

# Factors Affecting the Tunneling Behavior of the Western Subterranean Termite, *Reticulitermes hesperus* Banks<sup>1</sup>

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**Abstract:** Laboratory studies were conducted to determine factors that affect the tunneling behavior of the western subterranean termite (*Reticulitermes hesperus* Banks). Soil particle sizes between 2.36 and 0.84 mm prevented tunneling. Exposure to solid layers of calcium, magnesium, or zinc borate did not repel workers, but produced >87 percent kill within 10 d. No tunneling occurred in soils treated with the formulated termiticides tested at concentrations  $\geq 50$  ppm. Termites did not tunnel in soils treated with as little as 1 ppm bifenthrin or cypermethrin. Exposure to bifenthrin and cypermethrin deposits immobilized termite workers which prevented them from tunneling.

Termites failed to penetrate a layer of dead termites killed by earlier exposure to chlorpyrifos. Contact with dead termites resulted in 96 percent mortality of the tunneling workers. Workers readily penetrated layers of termites freshly killed by freezing, and some died within 24 h.

At 15°C, the distance tunneled by acclimated termites was significantly greater than those of unacclimated termites. At 21-32°C, the acclimation period did not affect the distance tunneled. Tunneling rates were significantly lower at 15°C than at 21, 27, or 32°C. Tunneling rates at 27°C were significantly greater than at 21 or 32°C.

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Most species of subterranean termites live deep within the soil, requiring workers to construct elaborate systems of tunnels to forage for food and water. The competence of a structural subterranean termite pest may, to a

great extent, be determined by its foraging behavior. Williams (1977) categorizes foraging in subterranean termites into two basic patterns: species which forage over a long distance to larger, more scattered pieces of litter and species which forage close to the nest, consuming small pieces of litter. *Reticulitermes* spp. in the western United States clearly fall into the first category.

The habit of random foraging may be a distinct advantage for *Amitermes*, *Heterotermes*, and *Reticulitermes* spp. occurring in open areas or in ranges of dense but widespread vegetation types. Studies by Su and others (1984) suggest that the Formosan subterranean termite (*Coptotermes formosanus* Shiraki) forages in a random network of tunnels. Within small plots, *Heterotermes aureus* (Snyder) was a random forager (Jones and others 1987). Descriptions of tunneling in the western subterranean termite (*Reticulitermes hesperus* Banks) of irregular reticulations with no particular plan also suggest a random foraging pattern (Pickens 1934).

Studies were conducted to determine various abiotic and biotic factors that might affect the random tunneling behavior of *R. hesperus*. The effects of soil particle size, borate compounds, soil treated with termiticides, the presence of dead termites, and temperature on tunneling behavior were studied.

## METHODS AND MATERIALS

### Size of Soil Particles

To determine the optimal particle size, termites were allowed to tunnel in soils with various particle sizes. Glass tubes (9 mm i.d. by 15 cm tall) were filled with soil with particle sizes ranging from 2.36 to 0.84, 0.84 to 0.42, 0.42 to 0.25, 0.25 to 0.18, and 0.18 to 0.15 mm (8 to 20, 20 to 40, 40 to 60, 60 to 80, and 80 to 100 mesh/in.). Twenty *R. hesperus* workers were placed in the bottom of each of five tubes for each range of particle sizes. The distance tunneled by the termites was recorded periodically for 24 h (Smith and Rust in press).

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### Influence of Borate Barriers on Tunneling

Termites were allowed to tunnel in glass tubes packed with a band of solid inorganic borate to determine its effect on their tunneling behavior. Calcium, magnesium, and zinc borate powders (provided by U. S. Borax Research, Anaheim, CA) were used in the study. The bottoms of glass tubes (9 mm i.d. by 15 cm tall) were closed with a cotton plug (1.8 cm long) and filled with 3.9 cm of untreated soil (20-100 mesh), a 1-cm thick layer of borate compound, and 6.5 cm of untreated soil. Cotton plugs (1.8 cm long) were placed in the top of each tube. The tubes were inverted, the cotton plugs were removed from the bottom, and 1.5-ml aliquots of water were added to the soils. This prevented the 3.9-cm untreated layer of soil from being contaminated with borate and termites from encountering any borate before the 1-cm layer. Twenty workers were placed in the bottom of each tube along with a small piece of paper towel to serve as food. The tubes were stoppered and set upright. Termites were allowed to tunnel for 48 h. The number of tubes in which termites penetrated the borate barrier and number of dead termites were counted at 48 h. Termites were removed from the tubes and placed in plastic Petri dishes (35 mm diameter by 10 mm tall) provisioned with a disk of clean paper towel. The dishes and termites were maintained at  $25 \pm 2^\circ\text{C}$  and 93 percent RH. The number of dead termites was counted 7 and 10 days after the termites were removed from the tunneling tubes. Five replicates were used for each of the three different borate compounds.

### Influence of Soil Termiticides on Tunneling

To determine the toxicity and repellency of soils treated with termiticides, termites were permitted to tunnel in glass tubes (9 mm i.d. by 15 cm tall) packed with untreated and treated soil (Smith and Rust in press). A 3.9-cm layer of untreated soil was gently packed into each tube followed by a 7.5-cm layer of treated soil. The tubes were inverted and 1.5-ml aliquots of water were added to the untreated soil. The water was drawn through the treated section with an air vacuum, allowing the soil to be uniformly moistened with minimal contamination of the untreated soils.

Twenty workers were introduced in the bottom of each tube. The distance tunneled by the termites was recorded after 48 h, at which time the termites were removed from the tubes and the number of moribund and dead insects was counted.

### Avoidance of Dead Termites

To determine the effect of dead termites on the tunneling behavior of *R. hesperus*, termites were allowed to tunnel in soil and encounter dead termites. Glass tubes (9 mm i.d. by 15 cm tall) were filled with 3.9 cm of soil at the bottom.

Next, a layer of ten dead termites was added, which had been exposed to either soil treated with chlorpyrifos at 500 ppm for 24 h (Smith and Rust in press) or frozen for 24 h. Exposure to treated soil simulates mortality caused by contact with a chemical barrier, and freezing simulates natural mortality. The dead termites were covered with 7.5 cm of soil. Twenty live workers were added to the bottom of each of five tubes per treatment and allowed to tunnel 24 h. At 24 h, the number of tubes in which the termites penetrated the barrier of dead termites and the number of termites rendered dead or moribund during the tunneling activities were recorded.

### Influence of Temperature on Tunneling

Studies were conducted to determine whether length of acclimation of termites to certain temperatures affected their rate of tunneling. To determine whether acclimation periods were important, 5 lots of 20 termites were held at 15, 21, 27, or  $32^\circ\text{C}$  or at room conditions ( $25^\circ\text{C}$ ) for either 12, 24, 36, or 48 h before testing. Glass tubes (9 mm i.d. by 15 cm tall) were filled with 11.4 cm of untreated soil. The soil was moistened with 1.5 ml of water. The tubes were placed in environmental chambers maintained at either 15, 21, 27, or  $32^\circ\text{C}$  for 12 h to condition the soil to the appropriate temperature. At the end of each termite acclimation period, twenty workers were placed in each of five tubes per temperature and allowed to tunnel for 12 h. The five tubes with acclimated termites and termites held at  $25^\circ\text{C}$  were placed back into the same acclimation chambers maintained at either 15, 21, 27 or  $32^\circ\text{C}$ . Distances tunneled were recorded at 12 h. Differences in the distances tunneled from each acclimation period and temperature were analyzed by an analysis of variance (ANOVA) using MSTAT: Micro Computer Statistical Program (Michigan State University, East Lansing), and means were separated by Tukey's HSD at the  $\alpha = 0.05$  level (Tukey 1953).

To determine the effect of soil temperature on tunneling rates, workers were permitted to tunnel in glass tubes (9 mm i.d. by 30 cm tall) filled with 25 cm of untreated soil and moistened with 3 ml of water. Groups of termites were acclimated at 15, 21, 27, and  $32^\circ\text{C}$  for 48 h. The tubes containing the moist soil were placed in environmental chambers maintained at either 15, 21, 27, or  $32^\circ\text{C}$  and allowed to condition for 12 h. After the 48-h acclimation period, twenty workers were placed in each of five tubes per temperature. The five tubes with termites were placed back at the original four acclimation temperatures. Distances tunneled were recorded at 2, 4, 6, 8, 10, 12, and 24 h. Tunneling rates at the four temperatures were determined by regression analysis of variance using MSTAT: Micro Computer Statistical Program (Michigan State University, East Lansing).

## RESULTS AND DISCUSSION

### Size of Soil Particles

Rates of termite tunneling in tubes filled with soils of different particle sizes were as follows:

Particle size (mm)	Distance tunneled Total cm (h) <sup>a</sup>	Rate $\pm$ 95pct CL <sup>b</sup> (cm/h)
2.36-0.84	0	0
0.84-0.42	24.9 (115)	0.22 $\pm$ 0.09
0.42-0.25	32.5 (115)	0.28 $\pm$ 0.11
0.25-0.18	28.8 (100)	0.29 $\pm$ 0.13
0.18-0.15	30.3 (100)	0.30 $\pm$ 0.21

<sup>a</sup> Total distance tunneled in (total cumulative hours)

<sup>b</sup> Mean rate with 95 pct confidence level in cm/hr

Consequently, soil with particle sizes  $\geq 0.15$  and  $\leq 0.84$  mm were used in subsequent studies to ensure optimal tunneling activity.

Size of the substrate particles is important in determining whether subterranean termites can tunnel through soil. Ebeling and Pence (1957) showed that soil with particle sizes ranging from 3.35 to 1.18 mm (6-16 mesh/in.) were impenetrable to *R. hesperus*, and soil with particle sizes ranging from 0.3 to 0.15 mm (50-100 mesh/in.) were penetrable. For the Formosan subterranean termite, *C. formosanus*, it was determined that penetration occurred in untreated substrates with particle sizes ranging from 0.8 mm to  $<0.2$  mm (20 to 80+ mesh/in.); particle sizes from 2.4 to 1.7 mm (8-12 mesh/in.) completely prevented penetration (Tamashiro and others 1987).

Substrates with particle sizes  $\geq 3$  mm were easily penetrated by the subterranean termites studied thus far; termites were able to move through the spaces made by these larger particles (Ebeling and Pence 1957, Tamashiro and others 1987). Soils that are impenetrable to termites have particles that are too large for termites to pick up in their mouths and move, yet with spaces between particles small enough so that termite headcapsules will not pass through. The size of particles that are penetrable to termites will vary with each species.

### Influence of Borate Barriers on Tunneling

*R. hesperus* workers penetrated 60 percent of the calcium borate barriers and 100 percent of the magnesium borate and zinc borate barriers. There was 42 percent mortality of termites exposed to calcium borate barriers at 48 h, but no substantial mortality with the other two barriers. Termite mortality was 87, 98, and 89 percent in tubes packed with calcium, magnesium, and zinc borates, respectively, 10 d after removal from the tubes. The solid borate layers were not repellent to worker termites, and contact with or ingestion of them caused significant latent mortality.

### Influence of Soil Termiticides on Tunneling

As shown in figure 1, termites did not tunnel into soil treated with bifenthrin or cypermethrin, regardless of concentration. No tunneling occurred in soil treated with chlordane, chlorpyrifos, fenvalerate, isofenphos, or permethrin at concentrations  $\geq 50$  ppm. In addition to preventing tunneling into treated soil, the above soil termiticide treatments also severely reduced tunneling activity in the untreated zone beneath the treated soil (fig. 1). Termites tunneled in soil treated with chlordane, chlorpyrifos, isofenphos, fenvalerate, or permethrin at concentrations  $\leq 10$  ppm. The most tunneling occurred in soils treated with chlorpyrifos and isofenphos, whereas very little tunneling occurred in the soils treated with chlordane, fenvalerate, or permethrin at these lower concentrations (fig. 1). After 48 h, termites were removed from the soil-filled tubes. The greatest mortality (76 to 100 percent) occurred in the tubes filled with soils treated with 100 to 500 ppm of bifenthrin or cypermethrin (table 1).

Termites did not tunnel in treated soil, especially in soil treated at high concentrations, yet substantial mortality occurred. Insecticide diffused from treated to untreated soil in concentrations high enough to kill or affect behavior. Movement of insecticide seems to be facilitated by water solubility or vapor pressure. Chlordane appeared to move the least distance, while bifenthrin and cypermethrin moved the farthest, because termites tunneled closest to soil treated with chlordane and never

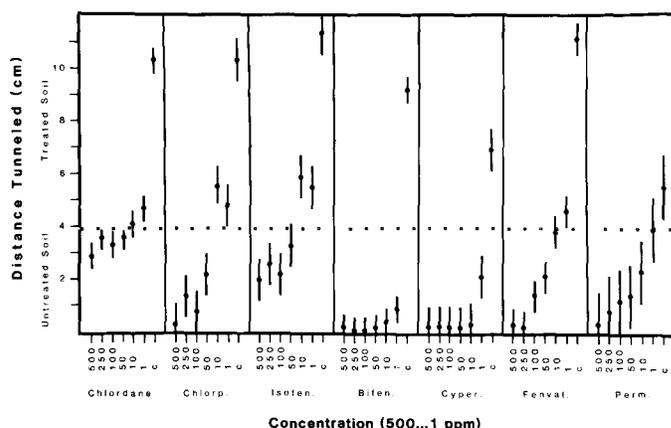


Figure 1--Average distance tunneled by *R. hesperus* underneath treated soils and in soils treated at 500 to 1 ppm with various termiticides. Dashed lines indicate common boundary of treated and untreated soil. Vertical bars represent standard errors of means. Chlorp. = chlorpyrifos, Isofen. = isofenphos, Bifen. = bifenthrin, Cyper. = cypermethrin, Fenval. = fenvalerate, Perm. = permethrin, c = control (Smith and Rust in press).

Table 1--Percent mortality of *R. hesperus* after tunneling 48 h in tubes filled with termiticide-treated soils. <sup>1</sup>

Termiticide	Concentration of termiticide (ppm)					
	500	250	10	50	10	1
Bifenthrin	100	100	76	29	2	9
Chlorpyrifos	90	95	85	51	10	42
Cypermethrin	50	27	1	5	0	44
Fenvalerate	32	4	52	0	0	2
Isofenphos	26	39	42	40	0	40
Chlordane	22	20	4	2	19	21
Permethrin	4	6	1	3	3	3

<sup>1</sup> Adapted from Smith and Rust (in press).

approached the soil treated with bifenthrin or cypermethrin (fig. 1). Apparently chlordane is not as repellent as bifenthrin and cypermethrin. At high concentrations less mortality occurred in soils under chlordane-treated soil than in soils under the bifenthrin and cypermethrin treatments, even though termite tunneling was severely reduced. This suggests that biologically active amounts of bifenthrin and cypermethrin were moving longer distances. At low concentrations, no significant mortality was produced underneath bifenthrin and cypermethrin treatments, and very little tunneling was observed in the untreated soil.

We hypothesize that the alpha-cyano pyrethroids produced sublethal effects which may inhibit tunneling, perhaps by temporarily paralyzing termites that contact them. Scott and Matsumura (1983) reported two types of pyrethroid action against cockroaches. Type I action was related to a factor which causes repetitive neuronal discharges, whereas type II action was related to suppressing repetitive discharge and causing immobility. The presence of an alpha-, may lead to type II action. Some pyrethroids with and without alpha-cyano moieties, such as type I and type II action (Scott and Matsumura 1983). This might explain why there was more tunneling in or underneath soils treated with fenvalerate and permethrin than there was underneath soil treated with bifenthrin or cypermethrin.

Factors causing repellency of termites to soils treated with insecticide are unclear. Su and others (1982) classified insecticides as type I or type II, according to behavioral responses of *C. formosanus*. Type I insecticides (e.g., pyrethroids) killed very few termites because the termites sealed or avoided tunnels that extended to treated areas, and therefore were considered repellent. Type II insecticide treatments (e.g., chlordane and chlorpyrifos) also killed few termites, but survivors avoided the treatment as dead and decaying termites accumulated in the tunnels, not in response to insecticide-treated media. Consequently, type II insecticides were not considered repellent.

In our studies with *R. hesperus* fenvalerate and permethrin performed like type I insecticides, killing very few termites and preventing tunneling in treated soil. The pyrethroids bifenthrin and cypermethrin, however, provided very different effects. They prevented tunneling by immobilizing the insects. Chlordane-treated soils performed as a type I insecticide, and chlorpyrifos did not elicit type I or type II responses. Termites tunneled into soils beneath the chlorpyrifos treatment, resulting in high mortality, but did not tunnel into the treated soil.

When soils were treated with high concentrations of all these termiticides, the amount of tunneling was negligible. Some tunneling occurred when soils were treated with lower concentrations. Biologically active amounts of insecticide move from the upper layers of treated soil to untreated soils below, causing reduced tunneling, mortality, and sublethal effects. This downward movement may increase the effective depth of the primarily surface applications of insecticides. Even though environmental exposure may reduce the initial levels (500 ppm) of soil treatments, it is likely that the soils will retain their repellency for a number of years. There is a considerable amount of research yet to be conducted on the factors that affect longevity and movement of insecticide in the soil, as well as the behavior of termites encountering these termiticide-treated soils.

#### Avoidance of Dead Termites

Workers of *R. hesperus* did not penetrate the layer of dead termites which were killed by exposure to chlorpyrifos, and there was 96 percent mortality from their tunneling efforts. Termites penetrated the layer of termites killed by freezing in 80 percent of the tubes and there was 30 percent mortality. In the untreated controls, workers tunneled to the top of all the tubes with only 5 percent mortality. The layer of termites killed with chlorpyrifos was not penetrated by *R. hesperus* workers; contact with the treated termites was enough to kill

significant numbers of termites within 24 h. The layer of "naturally" killed termites was not completely repellent to tunneling termites, but did produce some mortality. The appearance of dead and decaying termites may lead to the avoidance of treated areas as suggested by Su and others (1982). However, it may also be that toxicant on the dead termites also contributes to avoidance behavior in addition to mortality.

### Influence of Temperature on Tunneling

Regardless of acclimation periods, the tunneling rates of termites exposed to soil temperatures of 21, 27, and 32°C were not significantly different from the tunneling rates of unacclimated termites at these same temperatures. At 15°C, the distance tunneled by termites acclimated for 12, 24, 36, or 48 h was significantly greater than those tunneled by unacclimated termites (fig. 2).

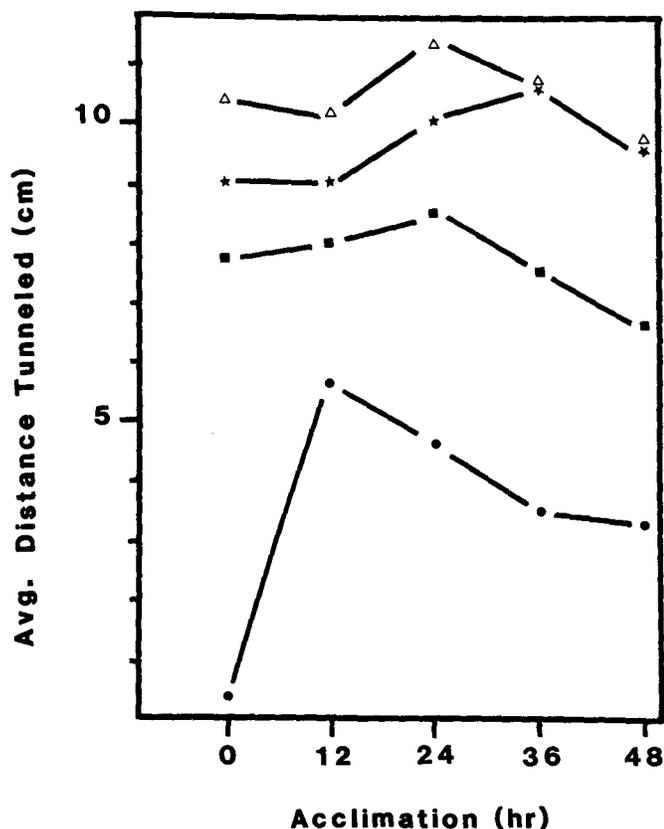


Figure 2--Average distance tunneled by *R. hesperus* at four different temperatures and five acclimation periods. Circle = 15.6°C, square = 21.1°C, star = 26.7°C, triangle = 32.2°C.

At 15°C, termite tunneling rates (0.1 cm/h) were significantly less than at 21, 27, or 32°C ( $F = 17.65$ ;  $df = 3$ ;  $P < 0.01$ ). At 21 and 32°C, there were no significant differences in termite tunneling rates (0.39 and 0.33 cm/h, respectively). Tunneling rates at 27°C were significantly greater than at 15 and 21°C ( $F = 70.58$ ;  $df = 2$ ;  $P < 0.01$ ) (fig. 3).

As termites forage from subterranean galleries where soil temperatures are relatively constant, worker termites will be exposed to elevated temperatures. The rates at which they tunnel will influence the time spent contacting treated soils and the time exposed to elevated and possibly lethal temperatures. The study suggests that termites leaving subterranean galleries do not require acclimation to the elevated surface temperatures, whereas returning termites may require time to acclimate to cooler subterranean conditions.

The above studies show that both abiotic and biotic factors affect the tunneling behavior of *R. hesperus*. Certain physical and chemical barriers have the ability to affect tunneling behavior most drastically. The potential effects of biotic factors such as temperature and of the appearance of dead termites in galleries on the performance of chemical barriers are evident but need further examination.

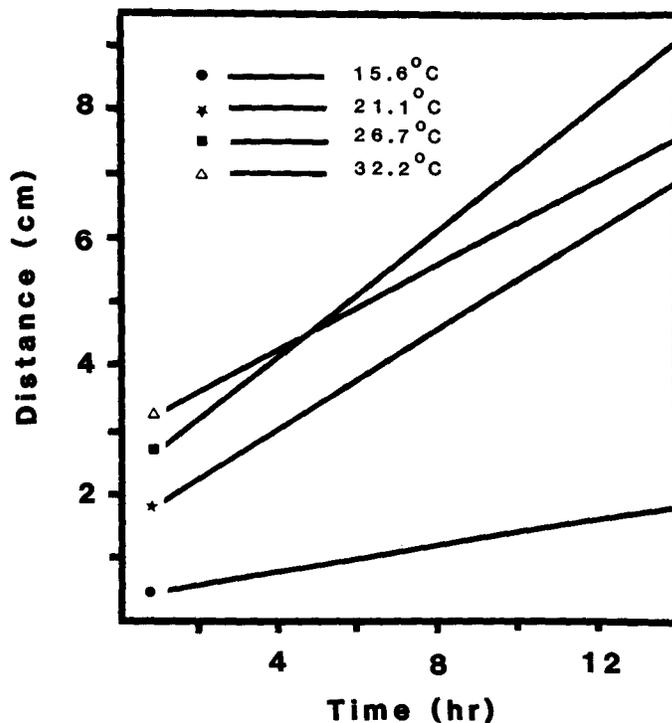


Figure 3--Regression lines of distance tunneled by *R. hesperus* at four different temperatures. 15.6°C,  $y = 0.44 + 0.10x$ ; 21.1°C,  $y = 1.46 + 0.39x$ ; 26.7°C,  $y = 2.21 + 0.49x$ ; 32.2°C,  $y = 2.98 + 0.33x$ .

## ACKNOWLEDGMENTS

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