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Application of Fractal Analysis for Tunnel Systems of Subterranean Termites (Isoptera: Rhinotermitidae) Under Laboratory Conditions

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ABSTRACT We used fractal geometry to analyze the morphology of the tunneling system of two subterranean termite species, *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki, and to evaluate the effect of termite species, and the presence of wood on the degree of intricacy of the tunnels represented by the fractal dimension (D), and on the abundance of tunnels ($\log K$). The differences in D and $\log K$, before and after termites reaching a testing chamber, were also examined. Results indicated that termite tunneling systems have a fractal structure because $-2 < D < -1$. The tunnel fractal dimension (D) was not significantly different between *C. formosanus* and *R. flavipes,* before or after reaching a testing chamber, suggesting that *C. formosanus* and *R. flavipes* created tunnels with the same degree of intricacy at all time periods. The abundance of tunnels, $\log K$, was higher before reaching a testing chamber, while termites were searching for food, than after regardless of the presence of wood or the species of termite introduced in the arenas. The utility of tunnel fractal dimension deserves further study, because it may provide new ways for understanding the functional implications of the branching patterns of termite tunnels in relation to optimum soil exploration by termites.

KEY WORDS termite tunneling, tunneling geometry, *C. formosanus, R. flavipes*

THE SUBTERRANEAN TERMITES of the genus *Reticulitermes* and *Coptotermes* are abundant in several geographic zones and some are serious threat to wood in structures wherever they occur (Su et al. 1993). The habit of these subterranean termites to build a net of numerous exploratory runways or tunnels has been observed by Williams (1977), and apparently is part of a systematic search for food. Quantification of the morphology of the tunneling system, as branching grows, is important in analyzing the progress of the tunneling. In spite of the economic impact that the tunnel construction has on dwellings, little is understood about the morphology and architecture of termite tunnels. Studies of subterranean termite tunnels have lagged behind because of the tedious and time-consuming labor involved in the quantifying and observing the tunnels in their natural environment. Wooden structures already attacked by termites, need to be destroyed to determine the branching pattern and the collection of information related to the tunneling system becomes an almost impossible task.

The calculus of fractals has been used in recent years to describe the morphology of plant root systems (Tatsumi et al. 1989, Fitter and Stickland 1991, Eghball et al. 1993, Lynch and van Beem 1993, Pages et al. 1993, Masi and Maranville 1998). Fractal analysis can be applied to a wide range of problems (Mandelbrot 1983) including the shapes of sea coasts, mountains and rivers, the intricacy of these shapes being quantified by fractal dimensions (D). A fractal is a set whose Hausdorff-Besicovich dimension exceeds the topological dimension (Mandelbrot 1983).

Fractal dimension refers to the existence of self-similarity arising from repeated iteration of a structural unit (Bernston 1994, 1996). The geometry of a root system is similar to the branching of the tunnels of subterranean termites, but fractal analysis has never been applied to termite tunneling systems. The advantage of applying fractal analysis to the study of termite tunneling systems is its relative simplification in estimating the fractal dimension, D. Consequently, information on the effect of a specific factor (such as the presence of wood) on tunnel construction is achieved faster than through the traditional routes of counting and measuring the length of the tunnels built by the termites.

The objectives of this study were to evaluate the tunneling system of two subterranean termite sp., *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki, by fractal analysis, and to examine the suitability of fractal analysis to describe the tunneling system of subterranean termites in the presence of wood.

Materials and Methods

In this investigation we used drawings taken from tunneling samples developed in artificial arenas and constrained our analysis of tunneling behavior to near two dimensions, even though the tunneling system of termites develops three-dimensionally in the soil.

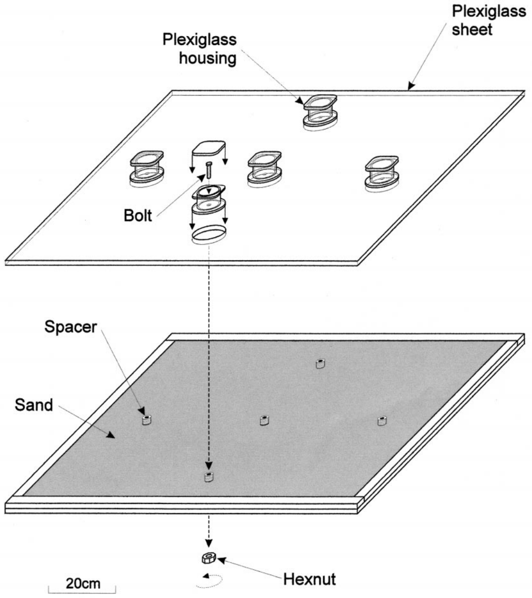

Fig. 1. Experimental arena constructed of two sheets of transparent Plexiglas, separated from each other by Plexiglas laminas placed between the outer margin.

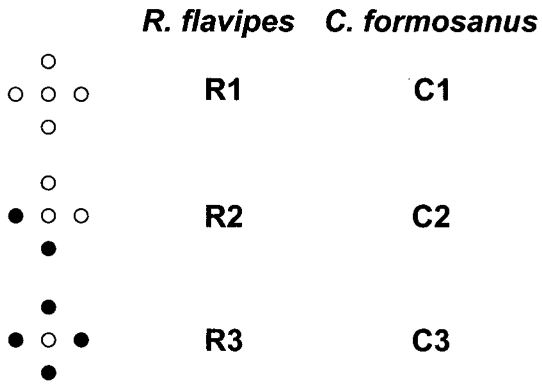

Fig. 2. Distribution of wooden discs among four testing chambers for each experiment. (O) Represents a testing chamber without wood.

Five foraging groups of the termites *R. flavipes* and *C. formosanus*, were collected from different field colonies in Broward County, FL, using the method described by Su and Scheffrahn (1986).

Termite Tunneling in Foraging Arena. Experimental arenas were constructed of two sheets of transparent Plexiglas (105 by 105 cm^2) separated from each other by four Plexiglas laminas (105 by 2.5 by 0.2 cm^3) placed between the outer margins (Fig. 1). The upper sheet had five clusters of four access holes or chambers each (1 cm diameter). Each cluster of access holes was fitted with Plexiglas cups with lids creating five chambers. Four of these chambers were equidistantly separated 30 cm from a center chamber. The center chamber was used to release termites and the other four represented potential foraging destinations or testing chambers (TC1, TC2, TC3, and TC4). The perimeters of the experimental arenas were held together with binder clips.

The 0.2 cm gaps between the Plexiglas sheets were filled with 2.1–2.4 kg of sifted sand (150–500 μm sieves; Play Sand Bonsal) and moistened with 500–600 ml of deionized water. Some of the testing chambers were left empty or received a wooden disc (*Picea* sp., 6.5 diameter, 0.3 thick cm^2; this type of wood has no attractive or repellent effect on termites) (Fig. 2). The discs were secured in the center of each hole by a screw (1-cm-diameter hole). The mean ± SD dry weight of each wooden disc was 2.2166 ± 0.1995 g.

After each experiment was run, three samples of moist sand were collected from each experimental arena, weighted, and dried for 24 h at 38°C, and reweighted to estimate the approximate moisture content in the sand, which ranged from 21 to 27%.

Wooden sticks of 3 cm were placed in the center chamber, followed by the release of 1,000 workers (undifferentiated larvae of at least the third instar) plus 100 soldiers or 10 soldiers for *C. formosanus* and *R. flavipes*, respectively, to simulate the approximate soldier field proportions of these species (Su and La Fage 1987; Su and La Fage 1999). Six experiments were run simultaneously, three each for *R. flavipes* (R1, R2, and R3), and three for *C. formosanus* (C1, C2, and C3, Fig. 2). Experiments R1 and C1 represented control conditions and no wood (0%) was added to any of the four testing chambers. Two testing chambers (TC3 and TC4) for *R. flavipes* (R2) and *C. formosanus* (C2) were filled with wooden discs (50% of testing chambers with wood, representing a total of 4,433.27 g. of wood in the arena), and the other two chambers contained sand. In experiments R3 and C3 of *R. flavipes* and *C. formosanus*, respectively, all four testing chambers were filled with wooden discs (100% of testing chambers full, representing an equivalent of 8,866.53 g. of total wood in the arena). All the experiments were conducted with the arenas in the horizontal position in a dark room at 27°C. Five replicates of each experiment were conducted ($n = 3$ treatments × 5 colonies × 1 replicate/colony = 15 experimental units) for each termite species.

Termite tunnels were recorded daily for ≈10 d, when termites reached the edges of the arenas. Tunnels were traced daily using different colors on a transparent plastic sheet placed on top of the experimental arenas. A digital photograph was taken of each of the resulting tunnels and the digital images were downloaded into the computer for later analysis (Picture Easy, Kodak Digital Science, Charlotte, NC).

Comparison of Tunneling by *C. formosanus* and *R. flavipes*. Data for each of the six experiments (C1, C2, C3, R1, R2, and R3) were further divided into two groups: before and after termites reached the testing chamber. The fractal dimension, *D*, and the abundance of tunnels, log *K*, were calculated for each experiment (6) and grouping (2) combination; totaling 12. We made comparisons among the six experiments to detect if the presence of wood and the time to reach the testing chambers affected the fractal dimension (D), or the abundance of tunnels (log K); and to determine if these two parameters differed between the two species of subterranean termites. Test of homogeneity was performed on the data to determine if the regression coefficients were homogeneous. If homogeneity was not established, a Kruskal–Wallis analysis of variance was used to detect if differences existed among the mean values of the fractal dimension, D, or the abundance of tunnels, log K. An approximate test of equality of means of the estimated slopes (fractal dimension, D), and the estimated abundance of tunnels, log K, was used to compare pairs of means for all the experiments (Sokal and Rohlf 1981).

Data Analysis and Statistical Methods. The method used in this study for fractal analysis (Maldebrot 1983) has been used successfully to quantify the root system morphology when root branching occurs (Tatsumi et al. 1989). To analyze the data with this model, we transformed the traced tunnels into black and white (Corel Photo Paint 9; Corel, Ottawa, Ontario, Canada); and the computer images of the arenas were divided into $(1/r)^2$ square grids of side r (Fig. 3), and the number of N (r) squares that were intersected by the tunnels constructed by the termites were counted (SPSS 1999). Three sizes of r (1.056, 2.112, and 4.356 cm) were tested. The values of log [N (r)] were plotted against log (r). When measuring N (r) at small values of r, if a straight line with negative slope (-D) is obtained, the interpretation is that the object is fractal and D is the fractal dimension ($1 < D < 2$) because

$$log\,[N\,(r)] \approx -D \log (r) + \log K,$$

where K is a constant.

Higher values of D indicate that the tunnels are touching more grid squares and therefore, D represents the degree of intricacy of the tunnels, i.e., greater branching structures that are more complex and greatly branched in contrast to those with lesser values which may be expected to be simpler and with less branching (Tatsumi et al. 1989). Higher values of log K indicate that more branches exist in the arena.

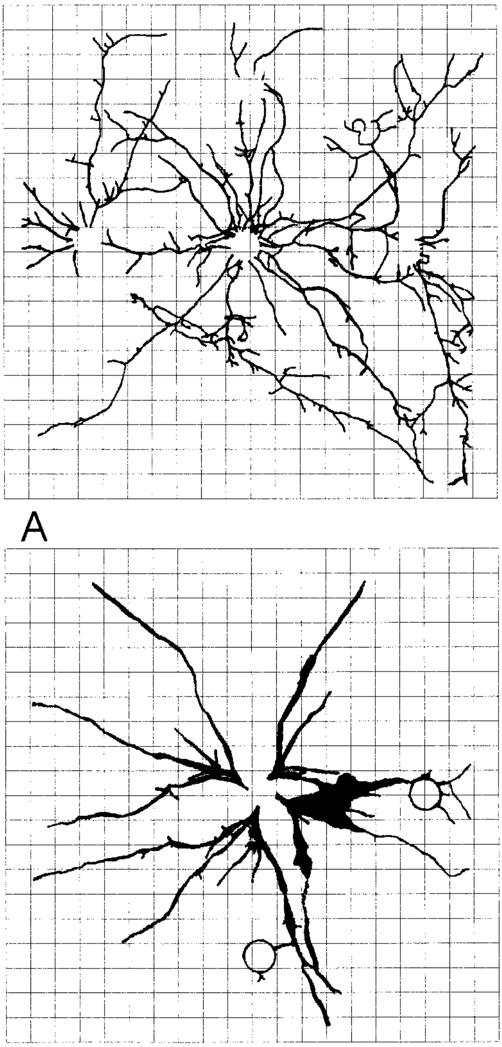

Fig. 3. Tunneling system of *R. flavipes* (A) and *C. formosanus* (B) with a grid of size r. The number of N (r) squares intersected by the tunnels are counted and log N (r) regressed against log r, to calculate the fractal dimension, D, and the abundance of tunnels (log K) based on the relation: log N (r) $\approx -D$ log r + log K. Black areas represent tunnels.

Results and Discussion

The results indicated that the tunneling systems of both termite sp. were characterized by the slope of the regression between log [$N(r)$] and log (r), where r ranged from 1.056 to 4.356 cm. This slope is an estimate of D, the fractal dimension. By definition, when D lies in the range of $1 < D < 2$, the fractal dimension describes a line (a transect across a surface; Mandelbrot 1983). Our values of D were in this range. This is an indication that both species of termites built almost straight line tunnels from the release chamber toward different points of the arena.

The average fractal dimension, D, was 1.301 ± 0.023 for *R. flavipes,* and 1.374 ± 0.021 for *C. formosanus,* before the termites reached the testing chambers (Table 1), and 1.100 ± 0.035 for *R. flavipes* and 1.116 ± 0.041 for *C. formosanus,* after reaching a testing chamber. These values were not significantly different (Approximate test of equality of means, $P > 0.05$), which is an indication that the overall intricacy of the tunneling system remained the same for both species either before or after reaching a testing chamber.

Overall, the abundance of tunnels, log K, was significantly higher before reaching a testing chamber than after. When comparing both termite species we found that before reaching a testing chamber, log K values for C. *formosanus* was higher than for *R. flavipes*

Table 1. Comparisons for the fractal dimension (D) and the abundance of tunnels (Log K) among six experiments with *R. flavipes* (R1: no wood; R2: 50% wood, and R3: 100% wood in the testing chambers) and *C. formosanus* (CI: no wood; C2: 50% wood and C3: 100% wood in the testing chambers), before (B) and after (A) termites reached the testing chambers (mean ± SD)

Experiments	Time	Fractal dimension	Abundance of tunnels
R. flavipes	B/A	D	Log K
R1	B	−1.314 ± 0.059a	4.024 ± 0.180f
R2	B	−1.275 ± 0.056a	3.935 ± 0.077e
R3	B	−1.314 ± 0.049a	3.940 ± 0.157eg
C. formosanus			
C1	B	−1.385 ± 0.041a	4.034 ± 0.098gh
C2	B	−1.388 ± 0.034a	4.073 ± 0.132h
C3	B	−1.350 ± 0.067a	4.027 ± 0.209fgh
R. flavipes			
R1	A	−1.070 ± 0.046a	3.728 ± 0.168d
R2	A	−1.092 ± 0.117a	3.672 ± 0.295cd
R3	A	−1.138 ± 0.044a	3.669 ± 0.151cd
C. formosanus			
C1	A	−1.135 ± 0.107a	3.479 ± 0.393b
C2	A	−1.069 ± 0.108a	3.321 ± 0.372a
C3	A	−1.144 ± 0.107a	3.613 ± 0.182c

B/A, Number of days before (B) and after (A) reaching any testing chamber. Means followed by different letters are significantly different (Approximate test of equality of means).

in the arenas when no wood were in the testing chamber (C1) and those with two wooden disks in the testing chambers (C2). After reaching a testing chamber, the abundance of tunnels, log *K* was higher for *R. flavipes* in arenas with no wooden disks in the testing chambers (R1) than for any other experiments. The lowest values of abundance of tunnels, log *K*, were found in those arenas loaded with *C. formosanus* without wood in the testing chambers (C1) and in those with two wooden disks (C2) (Approximate test of equality of means, Table 1). The log *K* parameter refers to tunnel abundance. Therefore, before reaching a testing chamber, *R. flavipes* and *C. formosanus* built more tunnels than after. However, after reaching a testing chamber, *R. flavipes* built more branches in those arenas without wood than those built by *C. formosanus* regardless of the presence of wood in the arena. This is an indication that *R. flavipes* and *C. formosanus* are not capable of detecting wood in sand over distance and their tunnel behavior is the same regardless of the presence of wood in the chambers. In general, when the tunneling system begins to grow, the cohort of termites produces more tunnels to acquire other food resources efficiently from the soil. When the tunneling system becomes larger (greater total length), the strength of the termite cohort decreases due to a decrease on termite density with distance. As a result, the abundance of tunnels, log *K*, decreases. This result agrees with what Masi and Maranville (1998) observed in sorghum root branching, which became more abundant in the top 35 cm portion of the root profile, enabling the plant greater soil exploration for water and nutrients than at a longer distance. However, our result contradicts the one reported by Reinhard et al. (1997) who worked with *R. santonensis* in an open smaller arena (20 by 20 cm) than those in our experiments (100 by 100 cm). Therefore, termites in Reinhard's experiments had a better chance to find the wood source. In our experiments the wooden disks were embedded inside the arena and placed at 30 cm away from the release center chamber. Evidently, the presence of sand around the wood and the longest distance to the source of wood, interfered with the termite detection of the wood. As a result, termites in our experiments did not detect any wood in sand over distance, which may be indicative of what occurs in the field, when termites search for wood underground.

The fractal dimension, *D*, was used to describe the architecture of the termite tunnels because it can describe the shape of a tunnel independently of its size. The use of log *K* (the abundance of tunnels) has the advantage to supplement *D* (the fractal dimension or degree of intricacy of the tunnels) in those cases were *D* values for different treatments are identical (Eghball et al. 1993). The two species of termites can be differentiated with regard to their ability to exploit the soil profile, even if the *D* values are similar.

Our results indicated that these termites were unable to detect wood in sand over distance. After reaching the testing chamber, the search ceased and the abundance of the tunnels decreased.

In conclusion, the principal features of our observations and analyses were that the abundance of tunnels (log *K*) was significantly higher for *C. formosanus* than for *R. flavipes,* and overall, log *K* was higher before reaching a testing chamber than after, but was not affected by the presence of wood.

This method of fractal analysis allows for an easy data collection and represents a new field of application to describe and simulate the architecture of the tunneling system of the subterranean termites. With the application of fractal analysis to quantify the tunneling system of the subterranean termites, it is possible to statistically compare their tunneling construction under different environmental factors such as temperature or humidity.

The utility of tunnel fractal dimension deserves further study, because it may provide new ways for understanding the functional implications that the branching patterns of termite tunnels have in relation to optimum soil exploration in relation to the spatial distribution of wood availability.

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