The Role of Toilet Paper in Studies of Desert Subterranean Termites (Isoptera) in Arizona, USA: A Substrate for Nondestructive Observations of Foraging Activity

by

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ABSTRACT

Toilet paper rolls were used as a substrate for nondestructive observation of the foraging activity of two termite species — *Heterotermes aureus* (Snyder) and *Gnathamitermes perplexus* (Banks) — in the Sonoran Desert of Arizona. Foraging was minimal during winter, increased in spring, was high but erratic in summer, and then was moderate in fall. *H. aureus* foraged within a temperature range of 7.6° to 47° C; *G. perplexus* foraged within a range of 9° to 49° C. Temperature had the greatest influence on the number of foragers appearing at the soil surface. Rainfall (and resulting soil moisture) greatly affected foraging in summer. The density of *H. aureus* foragers was estimated to be 4.31×10^6/hectare, while the density of *G. perplexus* was estimated to be about twice that of *H. aureus*. The density of *H. aureus* colonies was estimated to be 190.4 colonies/hectare with an estimated 22,632 foragers/colony and an average foraging territory of 12.5m^2. Together, these two species transported soil to the soil surface at a combined rate of 744.2kg/hectare/year. Both species brought up soil richer in clay and added significant quantities of organic carbon, nitrogen, PO_4, Na, Mg, and soluble salts, while *H. aureus* alone increased soil K.

Key Words: Isoptera, Rhinotermitidae, foraging behavior, foraging territories, foraging populations, alate production, soil movement, soil chemistry, *Heterotermes aureus* (Snyder), *Gnathamitermes perplexus* (Banks)

INTRODUCTION

As part of a symposium recognizing the contributions of Dr. Jeffery P. La Fage to termite biology, I was asked to present a brief synopsis of his accomplishments during the early years of his professional life. In 1971 Jeff and I began working on termites as part of research program funded by the National Science Foundation to study the ecology of the

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deserts of North America. One of the first assignments given to us by our professor, Dr. William L. Nutting, was to develop a method for studying foraging activity of the two predominant subterranean termites, *Heterotermes aureus* (Snyder) and *Gnathamitermes perplexus* (Banks), on one of our research sites. We wanted to find a substrate that was acceptable to these termites and would allow us to observe them without damaging them. We first tried partially burying birch (*Betula* sp.) tongue depressors. These small pieces of wood were occasionally visited by *H. aureus*, but upon removing the wood from the soil the termites were severely damaged. Next we tried placing 5- by 10- by 10-cm blocks of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) on the soil surface. Neither termite species seemed interested in this species of wood.

In 1971, Goeffrey Nutting, the son of Dr. Nutting, made a serendipitous discovery. He found a box of toilet paper that appeared to be "glued" to the concrete floor of a storage area where he was working. The "glue" was actually carton material made by *H. aureus*. Several thousand termites had completely infested this box of "processed wood." We brought the box into the laboratory to photograph and collect the termites for laboratory studies. We then began to wonder whether toilet paper would be a suitable substrate for our studies of termite foraging behavior. To find out, we wrapped 200 rolls of toilet paper with masking tape and placed them in two grids adjacent to the grids containing the blocks of Douglas-fir. The contrast in acceptability was startling! We had found our nondestructive sampling substrate for studying termites. We suspected that toilet paper suited *H. aureus* because it resembled wood on the surface of the soil. We supposed that *G. perplexus* favored toilet paper because it resembled seasoned cattle feces, a preferred food of this termite species. Once we had our substrate for observing the foraging activities of these termites, we established a site to study them around the clock, one day every week, for an entire year.

MATERIALS AND METHODS

Our studies were conducted on a research site provided by the Forest Service, United States Department of Agriculture, on its Santa Rita Experimental Range approximately 40km S of Tucson, Arizona, at an elevation of 950m. The annual rainfall at this site averages 330mm, half of which occurs during the summer monsoon. The remainder is frequently made up, less reliably, during the period from December through February. The area is a shrub-invaded, desert grassland ecotone, characterized by shrubs, cacti, and small trees.

To examine the physical factors affecting or regulating foraging of the two subterranean termite species we studied, we developed a modified bait-sampling method (La Fage *et al.* 1973). The standing vegetation at the test site had been removed 18 months before we set up the experimental grid. In addition, all superficial and partially buried wood had been raked off to eliminate any possible competition between natural wood and the sampling substrate (rolls of toilet paper). We established 16 contiguous grids, each containing 100 rolls of toilet paper placed at 1-m intervals.

Foraging activity of both *H. aureus* and *G. perplexus* was observed from October 15, 1971, to October 13, 1972. Observations were made on the same 12 (out of 16) grids during a 24-hour period each week, such that a different test area of 100 toilet paper rolls was examined every 2 hours. The order in which each grid was examined was randomized each week. No single roll was checked more than once a week. Four of the grids were left undisturbed for the entire year. Checking for termite foraging activity involved a quick examination of the underside of each roll of toilet paper. The species present and their approximate number were noted. If termites were not present, past activity of *H. aureus* and/or *G. perplexus* was also noted. Prior to each examination several environmental parameters were measured: temperature at the soil surface and at 1, 3, 5, 10, and 15cm below the soil surface, in a roll, and at the roll-soil interface. Rainfall was measured weekly (Haverty *et al.* 1974, La Fage *et al.* 1976).

Five size classes described the number of termites observed in or on each roll of toilet paper. The median value of each class was used as the estimate of the number of termites foraging at a particular roll. The accuracy of the estimates was determined at the end of the study by examining 211 rolls from the undisturbed test area, assignment of a size class in the field as usual, and counting the termites in the laboratory. When the relationship of the estimates and the actual counts was examined, we found a highly significant correlation for both *H. aureus* and *G. perplexus*. For *H. aureus* we underestimated the number of foragers and needed to adjust our estimates with the equation $Y = 2.04 + 1.875X$, where X was the estimated number of *H. aureus* foragers. For *G. perplexus* we found no significant difference between our estimates and the actual counts (Haverty *et al.* 1974, La Fage *et al.* 1976).

Seasonal trends in foraging were determined by summing the estimated numbers of termites for each species at the soil surface for all 12 test areas (over a 24-hour period) for each week of the 53 weeks of the experiment. These totals were plotted against mean daily temperature (roll-soil interface) for the 24-hour observation period and cumulative rainfall for the preceding week. Preliminary regression analyses indicated that these two parameters were the most useful in explaining the number of termites foraging at the soil surface. The relationship between number of foraging termites, mean daily temperature at the roll-soil interface and rainfall was determined by multiple linear regression of transformed variables (Haverty *et al.* 1974, La Fage *et al.* 1976).

A weekly record was kept of foraging intensity for each roll of toilet paper. From this, frequency tables of foraging-number size classes were developed that allowed calculation of the average number of foragers visiting each toilet paper roll during the year. The weekly records also provided a basis for observing the lateral spread of foraging territories. Rolls of toilet paper attacked on the first week of the study and rolls of toilet paper with large, mean weekly counts of termites were considered as colony foci. The lateral spread of territories from these points to new toilet paper rolls was followed weekly. Isolated rolls or groups of 2 or 3 rolls were arbitrarily included with larger aggregations if they were within 2m of the larger clump and appeared to represent a temporal expansion of the original foci. We assumed that each roll of toilet paper subtended $1m^2$ of territory, so that the spatial area of each foraging territory was calculated by summing the number of rolls of toilet paper occupied in each grouping. Unoccupied rolls within such groups were included in the occupied area because it seemed, at that time, unlikely that these rolls of toilet paper would be used by other colonies of *H. aureus*. The area subtended by each of the colonies on the periphery of the plot was multiplied by 2 because, on the average, only one half of each such colony would fall within the plot. In determining densities of colonies, we counted colonies entirely within the plot as whole colonies but, according to the preceding assumption, those divided by plot boundaries were counted as half-colonies (Haverty *et al.* 1975).

At the end of the foraging activity study, 211 rolls of toilet paper were collected from the 420 in the undisturbed strip, and all soil was carefully removed from the termite workings in them. Of these, 21 toilet paper rolls had been occupied solely by *H. aureus*, 158 solely by *G. perplexus*, 26 by both species, and only 6 remained untouched by termites. All soil from each roll of toilet paper was dried, weighed, and analyzed for 20 separate chemical and physical characteristics. For comparison with termite-modified soil, samples were collected from the top 5cm of undisturbed soil at four randomly chosen places, each equidistant between two adjacent toilet paper rolls (Nutting *et al.* 1987).

RESULTS AND DISCUSSION

Both *H. aureus* and *G. perplexus* foraged, or were at least at the soil surface, day and night throughout most of the year. *H. aureus* was not seen on only 2 of the 53 observation days. *G. perplexus* was not seen on only 4 of the 53 observation days. Foraging of both species was minimal from December through February. In the spring and fall foraging intensity was moderately higher than in winter. During summer, foraging was highest, but erratic. The number of foragers generally increased as temperature increased. From October through December (winter months) when the temperature at the roll-soil interface was consistently below 20° C, rainfall appeared to have a slight effect on increasing the number of foragers. However, during the hot summer months (June – September), even a slight amount of rain increased the numbers of foragers of both *H. aureus* and *G. perplexus*, provided the temperature did not rise above 33° C. Above 33° C, even with precipitation, foraging numbers of both species dropped sharply (Haverty *et al.* 1974, La Fage *et al.* 1976).

H. aureus foraged at the rolls of toilet paper within a temperature range of 7.6° to 47° C; *G. perplexus* foraged within a temperature range of 9° to 49° C. This range apparently restricts the time that can be spent foraging (Collins *et al.* 1973). Temperature also determined foraging intensity or the number of individuals at the surface per unit area. Plots of foraging intensity vs. environmental parameters indicated that the relationship was not a continuously increasing function. For *H. aureus* the number of surface foragers at any instant (Y) is best explained by the equation

$$\ln Y = -0.985 - 0.0761\ T + 2.928 \ln T + 0.327 \ln R$$

where T is the temperature at the roll-soil interface and R is daily rainfall. For *G. perplexus* the relationship is explained by the equation

$$Y = 2.39 + 0.31\ T - 0.11\ R - 0.01\ T^2 + 0.54 \ln R.$$

From these results it was apparent that foraging activity was not endogenously controlled; rather it is exogenously controlled by temperature and soil moisture (as reflected in daily rainfall) (Haverty *et al.* 1974, La Fage *et al.* 1976).

Foraging territories and colony size could not be determined for *G. perplexus* with the same methods that we used for *H. aureus*. *G. perplexus* occupied 96% of the rolls of toilet paper, including 53% of those used by *H. aureus*. *H. aureus* attacked 22.8% of the rolls of toilet paper on our plots. There were 26 complete and 12 partial colonies of *H. aureus* on the 40- by 40- m grid (Haverty *et al.* 1975). The average foraging area of a *H. aureus* colony was estimated to be 12.5 m^2 with 190.4 colonies/hectare. This foraging territory estimate agrees reasonably well with the estimate of 13.9 m^2 for *H. aureus* reported by Jones (1990a) from essentially the same site. Using the average number of termites visiting a roll of toilet paper and a ratio of subsurface: surface foragers of 114:1 (Bodine 1973), Haverty *et al.* (1975) speculated that the density of *H. aureus* was $4.31\text{X}10^6$ foragers/hectare; the number for *G. perplexus* was hypothesized to be about twice as many (Haverty *et al.* 1975, La Fage *et al.* 1976). Dividing the total number of termites by 190.4 colonies/hectare resulted in an estimate of 22,632 termites/colony (Haverty *et al.* 1975). Jones (1990b) used a mark/release/recapture technique and estimated colonies of *H. aureus* from this site to contain 45,000 to 300,000 foragers. Even though the estimates reported by Haverty *et al.* (1975) and La Fage (1976) were speculative, the mark/release/recapture technique for estimating populations of subterranean termites is controversial (Thorne *et al.* 1996). The accuracy of the estimates by either technique (Haverty *et al.* 1975; Jones 1990b) should be regarded cautiously.

On the undisturbed, 420-m^2 study plot, *H. aureus* foraged over 22.3% of the area and *G. perplexus* over 87.2%, while their territories overlapped on 12.3% of the area. *H. aureus* moved soil to the surface at a rate of 70.3kg/hectare/year, *G. perplexus* at a rate of 574.9kg/hectare/year, and the two species together on overlapping territories, 99.0kg/hectare/year. In sum both species transported soil to the surface at a combined rate of approximately 744.2kg/hectare/year (Nutting *et al.* 1987). Both species brought up soil richer in clay than the adjacent soil surface, and contributed to a small increase in soil pH, mainly by bringing lime to the surface. These two termite species, particularly *H. aureus*, also added organic carbon to the soil as a result of digesting plant material and depositing feces or saliva or both in their workings at the surface. But only *H. aureus* increased the quantity of nitrogen. Finally, both species contributed to a considerable increase in PO_4, Na, Mg and soluble salts, while *H. aureus* alone increased K. These nutrients incorporated into the soil by *H. aureus* likely remain in its workings for months or years, while nutrients added to the soil by *G. perplexus* are probably released within weeks or months by the first heavy rains (Nutting *et al.* 1987).

In summary, a serendipitous discovery in 1971 of the apparent preference of toilet paper by *H. aureus* led to the use of this rich source of cellulose as a substrate for studying the foraging behavior and populations of two of the predominant subterranean termites in southern Arizona. Rolls of toilet paper proved quite effective in attracting the foragers to a substrate in which their numbers could easily be estimated and played a significant role in studies of subterranean termite ecology in North America.

ACKNOWLEDGMENTS

The studies reported in this paper represent a time of tremendous camaraderie between Dr. William L. Nutting and his two graduate students, Jeffery P. La Fage and me. We attacked these studies as full partners. We discussed findings and observations during lunch nearly every day from the summer 1971 until early 1975. We worked in pairs to assess foraging activity, including working throughout the night. We occasionally had the help of others in examining termites in the rolls of toilet paper, including Richard V. Carr, Margaret S. Collins, Ruth D. Nutting, Marsha M. Haverty, and Wendy L. La Fage. Needless to say, we also had a great deal of fun discussing this work and observing the reaction of our audiences when we discussed toilet paper as a substrate for studying subterranean termites. Special thanks go to Geoff Nutting for making the initial discovery of the acceptability of toilet paper to *Heterotermes*. This discovery accelerated the progress of our research.

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