitative artificial nests. With the data obtained from the studies, it should be possible to predict the effectiveness of the nematode in combating insects of similar or related niches. All the studies were conducted at the Institute of Zoology, Academia Sinica and the Taiwan Sugar Research Institute, Taiwan, Republic of China between 1988-1990.

MATERIALS AND METHODS

Source of Nematode

The nematodes, *Steinernema feltiae*, used in this study were originally obtained from the USDA Horticultural Crops Research Laboratory, Fresno, California, propagated in larvae of the silk worm, *Bombyx mori* L., and stored in petri dishes as described by Hara and Kaya (1981). Counts were made by dilution.

Establishing Host Populations

Host termites were collected from colonies in the field and cultured in individual artificial nests at our laboratory for 12 months (*Coptotermes formosanus*) and 18 months (*Reticulitermes speratus*) before the experiments described below began. A glass container (50×50×8 cm) was used for each artificial nest. Inside each glass container a layer of sponge (8 cm hight) was placed in the bottom, a layer of sandy loam (8 cm high) was then placed on the top of the sponge layer, and 4-8 pieces of pine wood (30×11×3 cm) were placed perpendicularly on the soil layer from one side to another (Fig. 1). Thin, narrow pieces of sponge were placed on each side between the glass and the wood to hold the latter in place. A thin pad of cotton batting was placed over the top of the wood, and this was thoroughly soaked with water about twice a week. 5,000 termites including 4,500 workers, 300 soldier and 200 brachypterous reproductives were released into each of the prepared artificial nests. Termites placed in such nests become established in a few days.

Effect of Nematode on Termites

The infective stage (*i. e.*, the 3rd larval stage) nematodes of *S. feltiae* were tested to determine their effect on different castes of *C. formosanus* and *R. speratus*. One hundred host termites, including 40 workers, 20 soldiers, 20 reproductives, and 20 nymphs, were placed in individual sterile petri plates (100×15 mm) lined with 4 sheets of filter paper (*i. e.*, type I treatment) or 30 g of sterile soil debris (*i. e.*, type II treatment, composed of sandy loam and wood chips). Into each plate 2 ml of water containing approximately 5,000 nematodes of *S. feltiae* were added. Control plates contained 100 termites with 2 ml of distilled water. The plates were covered with a modified lid with 24 openings (1 mm in diam.) and incubated at room temperature (22-27°C) and 72-88% RH. The activities of the nematodes and those of the host termites in the plates were observed periodically under a dissecting microscope. Each day the dead termites were removed from the plates and washed with distilled water. The destruction of the infected termites and the development of the nematodes within the hosts were then observed under a phase contrast microscope.

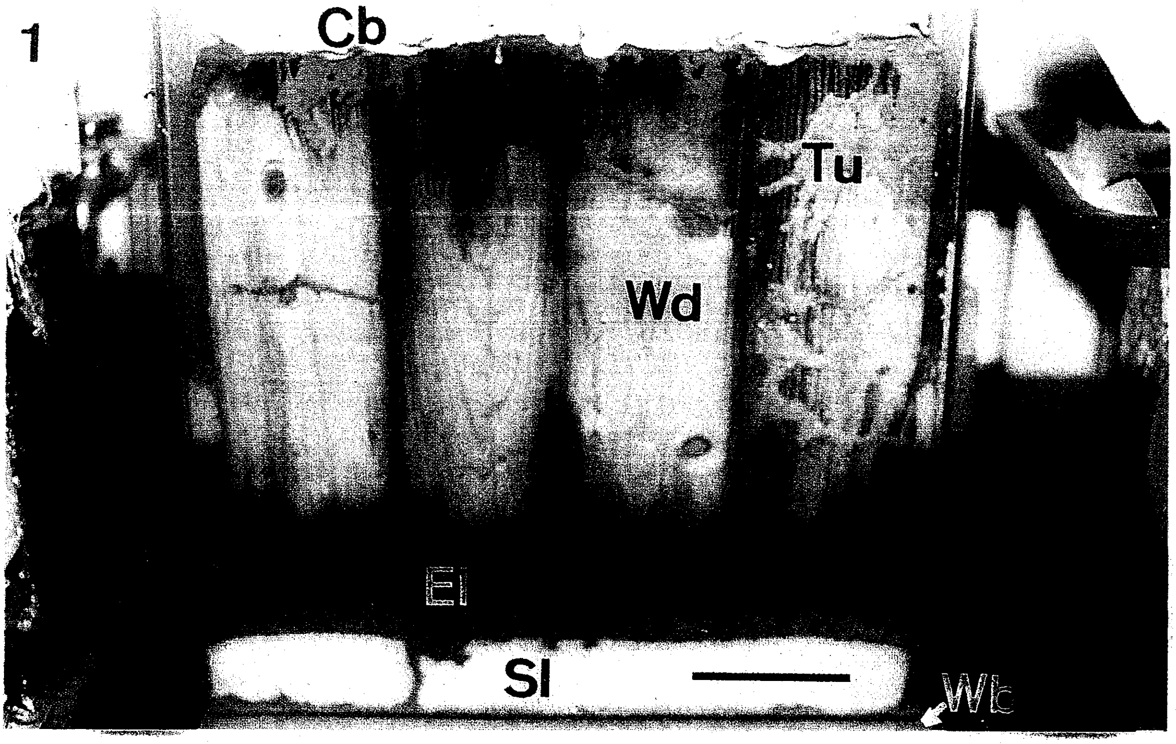


Fig. 1. The imitative artificial nest designed for establishing host populations (*C. formosanus* and *R. speratus*) and testing the susceptibility of the host termites to the entomogenous nematode (*S. feltiae*). Bar: 10 cm; Cb: cotton batting (as a water source) on the pine wood; El: soil (sandy loam) layer; Sl: sponge layer at the bottom of the nest; Tu: eastern tunnels (on the surface of the pine wood) built by the termites; Wb: water barrier outside and under the nest; Wd: pine wood (provided to the termites as a food source).

Nematode Infectivity to Hosts in Artificial Nests

The susceptibility of *C. formosanus* and *R. speratus* to the infective stage nematodes of *S. feltiae* was tested in the individual imitative artificial nests described before. Three mls of nematode suspension was added to one side of the soil layer (see El in Fig. 1) in each of the 24 experimental artificial nests. The six treatments consisted of 5×10^3, 1.5×10^4, 1.5×10^5, 5×10^5 and 1.5×10^6 nematodes per nest (Table 3). The 4 control nests contained 3 ml of distilled water without nematodes. The nests were incubated at room temperature (23-27°C) and 80-90% RH.

Seven days after the initial host exposure to *S. feltiae*, 14 nests (see Table 3) were carefully excavated, live and dead host termites collected and counted, and the dead termites removed to moistened petri dishes and dissected to verify nematode parasitism and determine nematode-associated host mortality. A host body containing developing *S. feltiae* adult or larval stages was considered to be parasitized. Twenty days after the initial nematode exposure, another 14 nests were excavated and nematode effectiveness at infecting host termites was examined in the same way.

RESULTS

Effect of Nematode on Constrained Termites

Two Formosan termites (*Coptotermes formosanus* and *Reticulitermes speratus*)

Table 1
Average cumulative mortality of verified nematodes infection of *C. formosanus* and *R. speratus* during 6 consecutive days after exposure to infective stage nematodes of *S. feltiae* (n=100 termites and 5,000 nematodes per plate)

Hours after initial exposure	Average cumulative mortality (%)					
	Type I plates (P1-P10)*			Type II plates (P11-P20)*		
	Cf**	Rs**	Mean (%)	Cf**	Rs**	Mean (%)
24	18.4	19.4	18.9	39.0	45.2	42.1
48	41.2	45.8	43.5	74.4	86.8	80.6
72	54.2	59.2	56.7	96.4	98.2	97.3
96	81.2	84.2	82.7	98.4	98.6	98.5
120	83.2	86.0	84.6	99.0	99.2	99.1
144	83.8	86.8	85.3	99.2	99.6	99.4

* Type I plates were lined with filter paper; Type II plates were lined with sterile soil debris.
** Cf=*C. formosanus*; Rs=*R. speratus*.

Table 2
The effect of *S. feltiae* on constrained *C. formosanus* and *R. speratus* in the petri plates and the development of the nematode within the parasitized termites (n=100 termites and 5,000 nematodes per plate)

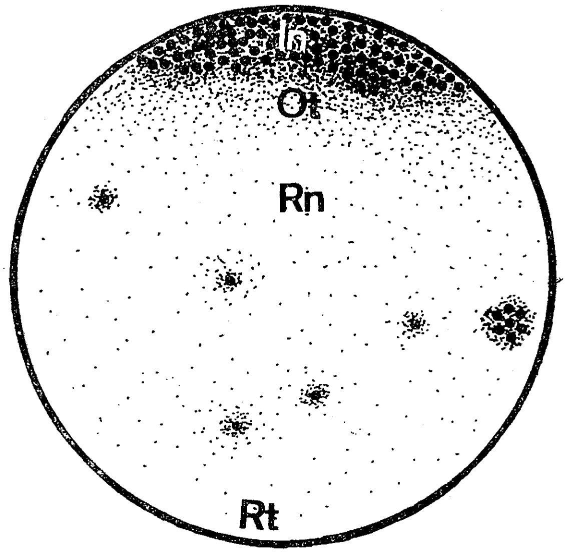
Hours	Experimental		Control
	Host termites	*S. feltiae*	(without *S. feltiae*)
0- 4	Active hosts were more or less clumped in the plates to avoid nematode attack (not seen in control plates)	Active larvae over general surface of host body; some larvae seen penetrating via the mouth of the hosts	Active termites seen wandering around the circumference in the plates
5- 24	Some hosts began to die; others were quiescent	Larvae which invaded the hosts clumped in host head, thorax, and abdomen	Nesting occurred in plates lined with soil debris (not seen in experimental plates)
25- 48	More dead termites seen and part of the host's tissue was decomposed; live hosts were weak and quiescent	Molting occurred, sperm or gonad primodium was developing in maturing nematodes within the hosts; larval skin shed, increased associated bacteria seen in infected hosts (not seen in control termites)	Active termites; increased nesting activities; no dead termites were seen thus far.
49- 72	More of the cadavers' tissue was decomposed; the still-living hosts (about 23%) were dying	Sexual maturity of males and females; development of ovoviviparous eggs in female	Active termites; 4 termites died of cannibalism
73-360	All tissues of the infected hosts were decomposed and depleted leaving only the decaying exoskeleton	Second generation larvae broke out of the mother's cuticle, left the hosts, and became infective-stage juveniles at about 115-140 h after the initial exposure	Active termites; about 97% of the termites were still alive and healthy at hour 360 and none of the dead termites died of nematode infection

were exposed to the infective stage of of nematodes *Steinernema feltiae* in two types of treatments in petri dishes (Table 1). The potential relationship between *S. feltiae* (and its associated bacterium, *Xenorhabdus nematophilus*) and the two host termites is that of host parasitism (Table 2). Nematode invasion occurred via the mouth of the hosts. Dissections showed that 24 h after exposure, the number of nematodes found in each infected termite ranged from 1 to 102. All castes of the hosts including; workers, soldiers, reproductives and all stages of nymphs, were susceptible to the nematode. A total of 95.7% of hosts in type I and 99.8% of hosts in type II died of verified nematode infection 15 days after nematode exposure. In contrast, 97% of the termites in the control plates were still alive and healthy on the 15th day, and none of the dead termites died of nematode infection.

Many more host termites were killed by nematode infection in type II plates (*i.e.*, lined with sterile soil debris) than in type I plates (*i.e.*, lined with filter paper) during the first 3 days after nematode exposure (Table 1). This result might be due to different host distribution patterns formed and different nematode locomotion methods with plates lined with different substrates. This conclusion was drawn from the following observations:

1) Most of the termites clumped together at one side of the type I plates soon after nematode exposure (Fig. 2-a). Such a clumped distribution, as observed under a dissecting microscope, greatly hindered the nematodes ability to make quick close encounters with the termites that were crowded inside the clump. Consequently, there was a decreased nematode-associated mortality of the hosts during the first few days.

In contrast, the more or less random distribution of the hosts in type II plates



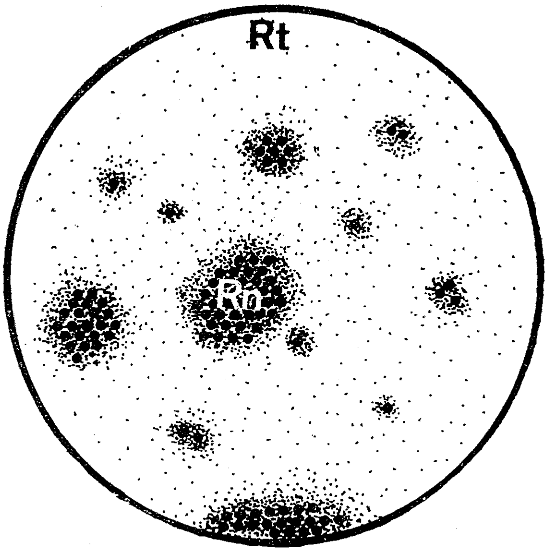


Fig. 2. General distribution patterns of host termites (*C. formosanus* and *R. speratus*) and nematodes (*S. feltiae*) in two types of treatments in petri plates. Size of plates: 100×15 mm; Circle "●": termites; Dot "·": nematodes; In: the inner sites of the termite clump; Ot: the outer sites of the termite clump; Rn: the site where nematodes were originally released; Rt: the site where termites were originally released.

(Fig. 2-b), which enabled the nematodes to make quick close encounters with most of the termites soon after the exposure. Consequently, host mortality increased during the first few days.

2) Soon after the nematodes were released onto the flat, moist filter paper in type I plates (Rn) they migrated to the circumference. Most of them were observed to move in either of two ways, by "waving" or by "bridging". In the former, the nematode waved both its anterior and posterior ends, in the latter, the nematode assumed an errect posture and waved its anterior end until it came into contact with another portion of the filter paper. The movement went on until the nematode reached one of the host's legs. Many nematodes were shaken off of the moving legs of the host before they could make successful movements toward the mouth of the host.

The moist, projecting surface of the soil debris in type II plates, favored nematode's "looping" movement. The nematode formed a loop by bending the anterior part of the body until contact was made near the posterior end. From this position nematodes were observed to propel themselves through the air at distances of 5-10 mm. In may cases the nematodes were able to leap directly onto the dorsal surface of the host body making a subsequent movement toward the mouth of the host easier.

The fact that most of the nematodes were able to clump at (or around) the host sites in both types of plates (Fig. 2) soon after the exposure, indicates a possible means of host detection rather than random encounters.

Nematode Infectiveness to Hosts in Artificial Nests

All castes of Formosan termites (*C. formosanus* and *R. speratus*) were exposed to the infective-stage nematodes of *S. feltiae* in individual artificial nests and were susceptible. However, host eggs were resistant to *S. feltiae* under conditions used in the experiments. Mean mortality of the experimental termites verified nematode infections 7 and 20 days after exposure was dose-related ranging from 4.8% at the lowest nematode dosage level to 97.9% at the highest dose (Table 3). No nematode-associated termite deaths occurred in the control nests.

Nematode-avoidance behavior in response to nematode migration was observed in the experimental nests during the testing period.

1) During the first 3 days after nematode exposure the termites gradually disappeared from the visible surface of the soil layer (El) and of the pine wood (Wd) in the nests (Fig. 1), where before usually numerous termites appeared on these surfaces. To determine the dispersion of the nematodes in the nests, 3–5 small pieces of wood and a small amount of soil were scraped randomly from the surface in each experimental nest. Microscopic observations revealed that within 25–40 h after the exposure, the nematodes had dispersed to most parts of the moist surfaces of the pine wood and sandy loam in the nests. These observations suggested that the experimental termites had been disturbed and were trying to avoid nematode attack by hiding in the tunnels inside the wood and the soil.

2) Another nematode-avoidance behavior began 3–4 days after nematode exposure: abnormal tunnel-extension (*i. e.*, extending earthen tunnels to the outer surface of the nest containers) occurred in the nests with higher nematode dosage levels (*i. e.*, from 1.5×10^5 to 1.5×10^6; see Table 3). The number of such abnormal tunnels per nest was dose-related ranging from 1 at the 3rd highest nematode dosage level to 7 at the highest dose. Some

Table 3
Percentage of mortality of verified nematode infection of *C. formosanus* and *R. speratus* in 28 artificial nests 7 days and 20 days after exposure to the infective-stage of *S. feltiae* (n=6,218–8,339 termites per nest)

Nematode dose per nest	% mortality of termites					
	7 days after exposure			20 days after exposure		
	Cf*	Rs*	Mean	Cf*	Rs*	Mean
1,500,000	94.9	96.5	95.7 a	97.4	98.3	97.9 a
500,000	62.4	67.1	64.8 b	71.5	76.7	74.1 b
150,000	41.7	43.4	42.6 c	42.6	47.9	45.3 c
50,000	14.0	15.8	14.9 d	16.7	17.5	17.1 d
15,000	8.4	10.3	9.4 d	9.1	10.8	10.0 d
5,000	4.7	4.9	4.8 d	5.8	6.3	6.1 d
Control**						

Means followed by the same letter are not significantly different ($p<0.05$; Duncan's (1951) multiple range test).

* Cf=*C. formosanus*; Rs=*R. speratus*.

** No nematode-associated dead termites were found in the control.

of these tunnels were even extended into the outside water barrier (Wb) outside and under the nest containers (Fig. 1). A partial excavation of 2 of the abnormal tunnels found that 47% (n=100) and 82% (n=100) of the termites within the tunnels where both parasitized. Nest excavations 7 days after nematode exposure found that numerous nematodes had dispersed through the tunnels inside the pine wood and the soil layer in the experimental nests. These observations suggested that the experimental termites were seeking a way to avoid nematode attack by extending their earthen tunnels outside of the nest.

No nematode-avoidance behavior was observed nor were the nematodes found in the control nests.

DISCUSSION

Infection tests have revealed that nematode invasion occurs via the mouth of the host termites. Since the mouthparts of all the hosts are a wide-open chewing type, it is not surprising that all castes and nymphal stages of the hosts are very susceptible to the infective-stage of *Steinernema feltiae*. In contrast, many Lepidopteran pests, such as adults of the cabbage worm (*Pieris rapae crucivora*), have a long proboscis that is too narrow to allow either passive or active invasion by the nematode (Wu and Chow, 1989). The nematode served as a carrier of the symbiotic bacterium, *Xenorhabdus nematophilus* (Lysenko and Weiser, 1974; Poinar, 1966; Poinar *et al.*, 1971; Poinar and Thomas, 1966, 1967; Thomas and Poinar, 1979), that was introduced into the host body and released in the head, thorax and/or abdomen of the host termites (Table 2). The bacterium multiplied quickly and was probably responsible for tissue decomposition and the death of the termites which occurred within 24–72 hours. Although host eggs were resistant to *S. feltiae* they could not live long without being taken care of by the still-surviving workers.

Most of the nematodes were able to clump at the host sites soon after the exposure indicating a possible means of detection rather than encountered through random movements. The three types of nematode locomotion found in the two types treatment methods in petri plates have suggested that the moist earthen and/or wooden substrates like those in the artificial nests favored nematode's movements toward the host body. Although host termites could detect the nematodes and seek ways to avoid nematode attack, a considerable number of the termites had already been invaded by the nematodes before they could run away from their nemesis.

Although nematode concentrations needed for the control of termites appeared to be high, an effective mortality response for termite control using this nematode was as high as 64.4–97.9%, or 106–211 nematodes per host termite in the imitative nests (Table 3). These mortality response levels and the recently reported reductions in nematode production costs, less than 1 US dollar per 50 millions (Bedding, 1981), indicated that field application for termite control should be attempted, especially in infested buildings where the nematodes could be applied directly to the tunnels of the nests. Such application may also be efficacious for the control of other susceptible pests that occupy similar niches and have similar substrates, temperatures and relative humidity for the nematode's movements and dispersion.

Acknowledgements: We greatefully acknowledge Mr. James E. Lindegren of the USDA Stored Products Laboratory of Fresno for mailing the original stock of *S. feltiae* and providing useful ideas to our endeavours.

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費爾地線蟲 (*Steinernema feltiae*) 對兩種臺灣產白蟻防治潛能之研究

吳輝榮　王次男　歐家斐
蔡如秀　周延鑫

將家白蟻 (*Coptotermes formosanus*) 及大和白蟻 (*Reticulitermes speratus*) 分別在培養皿及模擬人造蟻巢內曝露於費爾地線蟲 (*Steinernema feltiae*) 之第三齡幼蟲，發現只有白蟻的卵期不被線蟲寄主，其餘白蟻各階級成蟲及各齡幼蟲都會被該線蟲寄生。線蟲入侵的門徑只要是經由白蟻的口器，並於入侵後 24～72 小時內殺死寄主。將人造蟻巢曝露在不同劑量的線蟲下，每巢由 5,000～1,500,000 隻不等，於第 7 天和 20 天後分別檢視，發現平均感染致死率 (4.8～97.9%) 與線蟲劑量之高低成正比。進一步的研究和觀察，顯示蟻巢內的環境極適合該線蟲展開其擴散及對寄主的有效攻擊行動。雖然白蟻能擴建土隧道逃離線蟲集中區，但因爲線蟲隨卽擴及蟻巢各區域，使得白蟻受到嚴重的傷害。目前該線蟲大量生產的成本很低，將來若以高劑量的線蟲直接施用到白蟻巢內，讓線蟲自行感染巢內白蟻，在白蟻防治上將具有相當樂觀的潛能。