

Impacts of Soil Moisture Level on Consumption and Movement of Three Sympatric Subterranean Termites (Isoptera: Rhinotermitidae) in a Laboratory Assay

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J. Econ. Entomol. 98(3): 933-937 (2005)

ABSTRACT Three subterranean termite species, *Reticulitermes flavipes* (Kollar), *Reticulitermes tibialis* Banks, and *Reticulitermes virginicus* (Banks), were collected from locations in northern Indiana and tested under laboratory conditions to determine whether preferential differences exist among species. Foraging behaviors and location of all three species were studied using a linear, three-dimensional assay with a soil moisture gradient (5, 15, 25, 35, 45, and 55% moisture by weight) and quantified by 1) consumption weights and 2) location counts. In a 7-d period, *R. flavipes* and *R. tibialis* consumed almost twice as much filter paper as *R. virginicus*. No significant difference in feeding was attributed to moisture level for *R. tibialis*, but there were differences for *R. flavipes* and *R. virginicus*. In terms of location of harborage, there were clear patterns associated with moisture level, as predicted using a Poisson distribution. Results from consumption and location data show unique patterns among species, and illustrate species-specific variation in feeding location and nesting preference in response to moisture. There are significant differences in movement patterns, consumption, and mortality among Indiana *Reticulitermes* according to the laboratory assay. These findings contribute to the overall understanding of midwestern *Reticulitermes* termites.

KEY WORDS *Reticulitermes tibialis*, *Reticulitermes flavipes*, *Reticulitermes virginicus*, foraging, soil moisture

SUBTERRANEAN TERMITES, PARTICULARLY those in the genus *Reticulitermes*, are the most destructive and economically important urban pests in the United States (Su and Scheffrahn 1990). Urbanization throughout the country has changed natural termite habitats mainly through irrigation and landscaping. These changes have made environments conducive to termite activity, particularly because of the influence these practices have on water content, relative humidity, and temperature (Haagsma and Rust 1995). In locations where cold winters occur, termites are able to maintain activities throughout the winter by taking advantage of the human practice of structural heating. This consistent environment, in and around structures, makes it possible for termites to remain active year-round by using warm, moist areas (Mallis 1982, Pearce 1997).

Five subterranean termite species comprise the majority of the U.S. termite control market (Su and Scheffrahn 1990, Austin et al. 2002). Three of the five species, *Reticulitermes flavipes* (Kollar), *Reticulitermes tibialis* Banks, and *Reticulitermes virginicus* (Banks), can be found in northern Indiana. *R. tibialis* and

R. flavipes are regarded as common termites in the United States and North America (Snyder 1934, Weesner 1965, Mallis 1982, Nutting 1990, Metcalf and Metcalf 1993). According to Nutting (1990), the distribution of *R. virginicus* is limited to the southeastern United States, with northerly limits reaching just the southern portions of Illinois, Indiana, and Ohio. Because *R. virginicus* has been recovered in natural environments in northern Indiana (Ye et al. 2004), it is probable that their natural range is much further north and inland than indicated by earlier distribution maps and records.

No studies have compared the habitat preference of different *Reticulitermes* species from Indiana. It was thought that termites found in the Great Lakes region were transported into cities in wood and soil (Behr 1973). Northern Indiana region is characterized by high moisture from Lake Michigan and sandy soils, which are known to be a favorable environment for termites. Moisture content of food is usually directly dependent upon the moisture content in the soil and food that is usually in or on the ground (Williams 1934). *R. flavipes* and *R. virginicus* were reported as being very resistant to dehydration, and *R. tibialis* as most resistant (Snyder 1934, 1954). Therefore, we hypothesize that *R. tibialis* would be better suited to survive at lower moisture levels.

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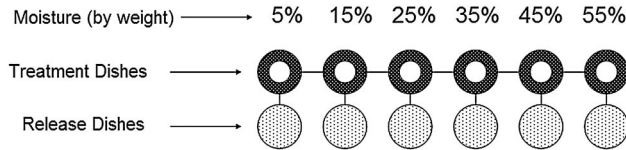


Fig. 1. Experimental assay used to test termite foraging across a soil moisture gradient. Clear plastic dishes were connected with Tygon tubing and prepared with a moisture gradient of 5–55%. Treatment dishes contained a sand:vermiculite mixture (dark circles) and filter paper (white circles), and release dishes contained only sand (light circles). Each treatment dish was connected to a release dish by Tygon tubing so that termites were able to move freely between all dishes for 7 d.

The overall objectives of this project were to determine the influence of soil moisture level on food consumption and movement for colonies of *R. flavipes*, *R. tibialis*, and *R. virginicus*. Our specific objectives were to 1) determine moisture levels that favor the highest consumption of resources for each species, 2) determine moisture levels preferred for harboring among species, and 3) infer ecological differences in foraging behavior between species that can be further tested under field conditions.

Materials and Methods

Termite colonies were collected between May and October 2003, brought back to the laboratory, sorted, and placed into colony boxes. *R. tibialis* and *R. flavipes* colonies were collected from Tippecanoe County, Indiana, ≈ 1 mi from each other. Termites were collected in traps that were installed in the ground close to structures where evidence of termite activity was noted previously (Jones 2003). Traps were buried polyvinyl chloride pipe containing corrugated cardboard bait. Both species were collected from highly urbanized areas that had structures situated within a few feet of collection sites.

R. virginicus colonies were collected from Jasper-Pulaski Fish and Wildlife Area, in Jasper County near Medaryville, Indiana. The location can be described as a conservation area that has been highly modified with intentional burns. Termite-infested wood (oak, *Quercus* spp.) was gathered and taken back to the laboratory for sorting. Termites were identified using soldier morphological characteristics (Snyder 1934, Miller 1949, Weesner 1965, Nutting 1990, Scheffrahn and Su 1994) and later confirmed by mitochondrial DNA analysis (Ye et al. 2004).

Termites were sorted by the method of Tamashiro et al. (1973) to separate the healthy termites from the cardboard or wood in which they were collected. Laboratory colonies of all species were kept in plastic sweater boxes (31.75 by 25.56 by 9.68 cm) with a mixture of moist sand, vermiculite, and wood. Boxes were kept in 24-h darkness at $25 \pm 1^\circ\text{C}$ and $50\% \pm 10\%$ RH. Colonies were checked and maintained weekly to ensure that moisture and food resources were not declining. Termites were kept in the laboratory for no more than 1 mo before testing.

Each of six clear plastic release dishes (5 cm in diameter and 50-ml volume) was connected by tubing to a feeding dish, with all feeding dishes connected to

each other as indicated in Fig. 1. Treatment dishes contained 25 ml of 1:1 sand:vermiculite mixture (Haverty 1979) at a specific moisture level (5–55%); release dishes contained dry sand only. Deionized water, at five different amounts, was stirred into the sand:vermiculite mixture, so that throughout each treatment dish, constant moisture could be achieved. Assays were allowed to run at moisture levels of 5, 15, 25, 35, 45, and 55% (moisture by weight) for the experimental period. Moisture levels $>55\%$ resulted in supersaturation of substrate. Lids and tubing fit snug to minimize water evaporation.

Fifty termites were placed into each release dish: 49 workers of at least the third instar and one soldier, totaling 300 termites per replicate. Termites were chosen randomly and counted while aspirated into groups of 50 before being released into their respective release dishes. *R. flavipes* and *R. tibialis* were replicated 16 times each and *R. virginicus* was replicated 15 times. The placement order of assays was randomized to minimize any environmental variability. The observer who recorded data did not know the identity of the colonies.

A single disc of filter paper (Whatman no.1, 42.5 mm in diameter) was placed on top of the substrate surface as a food source in each treatment dish. Dry filter paper weights were recorded to quantify consumption at each moisture level over the test period. Before weighing, filter papers were dried in a 40°C oven for 48 h. In addition to placing filter paper in a drying oven at takedown, final counts were recorded for all surviving termites, paying particular attention to where termites were distributed within each arena. This was known as location data.

Tests were run for 7 d under a photoperiod of 12:12 (L:D) h at $26 \pm 2^\circ\text{C}$ and $50 \pm 10\%$ RH. According to previous observations and control experiments, there were no indications that any of these external variables affected the results of the tests. A water loss control replicated six times indicated that there was no difference in moisture loss for any treatment dish. Controls were set up identical to bioassay, but without termites, and dishes were weighted before and after 7 d.

All assayed termites were similar in size (i.e., similar instars) and were held in the laboratory for similar times, fed the same diet, and maintained through identical regimens before assaying. The variables evaluated were species, moisture level, and either consumption or location count. Consumption was quantified by

Table 1. Pooled consumption and survival data (means ± SEM) independent of moisture level for each replicate

Species	Filter paper consumption (mg)	Surviving termites (from 300)
<i>R. flavipes</i> (n = 16)	49.32 ± 3.08a	251.63 ± 7.60ab
<i>R. tibialis</i> (n = 16)	42.93 ± 2.59a	261.56 ± 5.77a
<i>R. virginicus</i> (n = 15)	29.44 ± 2.66b	234.73 ± 8.54b

Means within a column followed by the same letter are not significantly different (Fisher's LSD *t*-test, $\alpha = 0.05$).

the difference in dry filter paper weight pre- and posttest (milligrams), and location count was quantified by the number of termites recovered from a particular treatment dish/particular moisture level. Consumption and location counts were analyzed using generalized linear modeling and analysis of variance (ANOVA); the effects of both species and moisture were compared using Fisher's least significant difference (LSD) *t*-test method (SAS Institute 2001). To correct for a Poisson distribution, a square-root transformation of the location data were used to normalize the data before ANOVA (Zar 1974).

Results

General Observations. Upon release, termites immediately explored the surroundings of the dry release dishes and moved through tubing into the treatment dishes. Abandonment of the release dish would normally occur, but whether termites blocked the entrance back to the release dish was variable between species. All species exhibited the behavior of physical partitioning by sealing the tube with substrate to separate different moisture levels. This occurred in 94% of *R. flavipes* replicates, 60% of *R. virginicus* replicates, and 13% of *R. tibialis* replicates.

When a treatment dish with adequate moisture was entered, termites tunneled down to the bottom edge, circled around, and transported substrate to the surface. Termites would live at the bottom 3 mm without substrate, directly on the plastic dish bottom.

Consumption Study. Species was found to be a significant effect ($F = 11.71$; $df = 2, 44$; $P \leq 0.0001$) in the amount of filter paper that was consumed overall for the study. *R. flavipes* and *R. tibialis* consumed ≈1.5-fold more filter paper than *R. virginicus* over the course of 7 d (Table 1).

There were significant differences ($F = 2.19$; $df = 7, 274$; $P = 0.056$) in consumption based on moisture level among the species. *R. flavipes* consumed the most filter paper in the 25 and 55% moisture dishes, and had the lowest consumption in the five and 35% dishes ($F = 8.33$; $df = 5, 90$; $P = 0.005$). *R. tibialis* spread consumption equally among all treatment dishes ($F = 0.67$; $df = 5, 90$; $P = 0.649$). Moisture had a significant influence on consumption of *R. virginicus* ($F = 3.39$; $df = 5, 84$; $P = 0.007$). Consumption was significantly greater in the 25 and 45% moisture dishes compared with the 5 and 55% moisture dishes ($F = 15.48$; $df = 5, 84$; $P = 0.0002$), as indicated in Fig. 2.

Location Study. There was no difference among different species in terms of the location where they were recovered ($F = 1.33$; $df = 7, 274$; $P = 0.265$) (Fig. 3). However, moisture was a highly significant ($F = 16.32$; $df = 7, 274$; $P < 0.0001$) indicator of where termites were located within the assay. Treatment dishes for *R. flavipes* showed that termites harbored in the 5, 15, and 25% dishes significantly less than 35, 45, and 55% moisture dishes ($F = 123.61$; $df = 5, 90$; $P < 0.0001$). Results were similar for *R. tibialis*, which also resulted in a significant difference between the three highest moisture levels compared with the three

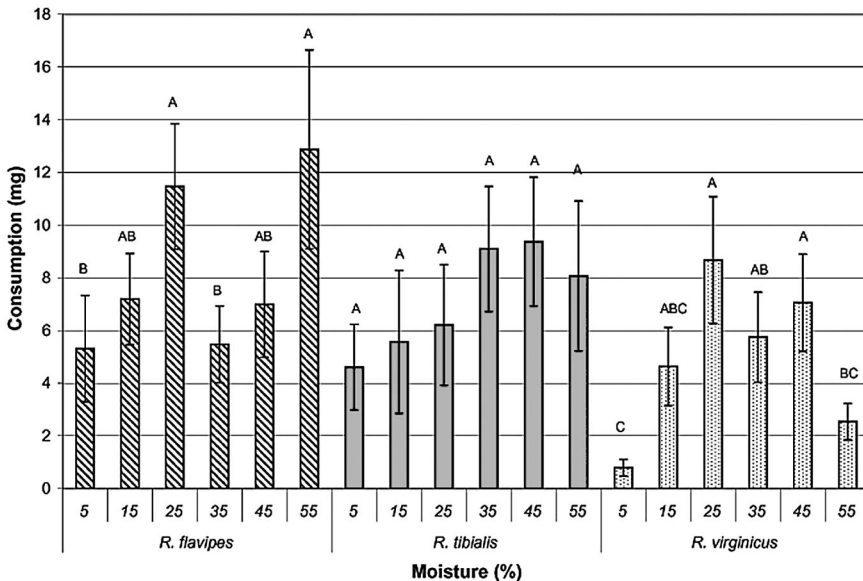


Fig. 2. Comparison of consumption (mean ± SEM) by 300 termites at six soil moisture levels over the 7-d assay. Different letters indicate significant differences ($\alpha = 0.05$) within species using Fisher's LSD *t*-test.

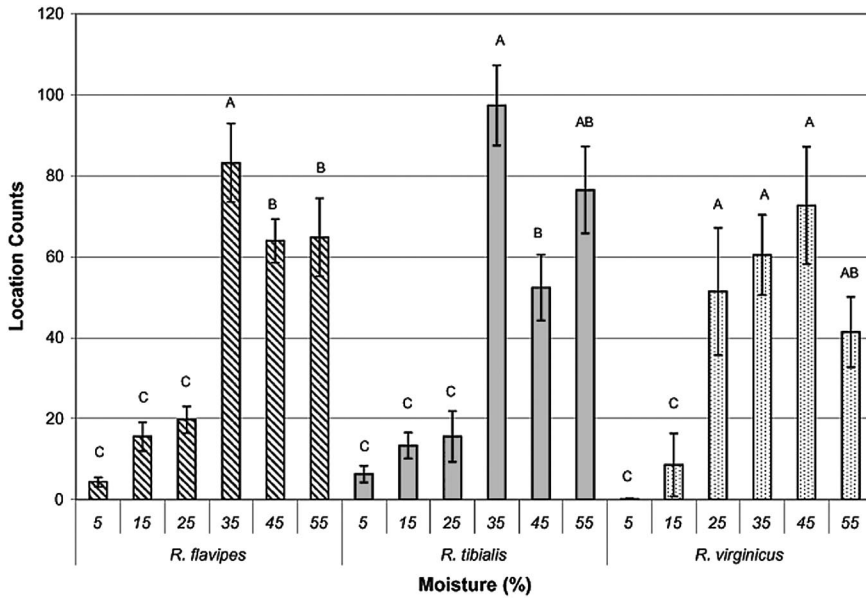


Fig. 3. Comparison of the location (i.e., soil moisture level) where termites (mean \pm SEM) were recovered at the end of the 7-d assays. Different letters indicate significant differences ($\alpha = 0.05$) within species using Fisher's LSD *t*-test.

lowest moisture levels ($F = 109.52$; $df = 5, 90$; $P < 0.0001$). *R. virginicus* harbored in the 5 and 15% dishes significantly less than the 25, 35, 45, and 55% moisture dishes ($F = 1.45$; $df = 5, 84$; $P < 0.0001$). Occasionally, termites of each species were found living satisfactorily in release dishes, which contained dry sand. Of the 282 release dishes used in this experiment, six were used as harborage (2%).

Consumption and Location Compared. At time of takedown, it was discovered that consumption location data were inconsistent with that of final recovery location (harborage data) and that termites were not eating where they were harboring. This was indicated by tunnels and active termites in the tunnels, or at the bottom of the treatment dish. For all species, the highest consumption was not the treatment dish where the majority of workers were recovered at the end of 7-d trials. Of the three species, *R. virginicus* had the most similar consumption and location patterns, suggesting that they may have preferred to feed where they harbored.

Mortality. There were significant differences in survival between species ($F = 3.36$; $df = 2, 44$; $P = 0.044$). *R. tibialis* had the lowest mortality rate of 13%, and *R. virginicus* had the highest mortality rate of 22%. Neither species, however, was significantly less than that of *R. flavipes*, which had a 6% mortality rate (Table 1).

Discussion

This study compared three *Reticulitermes* species that were collected from northern Indiana in an assay with six moisture levels. Termite foraging would be expected to vary according to the moisture preferences for a species. We show that patterns of feeding

and patterns of movement within a moisture-graded assay are unique to each of the three species. From this experiment, it was also apparent that termites will consume resources at a location with adequate moisture levels regardless of the distance to their harborage.

The results from our location/movement study are consistent with the findings of Su and Puche (2003) who found that early tunneling behavior was concentrated in areas of higher moisture levels, but after 7 d, termites were not affected by moisture gradients. They hypothesized that termites were able to transfer sources of moisture to the lower moisture substrate, thus negating the artificial gradient (Su and Puche 2003). We found that termites were able to transfer moisture with them into drier areas to control and "customize" their environment.

The current study showed that termites do not always feed at a single area and will not necessarily concentrate at a single harborage. In field-monitoring situations using both mark-release-recapture methods and molecular techniques, it has been shown that termite colonies distribute individuals to many different feeding locations within a given area (Thorne et al. 1996, Vargo 2004). Termites have been known to forage >10 m in laboratory studies (Suarez and Thorne 2000), so it is possible that this assay can be expanded to reflect realistic distances in nature.

This study confirms that moisture significantly influences termite movement; however, the location in which they harbor is not entirely associated with consumption. Thus, although termites may forage for food resources randomly, their occurrence and harborage location are dependent on environmental factors, particularly moisture.

Of the three species, *R. flavipes* is known in the Midwest for its pest status. This is likely due to its extensive distribution, attraction to urban habitats, and its consumption capabilities. *Reticulitermes virginicus* and *R. tibialis* also maintain structural pest status, but to a greater extent in different states and geographical locations (National Termite Survey 2003). The hypothesis that *R. tibialis* would be better able to survive in a lower moisture level was not supported in this study.

Despite the importance of termites in natural environments, there is limited research regarding *R. tibialis* and *R. virginicus* in the Midwest. Foraging ecology of Indiana termites is currently not well understood. European subterranean termites that are morphologically similar have been found to have differences in behavior, genetic structure, colony formation, and resistance to pesticides (Clement et al. 2001). Knowledge of the relationship that exists between termites and their environment has significant scientific and practical value in terms of understanding both basic termite ecology and termite management. More efficient use of termiticides through baiting can be accomplished by understanding the species-specific behaviors that affect termite movement into different locations. Information about the niche that each species occupies can help in the planning of the placement of bait formulations. Being aware of conditions that are conducive to termite infestations can assist in quicker detection and more effective elimination. In this regard, the findings presented here represent a significant step forward in our understanding of sympatric Indiana *Reticulitermes*.

Acknowledgments

We acknowledge Catina Ratliff for help with fieldwork, editorial, and technical assistance; Jesse Hoteling and Brian Judt for help with termite collections; Jonathan Neal, Rick Foster, Chow-Yang Lee, and Changlu Wang for helpful discussions; and Doug Richmond and Grzegorz Buczkowski for review of manuscript drafts. We thank Dow AgroSciences, Bayer, and BASF for partial research support. This is journal article No. 17593 of the Agriculture Research Program of Purdue University, West Lafayette, IN.

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