

Influence of Guidelines and Passageways on Tunneling Behavior of *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) (Isoptera: Rhinotermitidae)

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Tunneling behavior of laboratory-maintained cultures of Reticulitermes flavipes (Kollar) and R. virginicus (Banks) was examined to determine (1) if the termites build tunnels along preexisting wires or tunnels, and (2) whether tunnels are arranged to optimize search efficiency. Tunnel patterns were considered optimal if, for the number of tunnels present, the maximum area was explored. Termites entered either control arenas or arenas in which they encountered a wire or a pre-formed tunnel. Analyses revealed that R. flavipes and R. virginicus almost always follow pre-formed tunnels, but do not follow wires as readily. Within each species, the distributions of tunnels in treatment arenas were different from distributions in control arenas, most often when pre-formed tunnels were the treatment. Optimal tunnel arrangements in control arenas were found in 62% of R. flavipes patterns with 2 tunnels and in 43% of R. virginicus patterns with 2 tunnels. None of the 3-tunnel patterns from control arenas of R. flavipes and 29% of those of R. virginicus had optimal arrangements. Overall, the spatial arrangement of tunnels in control arenas was significantly different between R. flavipes and R. virginicus.

KEY WORDS: *Reticulitermes flavipes*; *R. virginicus*; Rhinotermitidae; tunneling behavior; termites.

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INTRODUCTION

Termites are cryptic, eusocial animals that feed on wood and other material containing cellulose. Although they are most abundant in tropical regions, termites also occur in temperate regions (Michener and Michener, 1951; Robinson, 1996). In their natural habitats, termites are vital in the recycling of cellulose through the breakdown of vegetation (Michener and Michener, 1951). Only about 10% of the 2400 known termite species are pests (Robinson, 1996). In the United States alone, however, the four or five species of pestiferous termites found in urban and rural habitats cause structural damage costing over \$1 billion per year (Robinson, 1996). Despite their economic importance, there is still little information concerning the life history and sociobiology of termite colonies. This lack of understanding is partly due to the cryptic lifestyle and social organization of termites that makes them difficult to study. Therefore, more studies are needed for examining the social structure, foraging activity, and colony boundaries of subterranean termite colonies.

Some aspects of foraging or search behavior have been studied for several species of termites. An extensive examination of the underground gallery system of a *Macrotermes bellicosus* (Smeathman) colony in Africa revealed that the termites built many more foraging tunnels when the colony was in need of food (Lys and Leuthold, 1991). This is an example of how the needs of the colony may affect tunneling behavior. Southeast Asian species of *Macrotermes*, *Longipeditermes*, and *Hospitalitermes* follow preexisting guidelines found in the open field and respond to gravitational forces as they forage above ground in the open air without the cover of shelter tubes (Jander and Daumer, 1974). Studies of foraging activity and foraging population size have been performed with the subterranean termites, *Reticulitermes* spp. and *Coptotermes formosanus* Shiraki (Su and Scheffrahn, 1988; Su *et al.*, 1984, 1993; Haagsma and Rust, 1995; Forschler and Townsend, 1996). These studies employed wood-filled inspection ports or wooden stakes placed in urban areas in order to define foraging territories. Other termite studies have addressed tunneling response to physical obstacles (Goldberg, 1973, for *R. santonensis* Feytaud), termiticide barriers (Forschler, 1994, for *R. flavipes*), and the presence of a food source (Reinhard *et al.*, 1997, for *R. santonensis*) or vegetative cover (Jones *et al.*, 1987, for *Heterotermes aureus* Snyder)). Only one study thus far, however, has been focused on actual spatial patterns of search behavior of the eastern subterranean termite, *R. flavipes* (Kollar), in the absence of stimuli or barriers (Robson *et al.*, 1995).

Subterranean termites in the genus *Reticulitermes* occur in North America, Europe, and Asia and are the most economically important insect

pests in the United States (Robinson, 1996). There are at least 6 species of *Reticulitermes* in the U.S. (Weesner, 1965; Haverty *et al.*, 1996). Subterranean termites live in the soil and infest wood that is in contact with the soil. Sometimes they build shelter tubes above ground to travel to other sources of cellulose, such as the wooden structures of buildings (Robinson, 1996). *Reticulitermes* also occupy a diffuse and transient network of underground tunnels often connecting two or more feeding sites, making exact nest and feeding sites difficult to locate and differentiate.

Examinations of spatial patterns originating from nest sites of subterranean termites are difficult unless bioassays are performed in artificial arenas where termites and their tunnels are visible. Robson *et al.* (1995) used an upright "cell" that was filled with moistened sand in which termites could build tunnels. They found that patterns of termite tunnels were often arranged optimally, i.e., for the number of tunnels present, the maximum area of the foraging arena was explored.

In the present study artificial arenas, similar to those of Robson *et al.* (1995), were used to examine the spatial patterns of search behavior of *Reticulitermes flavipes* and *R. virginicus* (Banks). The primary objective of our study was to ascertain whether the presence of guidelines (i.e., wires) and passageways (i.e., impressions left by wires) positioned within arenas affects the tunneling behavior of termites. Furthermore, we wished to determine whether the two termite species differ in their tunneling behavior. As a consequence of the experimental design, it was also possible to examine the resulting tunnel patterns relative to random and optimal search patterns.

METHODS

Reticulitermes were collected in the summer and fall of 1997 from Termite D-Tecktor (Pestban Pest Control, Florida) detection devices placed near a residential building in Spalding County, Georgia. Soldier characteristics were used to identify the termites to species (Su and Scheffrahn, 1994). Termites were maintained inside clear, plastic containers: circular (dia. = 15 cm, $H = 4.5$ cm) or rectangular ($L \times W \times H = 27 \text{ cm} \times 20 \text{ cm} \times 10 \text{ cm}$). Moistened Whatman No. 1 filter paper, several slats of wood (approx. $12 \text{ cm} \times 3.7 \text{ cm} \times 0.3 \text{ cm}$), and sawdust were provided as food and shelter. Termite containers were kept in an environmental chamber in total darkness at a constant 25°C until used in this experiment. Three laboratory cultures each of *R. flavipes* and *R. virginicus* were used. Experiments were performed at the University of Georgia from 27 April 1998 through 22 July 1998 under ambient laboratory conditions.

Test Arena Assembly

Test arenas were constructed using two square pieces of Plexiglas (21.5 cm \times 21.5 cm; 3 mm thick). Plexiglas strips (1.2 cm wide, 3 mm thick) were affixed along the bottom and two sides (and not the top) of one of the two Plexiglas squares (Fig. 1). A hole (dia. = 0.5 cm) was drilled through the center of this same Plexiglas square. The Plexiglas squares were clasped together with six 5-cm binder clips (two clips on each side and two on the bottom) and were balanced upright on the flat sides of the two clips at the bottom. The center hole was plugged, and arenas were filled with 125 g of air-dried, sifted (3 mm mesh) sandy loam. Each arena was then laid flat by placing the Plexiglas square with the hole and edges against the countertop. Clips were removed, and the top (solid) Plexiglas square was carefully lifted and set aside. In order to moisten the soil in the arena, distilled water (17 ml) was released from a disposable pipette to distribute droplets uniformly onto the soil. Arenas were then reassembled and balanced upright. At least 20 min passed before an arena with newly wetted soil was

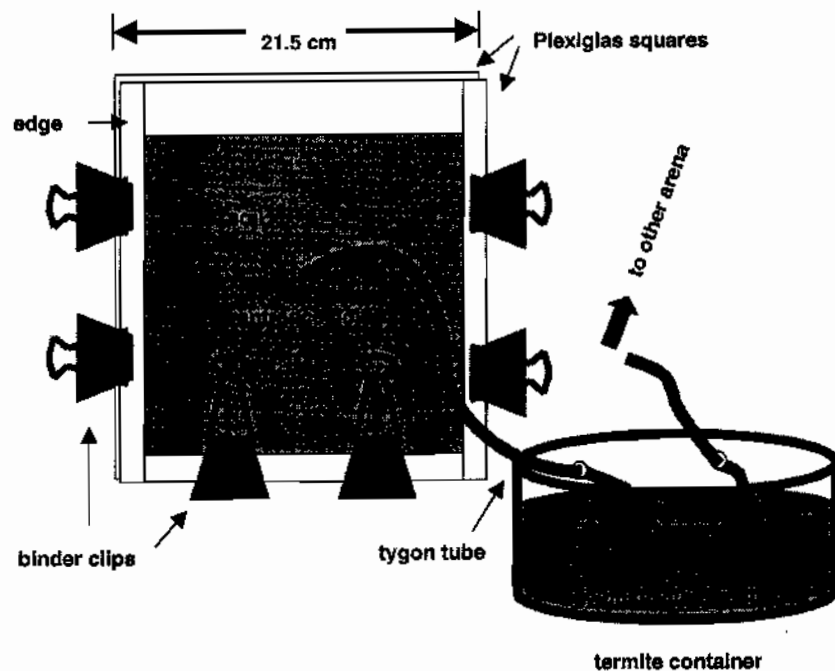


Fig. 1. Experimental design of foraging arenas for bioassays using *R. flavipes* and *R. virginicus*.

used in the bioassay. This delay allowed moisture from each water droplet to dampen the surrounding soil. A strip of Parafilm "M" (American National Can, Chicago, IL) was stretched across the top of the arena to reduce moisture loss and to confine any termites that ventured to this area during the bioassay.

Experimental Procedure

Placement of wires and formation of tunnels into the soil of the arenas in four different compass directions (North, South, East, and Southwest) constituted the eight treatments. To create an arena for a wire treatment, a copper wire (dia. = 1.5 mm, L = 9 cm) was placed in the arena while it was lying flat and before water was added to the soil. The soil covering the entrance hole was cleared at this time. The wire was carefully lined up along one of the four designated directions with one of its ends lying slightly inside the edge of the entrance hole (Fig. 2, dotted lines), and was gently pressed into the soil by replacing top Plexiglas square. To create a tunnel,

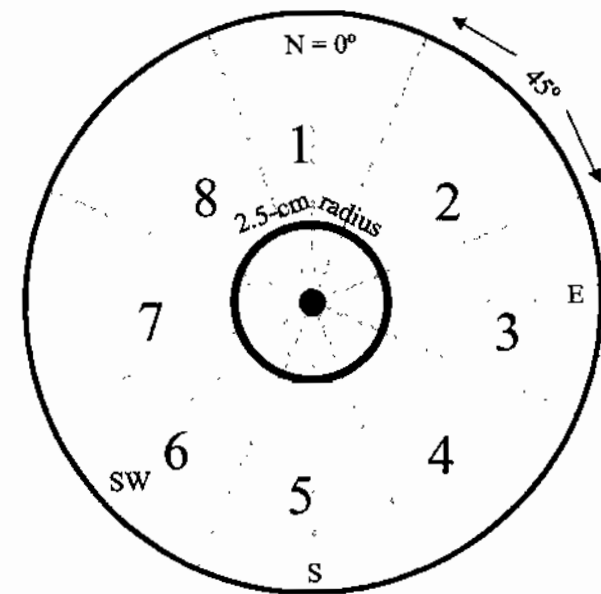


Fig. 2. Distribution of wedges (1-8) used for determining position of termite tunnels, and location of focal directions (N = north, S = south, E = east, and SW = southwest) within wedges.

the wire was placed into the arena as just described. After water had been applied, the wire was removed, leaving behind a well-formed tunnel of the same dimensions as the wire.

In order to get the termites from their culture and into the arena, tygon tubing (internal dia. = 3 mm) was used as a passageway between the plastic containers and the test arenas (Fig. 1). Two holes were melted into the tops of the plastic termite containers so that tubes ($L = 30$ cm for small containers; $L = 50$ cm for large containers) could be extended from inside the container, through the top of the container, and into the arenas via the entrance holes. In this manner, a treatment and a control arena could be used simultaneously with a tube running to each arena (Fig. 1). However, in some cases termites did not utilize both tubes. Therefore, when termites would only travel through one tube, treatment and control tests were performed on consecutive days, in random order.

Once termite containers were connected to the test arenas filled with moistened soil, termites were allowed to enter the arenas and remain undisturbed for 24 hr. After this time, the top of the arena was drummed firmly and repeatedly with the researcher's fingers so that the alarmed termites would abandon the arena by running out through the tube and back into the plastic containers. When all termites had left the arena, the tube was disconnected from the arena and pinched with a $\frac{1}{2}$ " binder clip to prevent termites from exiting the termite container. Tygon tubes remained with the plastic boxes. The top Plexiglas square was then removed from the arena, and tunnels made by the termites were traced onto clear transparency film. The entrance hole, wire or pre-formed tunnel, and edges of the arena were also traced as reference points. Between uses, the arenas were scrubbed in warm soap and water, and the wires were rinsed in 70% alcohol and then washed. Plexiglas tops and bottoms for the arenas were randomly matched with each other and randomly used for different treatments and controls.

At least three replications for each treatment and control were performed for each termite culture. In four instances, four replications were performed. The order of replication and treatment was randomly chosen. Termites tunneled in the treatment and control arenas simultaneously for 127 out of the 148 paired tests. However, only one of the six cultures (a *R. flavipes* culture) utilized both tubes at the same time for every replication of all eight treatments and respective controls.

Statistical Analyses

The transparency tracings of the tunnel patterns formed by the termites were photocopied onto paper so that the tunnel patterns from treatment

and control tests could easily be measured, analyzed, and compared. Data from the three cultures of each *R. flavipes* and *R. virginicus* were combined for each treatment and its respective control, resulting in 9 or 10 replications for each treatment. Analyses were performed for each species.

All treatment patterns were examined to determine whether or not termites constructed tunnels along wires or traveled through pre-formed tunnels. Termites were considered to have followed the wire or pre-formed tunnel only if they moved through the soil from the point of entry into the arena exactly alongside the wire or within the pre-formed tunnel for the total length of the wire or tunnel. Log-likelihood ratio tests were performed to determine if the termites followed the wires or tunnels significantly more often than by chance.

To make comparisons between each treatment and its respective control, a circle was divided into eight equal wedges (45° each) (Fig. 2) and was copied onto a transparency to use as an overlay. By using these eight divisions, each experimental direction (N, S, E, and SW) could be centered in one of the wedges (Fig. 2). The wedge in which a treatment direction occurred was regarded as the "focal wedge" for each respective treatment (Fig. 2). The transparency with the circle was placed over the tracings of each tunnel pattern. The center of the divided circle was aligned with the entrance hole of the test arena copied onto each tunnel pattern. All termite tunnels were then scored as occurring in the wedge in which they reached or crossed a 2.5-cm radius. For any tunnel pattern, if more than one tunnel passed the 2.5-cm radius in the same wedge, it was counted only one time. Likelihood ratio chi-square tests were used to determine if the distribution of tunnels among the 8 wedges in each treatment was different from its respective control.

In order to address whether foragers of *R. flavipes* or *R. virginicus* naturally prefer to tunnel in a particular direction, the 8 wedge distributions were considered as compass directions. Using Likelihood ratio chi-square analysis, wedge data results from all control arena tests were combined for each species to determine directionality of species-specific tunnel patterns in the absence of guidelines and passageways.

Circular statistics were performed on angular data. Tunnel angles were measured using the entrance hole of the vertical arena as the center point of reference and aligning a protractor such that 0° was in the 12-o'clock position (Fig. 2). Robson *et al.* (1995) found a positive correlation between angles measured at 1, 5, 10, and 20 cm along a tunnel from the point of origin. Thus, in order to standardize angle measurements in this study, the angle of each tunnel was measured by aligning the entrance hole with the point at which the tunnel crossed at a radius of 2.5 cm (Fig. 2). The angle reading was taken moving in a clockwise direction from 0° . Further

branching of tunnels that occurred beyond the 2.5-cm radius was not included. Rayleigh tests for determining one-sidedness were performed for each treatment and control, and for all controls combined (Batschelet, 1981; Fischer, 1993). Mean angles and angular deviations were also calculated (Batschelet, 1981, 1965).

And finally, an analysis using binomial probability was performed to determine if termites constructed tunnels in arrangements that denote optimal search behavior as described by Robson *et al.* (1995). An optimal pattern would be one that minimized search overlap and maximized the area searched based on the number of tunnels built. An optimal pattern for two tunnels originating from the entrance hole would have the two tunnels occurring in opposing directions (180° apart). When there were three tunnels, the tunnels would be spaced at 120° intervals. And four tunnels would occur at 90° intervals. For this analysis, a circle was divided into 12 equal sectors (30° each). Therefore, the probability (p) of 2 tunnels lying in opposing sectors is $\frac{1}{12} \times \frac{1}{12} = 0.0833$, because the first tunnel could be in any sector, but the second could occur in only one other, i.e., the opposing sector. For a 3-tunnel system, the probability of an optimal arrangement is $\frac{1}{12} \times \frac{1}{12} \times \frac{1}{12} = 0.0139$ (Robson *et al.*, 1995). Each pattern tracing was aligned with one tunnel lying in one of the 12 sectors, which then served as a reference point. The position of the other tunnels was determined from this reference tunnel. The frequency of the occurrence of the optimal pattern (k) was recorded for each tunnel system. The expected frequency of a pattern with the given probability (P) occurring k times out of the total number of observations (K) was calculated by $P = p^k (1-p)^{K-k}$ (Robson *et al.*, 1995). Using this expected frequency (P), a binomial probability (P) was calculated as: $P = P \times [k! / K! (K-k)!]$ (Ott, 1988). Resulting P values were compared to a critical value of $P < 0.05$.

RESULTS

In most individual tests, termites constructed 3 or fewer tunnels; in rare cases they built 4–6 tunnels. In 73 of 74 tests with pre-formed tunnels, termites followed the entire length of the tunnels (the exception being a test with *R. flavipes*, South Tunnel treatment). Termites followed the entire length of the wire in 22–80% of the wire treatments (Table I). The number of tests in which *R. flavipes* followed the East Wire (80%) was significantly higher than the number of tests in which the termites did not follow the wire (expected frequency = 0.5). *R. virginicus* also followed the wire most often (56%) when the wire was in the East position. The North and South-

Table I. Number of *R. flavipes* and *R. virginicus* that Followed Entire Length of Wires Placed in Four Compass Directions in Foraging Arenas

	Wire direction				Total
	North	South	East	Southwest	
<i>R. flavipes</i>					
No. tests	9	10	10	9	38
No. that followed	3	4	8	3	18
Test statistics ^{a,b}	$\chi^2 = 1.02$ $P < 0.9$	$\chi^2 = 0.40$ $P < 0.9$	$\chi^2 = 3.85$ $P < 0.05$	$\chi^2 = 1.02$ $P < 0.9$	$\chi^2 = 0.11$ $P < 0.9$
<i>R. virginicus</i>					
No. tests	9	9	9	9	36
No. followed	2	4	5	4	15
Test statistics ^{a,b}	$\chi^2 = 2.94$ $P < 0.1$	$\chi^2 = 0.11$ $P < 0.9$	$\chi^2 = 0.11$ $P < 0.9$	$\chi^2 = 0.11$ $P < 0.9$	$\chi^2 = 1.0$ $P < 0.9$

^a $df = 1$ for all tests.

^bLog-likelihood ratio tests were used for each direction; chi-square analyses were used for total values.

west Wires were followed least often by *R. flavipes* (33%), and the North Wire was followed least by *R. virginicus* (22%) (Table I).

The distributions of *R. flavipes* tunnels in treatment tests were significantly different from the distribution in controls in the South, East, and Southwest Tunnel treatments (Table II). The distributions of *R. virginicus* tunnels in East Wire, and East and Southwest Tunnel treatments, were significantly different from the distributions in respective controls (Table III).

Examination of number of tunnels in each wedge in all control tests combined for each species revealed that the two *Reticulitermes* species differed significantly in tunnel distribution ($N = 303$, $\chi^2 = 14.808$, $df = 7$, $P = 0.039$) (Fig. 3). The control tunnel patterns of both *R. flavipes* and *R. virginicus* showed that the highest proportions of tunnels were directed toward the west (wedge 7) (24.7% and 23.3%, respectively) (Fig. 3). Both species also constructed tunnels toward the east (wedge 3) (20.7% and 16.3%, respectively) in relatively high proportions. However, *R. virginicus* tunneled more southward (wedge 5) (17.1%) than eastward, while *R. flavipes* excavated only 6.9% of the tunnels southward (Fig. 3). The least traveled directions were north for *R. flavipes* (2.3%) and northeast (wedge 2) and northwest (wedge 8) for *R. virginicus* (6.2%) (Fig. 3). The difference in tunnel distribution between species is apparently due to the proportion of tunnels in the directly north and south directions (wedges 1 and 5, respectively). When either of these variables is removed from analysis, no significant difference is detected in tunnel distribution between species. The proportion of tunnels lying in wedge 1 and 5 for *R. virginicus* are at least 2–3 times higher than the proportion in these wedges for *R. flavipes*.

One reason for this difference is that one of the *R. virginicus* cultures excavated nearly $\frac{1}{2}$ its tunnels downward (14 out of 39 tunnels). The other *R. virginicus* cultures had 4 out of 37 tunnels and 4 out of 53 tunnels directed downward.

Circular statistics for *R. flavipes* revealed uniform (or random) distributions of tunnels except for patterns resulting from tests with the South Tunnel, Southwest Tunnel, and all the controls combined (Tables IV). The mean angle of all controls revealed a tendency of *R. flavipes* to build tunnels in a southern direction (183.89°) (Table IV). Tests using *R. virginicus* revealed that the tunnel patterns from only the East Tunnel treatment and all the controls combined were significantly different from random (Table V). For this species, the mean angle for all controls was in a southwestern orientation (215.37°) (Table V).

The binomial probability for all of the 2-tunnel patterns from *R. flavipes* controls combined revealed a significant occurrence (62%) of optimal patterns (Table VI). Separate analyses of *R. flavipes* controls from each treatment category were all significant except the control tests conducted in conjunction with the North and Southwest Tunnel treatments (Table VI).

Table II. Distribution of *R. flavipes* Tunnels into Eight 45° Wedges (Fig. 2) of Foraging Arenas for Treatments with Wires or Tunnels and Their Respective Controls

Treatment (no. tests)	No. tunnels in wedges of foraging arena ^a								Total tunnels	Log-likelihood test
	1	2	3	4	5	6	7	8		
North Wire (9)	4	1	4	3	2	2	3	1	20	$G = 9.8, df = 7$
Control	0	3	3	1	2	2	6	3	20	$P = 0.20$
South Wire (10)	0	2	4	1	6	3	3	2	21	$G = 6.8, df = 6$
Control	0	0	6	3	2	3	5	2	21	$P = 0.34$
East Wire (10)	1	1	8	0	1	4	2	5	22	$G = 13.3, df = 7$
Control	0	1	7	4	0	2	7	2	23	$P = 0.07$
Southwest Wire (9)	1	1	5	1	1	5	3	1	18	$G = 2.7, df = 7$
Control	1	1	5	4	3	5	5	3	27	$P = 0.92$
North Tunnel (9)	9	1	2	5	2	4	3	1	27	$G = 12.5, df = 7$
Control	2	2	7	2	0	4	3	0	20	$P = 0.08$
South Tunnel (10)	1	1	0	0	10	0	0	1	13	$G = 29.2, df = 7$
Control	0	4	4	4	2	3	6	2	25	$P = 0.001$
East Tunnel (9)	0	0	9	0	1	1	3	1	15	$G = 13.5, df = 6$
Control	1	2	3	3	2	3	6	0	20	$P = 0.04$
Southwest Tunnel (9)	0	0	4	2	0	9	1	1	17	$G = 21.7, df = 6$
Control	0	2	1	7	1	1	6	0	18	$P = 0.001$

^aFocal wedges are indicated in boldface.

Table III. Distribution of *R. virginicus* Tunnels into Eight 45° Wedges (Fig. 2) of Foraging Arenas for Treatments with Wires or Tunnels and Their Respective Controls

Treatment (no. tests)	No. tunnels in wedges of foraging arena ^a								Total tunnels	Log-likelihood test
	1	2	3	4	5	6	7	8		
North Wire (9)	5	1	1	1	4	4	3	0	19	$G = 7.8, df = 6$
Control	1	2	5	2	2	3	5	0	20	$P = 0.25$
South Wire (9)	3	1	3	4	4	3	1	1	20	$G = 5.5, df = 7$
Control	1	1	4	2	1	3	3	0	15	$P = 0.60$
East Wire (9)	1	0	8	0	3	2	3	0	17	$G = 14.0, df = 6$
Control	1	0	1	2	5	0	5	1	15	$P = 0.03$
Southwest Wire (9)	0	1	4	2	1	6	2	1	17	$G = 9.9, df = 7$
Control	2	0	2	2	2	1	3	0	12	$P = 0.20$
North Tunnel (10)	10	0	1	2	3	2	2	0	20	$G = 13.9, df = 7$
Control	2	3	1	1	3	3	4	2	19	$P = 0.05$
South Tunnel (9)	3	0	1	0	9	0	0	1	14	$G = 11.7, df = 6$
Control	1	1	2	0	4	1	4	1	14	$P = 0.07$
East Tunnel (9)	3	0	9	0	2	1	2	0	17	$G = 16.9, df = 7$
Control	1	1	2	3	1	3	2	3	16	$P = 0.02$
Southwest Tunnel (9)	4	1	3	0	0	9	0	1	18	$G = 22.0, df = 7$
Control	1	0	3	2	4	2	4	1	17	$P = 0.003$

^aFocal wedges are indicated in boldface.

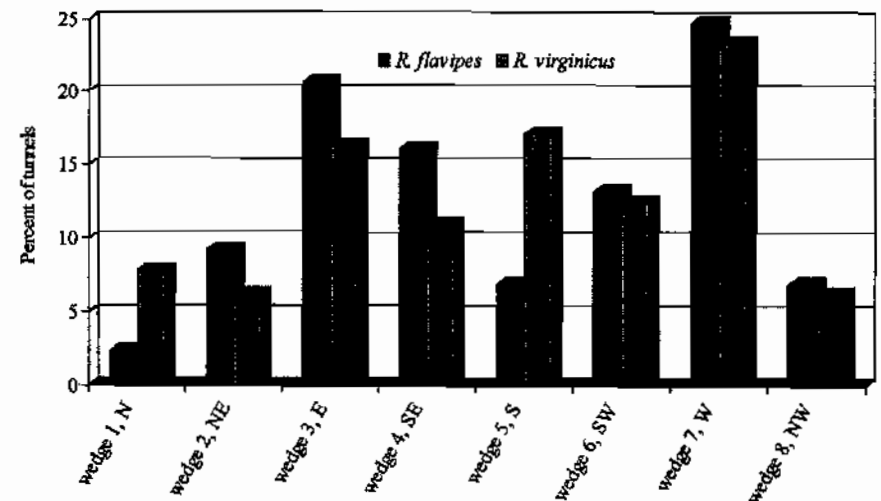


Fig. 3. Percent of tunnels from all control patterns of *R. flavipes* ($N = 174$) and *R. virginicus* ($N = 128$) observed in each of eight wedges representing different compass directions.

Table IV. Circular Statistics for Angles from Tunnel Patterns of Treatments and Controls of *R. flavipes* Foraging Bioassays

Treatment (no. of angles)	Mean vector length	Mean angle \pm angular deviation	Rayleigh test
North Wire (22)	0.10	317° \pm 77°	ns
Control (24)	0.30	85° \pm 68°	ns
South Wire (21)	0.31	168° \pm 67°	ns
Control (22)	0.25	179° \pm 70°	ns
East Wire (28)	0.07	316° \pm 78°	ns
Control (24)	0.21	138° \pm 72°	ns
Southwest Wire (22)	0.20	207° \pm 72°	ns
Control (29)	0.22	208° \pm 71°	ns
North Tunnel (30)	0.06	328° \pm 79°	ns
Control (20)	0.27	93° \pm 69°	ns
South Tunnel (14)	0.48	168° \pm 58°	$P < 0.05$
Control (28)	0.06	145° \pm 78°	ns
East Tunnel (15)	0.33	98° \pm 66°	ns
Control (23)	0.14	196° \pm 75°	ns
Southwest Tunnel (18)	0.49	207° \pm 58°	$P < 0.05$
Control (21)	0.26	182° \pm 70°	ns
All controls (191)	0.13	183.89° \pm 75.58°	$P < 0.05$

Table V. Circular Statistics for Angles from Tunnel Patterns of Treatments and Controls of *R. virginicus* Foraging Bioassays

Treatment (no. of angles)	Mean vector length	Mean angle \pm angular deviation	Rayleigh test
North Wire (21)	0.25	241° \pm 70°	ns
Control (19)	0.05	184° \pm 79°	ns
South Wire (23)	0.31	167° \pm 67°	ns
Control (16)	0.18	188° \pm 73°	ns
East Wire (17)	0.36	133° \pm 65°	ns
Control (15)	0.35	207° \pm 66°	ns
Southwest Wire (21)	0.30	199° \pm 66°	ns
Control (12)	0.15	205° \pm 75°	ns
North Tunnel (20)	0.24	338° \pm 70°	ns
Control (19)	0.18	263° \pm 73°	ns
South Tunnel (15)	0.42	185° \pm 62°	ns
Control (15)	0.20	237° \pm 73°	ns
East Tunnel (15)	0.52	86° \pm 56°	$P < 0.05$
Control (18)	0.06	243° \pm 79°	ns
Southwest Tunnel (17)	0.18	235° \pm 74°	ns
Control (19)	0.20	197° \pm 73°	ns
All Controls (133)	0.15	215.37° \pm 74.70°	$P < 0.05$

Table VI. Number of Optimal Search Patterns When *R. flavipes* Built only 2 Tunnels in Foraging Arenas

Treatment	Total no. of patterns	No. of optimal patterns	P value ^a
North Wire	2	0	0.84
Control	4	3	2.0×10^{-3}
South Wire	5	2	0.05
Control	8	4	2.0×10^{-3}
East Wire	5	3	5.0×10^{-3}
Control	5	5	4.0×10^{-6}
Southwest Wire	3	0	0.77
Control	6	4	6.0×10^{-4}
North Tunnel	1	0	0.92
Control	6	2	0.07
South Tunnel	5	3	5.0×10^{-3}
Control	7	5	7.1×10^{-5}
East Tunnel	6	5	2.2×10^{-5}
Control	5	3	5.0×10^{-3}
Southwest Tunnel	2	0	0.84
Control	1	0	0.92
All Controls	42	26	3.6×10^{-18}

^a P value obtained from tests of the binomial probability.

Analyses for each *R. flavipes* treatment revealed that 2-tunnel patterns from East Wire and South and East Tunnel treatments had in a significant number of optimal arrangements (Table VI). Of the *R. virginicus* 2-tunnel control patterns combined, a significant 43% were in optimal arrangements (Table VII). When analyzed separately according to treatment category, only the patterns from *R. virginicus* controls conducted along with the East Wire, Southwest Wire, and East Tunnel had significant numbers of optimal arrangements (Table VII). The treatments of South Wire and South and East Tunnel also yielded significant numbers of optimal patterns. Analyses of 3-tunnel patterns for *R. flavipes* (treatments = 18; controls = 21) revealed a significant number of optimal patterns only for the treatment with North Wire (2 out of 3, $P = 0.02$). None of the *R. virginicus* treatments with 3-tunnel patterns ($N = 20$) had significant numbers of optimal patterns. However, a significance value was obtained for the controls conducted along with the South Tunnel (2 out of 2, $P = 0.007$) and all of the *R. virginicus* controls combined (4 out of 14, $P = 0.02$).

DISCUSSION

The subterranean termites *R. flavipes* and *R. virginicus* tend to take advantage of the presence of guidelines (wires) and passageways (tunnels)

Table VII. Number of Optimal Search Patterns When *R. virginicus* Built only 2 Tunnels in Foraging Arenas

Treatment	Total no. of patterns	No. of optimal patterns	P value ^a
North Wire	2	0	0.84
Control	5	2	0.05
South Wire	5	3	5.0×10^{-3}
Control	8	2	0.12
East Wire	6	2	0.07
Control	8	5	2.0×10^{-4}
Southwest Wire	5	1	0.29
Control	5	3	5.0×10^{-3}
North Tunnel	3	1	0.21
Control	5	1	0.29
South Tunnel	3	2	0.02
Control	6	2	0.07
East Tunnel	4	3	2.0×10^{-3}
Control	5	3	5.0×10^{-3}
Southwest Tunnel	5	2	0.05
Control	5	2	0.05
All Controls	47	20	2.4×10^{-10}

^aP value obtained from tests of the binomial probability.

as they forage through soil (Tables I–III). Our experimental treatments encouraged the termites to excavate tunnels in directions that they did not tend to go towards in controls (Tables II and III) (Fig. 3). *Reticulitermes flavipes* traveled along almost half (47%) of the wires that they encountered, and traveled through 97% of the pre-formed tunnels (Table I). Likewise, *R. virginicus* followed 42% of the wires and 100% of the pre-formed tunnels (Table I). It appears that the termites were most likely to follow wires if they were oriented parallel to the horizon (east). This direction for tunnel building may be a natural orientation for these subterranean termites.

Our results demonstrate that guidelines and passageways in the soil can affect the tunneling behavior of subterranean termites. Work of Jander and Daumer (1974) also found that termites foraging in open-air follow guidelines in the field, albeit these termites forage aboveground. Laboratory termites follow along the sides of a container unless obstacles are placed in their paths (Goldberg, 1973). In our experiment, however, the wires were not obstacles or barriers, and the termites could begin tunnel building at any point along the circumference of the entrance hole to the foraging arena.

From a biological point of view, perhaps taking the easy route by following a guideline or pre-formed pathway may optimize termite search

efficiency. Suppose that the wire encountered by the termites in our bioassay were a root or fallen limb, and that a pre-formed tunnel were the impression of a decomposed root or an old cavity where a worm had burrowed. Following a root or existing tunnel may expedite the discovery of sources of cellulose in the soil or at the soil surface. Using this strategy, the colony would reduce the amount of time and energy exerted in digging through the soil, while increasing the likelihood of finding a valuable resource.

Termites may encounter the tunnels of other termites that are still in use or have been abandoned. Entering the active tunnels of conspecifics or other species may lead to aggressive encounters. Although such confrontations may be costly, winners may reap the reward of access to a valuable resource at the end of the tunnel. It would be interesting to investigate how readily termites will enter tunnels made by other termites, depending on the species or colony that made the tunnel, the age of the tunnel, or whether other termites were present in the tunnel.

Considering only the controls, termites constructed more tunnels directed along the horizontal (toward the east and west) and less tunnels heading north (Fig. 3). The paucity of tunnels directed upward is reflected by the fact that the mean angle for the combined controls for each species in south and southwest directions for *R. flavipes* and *R. virginicus*, respectively. Although both termite species tended to build tunnels in downward directions, *R. flavipes* built less than half as many tunnels directly down (6.9%) as did *R. virginicus* (17.1%), which accounts for the statistically significant difference between the two species. However, the apparent statistical difference in tunneling behavior between species may be an artifact of the behavior of only one culture. Further comparison of tunneling behavior between the two species is necessary before concluding that *R. virginicus* orients tunnels downward more than *R. flavipes*.

In view of the fact that termites in this study showed an overall tendency to spread out in horizontal and downward directions more than they did in upward directions (Fig. 3), it is possible that they respond positively to gravity. Work by Becker (1981, 1989) concludes that the general direction of tunnel building of the rhinotermitid termite *Heterotermes indicola* (Wasmann) is influenced by magnetic and electric fields, while other biological factors are also important. We did not account for such abiotic factors as geomagnetic or electric fields. Future investigations to address this tendency to move downward might include rotating or tilting vertical arenas or platforms, as did Jander and Daumer (1974). Also, using horizontal arenas rather than vertical ones would eliminate the ability of the termites to move in a downward direction.

Circular statistical analysis of the angles of the termite tunnels showed uniform or random distribution of tunnels in the arenas. This indicates that

even when the termites were influenced to create tunnels in a treatment direction, other tunnels were spread out around the arena. In only 3 treatments (*R. flavipes*: South and Southwest Tunnel; *R. virginicus*: East Tunnel) were the angles significantly clustered in a "homing" direction. The uniformity of tunnels may represent the ability of the termites to minimize search overlap. However, once all angles from control sets were combined for each species, circular analyses then revealed a statistical difference from uniformity. The analyses of the control patterns, therefore, appear contradictory. Using larger sample sizes (N) in these combined data sets allowed for the detection of differences that were not apparent with the smaller sample sizes. Thus, we must consider the mean angles and the patterns and trends that we observed in our other statistical analyses. We can then conclude that the combined data reflects the tendency of the termites to avoid moving upward and to prefer to tunnel horizontally and downward. Such a tendency would yield the overall nonuniform circular distribution of angles that we found.

Our examination of termite tunneling behavior did not include recording the sequence of tunnel building over time. Thus, the evaluation of optimal patterns after 24 hr of tunnel building assumes that the termites made a "decision" at the beginning of the time period about how many tunnels they were going to build and where to build them. Nonetheless, optimal tunnel arrangements were revealed in many of the 2-tunnel patterns, with more occurring in the *R. flavipes* tests than in the *R. virginicus* tests (Tables VI and VII). There were fewer optimal patterns when 3 tunnels were constructed. However, suppose a 3-tunnel pattern had originated as 2 tunnels in opposing directions, and then the third tunnel was added later. The third tunnel may have been built in a direction perpendicular to the opposing tunnels, the most "optimal" position in this case. Yet, our method of evaluation does not take into consideration such an optimal maneuver. Therefore, a better model that calculates strategic moves based on previous ones would be a more informative tactic for evaluating optimal search patterns.

For our purposes, the evaluation of optimal distribution of tunnels allowed us to determine that (in some cases) even when termites were influenced to follow a treatment direction, they continued to create tunnel arrangements that were considered optimal (Tables IV and V). Robson *et al.* (1995) also found that *R. flavipes* built optimal patterns using sand-filled arenas. In the absence of any stimuli in the environment, such as moisture gradients, food odors, or trail pheromone, termites may have an innate tendency to construct search patterns that can be considered optimal. Optimality theory assumes that "natural selection is an optimizing agent, favouring design features of organisms which best promote an individual's propa-

gation of copies of its genes into future generations" (Krebs and Davies, 1997). Accordingly, organisms displaying optimality would have behavior patterns that are considered efficient. For social animals that must leave the nest in search of food and new nest sites, such as termites, the adoption of an optimal foraging strategy would minimize the energetic costs to the colony. For example, foraging behavior would be optimal when it minimizes the amount of energy exerted by the colony to get to resources, when it increases the success rate of finding resources (especially resources of high quality), and when it reduces the risk of predation and parasitism or exposure to pathogens. The termites in this study were tested in a two-dimensional environment. In their natural three-dimensional world, such uniform or optimal patterns may not exist at all or may not occur in the same frequency as in our laboratory examination.

There are certainly many cues that are used by termites in the natural world to assist them in locating cellulose. The tactile and chemical signals that termites encounter may alter the innate behavior that regulates them in the absence of such signals. This experiment was designed to minimize the affect of any chemical cues, while exploring the affects of tactile ones. Future studies might investigate the affects of both tactile and chemical cues on the search behavior of termites and on their ability to locate food in a cost-efficient manner.

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