Monthly Fluctuation of Termite Caste Proportions (Isoptera) within Fire Ant Mounds (Hymenoptera: Formicidae)

by

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ABSTRACT

Monthly abundance and caste proportions of subterranean termites (*Reticulitermes* spp.) inhabiting red imported fire ant (*Solenopsis invicta* Buren) mounds were recorded during 1999 and 2000 from a relatively undisturbed forest edge in Tuskegee, Alabama. Temperature data were also recorded at these mounds; mean air, soil, and mound temperatures followed a sine model over the course of the year. During the late fall and early winter, relative proportion of young workers (< 3rd instar) increased from 0 to 66.7% of the sample from the previous month, suggesting a change in temperature requirements among termite castes. These data demonstrate that *Reticulitermes* spp. termites are found year-round inhabiting active fire ant mounds, although relative caste proportions change seasonally. The hypothesis of a minimum soil temperature determining the movement of young *Reticulitermes* spp. workers into fire ant mounds (or other locations of increased temperature) is discussed.

Keywords: *Solenopsis invicta*, *Reticulitermes* spp., temperature dependence, niche exploitation

INTRODUCTION

Interactions between ants and other insects have been examined in many areas of insect ecology (Collins & Markin 1971, Whitcomb 1974, Banks *et al.* 1985, Vinson & Scarborough 1991). The interactions of termites and ants are even more interesting because they place two (or more) eusocial species in intimate contact (Longhurst *et al.* 1978, Redford 1984, Wojcik 1986, Wells & Henderson 1993, Crist & Friese 1994, Cornelius & Grace 1995, Cornelius *et al.* 1995, Jaffe *et al.* 1995, Leponce *et al.* 1999). Increasingly, field data suggest that there may be more to these interactions than mere predation of termites by ants (Jaffe *et al.* 1995, Shelton *et al.* 1999).

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The red imported fire ant, *Solenopsis invicta* Buren, has been a well known pest throughout the Southeastern United States for almost 70 years (Lofgren 1986). *Solenopsis invicta* build mounds that disturb the aesthetic qualities of many open areas, and are important medical and veterinary pests when they come into contact with man and domesticated animals. However, *S. invicta* mounds may harbor other insects, such as termites (*Reticulitermes* Holmgren spp., Shelton *et al.* 1999) and cixiids [*Oliarus vicarius* (Walker), Sheppard *et al.* 1979]. The ecological interactions of *S. invicta* with these facultative myrmecophiles may provide further knowledge of how (and why) these species interact, and what methods are used by the myrmecophiles to avoid ants within the nest.

Shelton *et al.* (1999) showed that during spring months, up to 25% of active *S. invicta* mounds close to forest edges may be infested with either the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar), or the dark Southern subterranean termite, *R. virginicus* Banks (*sensu* Su & Scheffrahn 1994). During the spring, termite caste proportions within *S. invicta* mounds favor nymphs (pre-alates) instead of workers. It was hypothesized that there was a resource available in the mounds that was unavailable elsewhere, specifically areas of increased temperature or moisture compared to other habitats (wood or non-mound soil). Only temperature was significantly greater in the mounds, leading Shelton *et al.* (1999) to conclude that the nymphs were using the mounds as 'incubators', speeding up development into alates.

The objective of this study was to estimate the seasonal variation of termite occurrence (both of presence/absence and caste proportion) in *S. invicta* mounds over the course of a year. We show the relationship of termite caste proportions with temperature in the environment. Information from our prior study predicted that *Reticulitermes* spp. occurrence in *S. invicta* mounds should cease following flight periods for alates (spring to early summer). We address this question by examining *S. invicta* mounds on a monthly basis, recording mound, nearby soil, and ambient air temperatures. Termite caste proportions were also determined for mounds containing termites.

METHODS AND MATERIALS

A single site comprised of a forest opening near a small (~1 acre) pond in Tuskegee, AL (Macon county) was sampled monthly during 1999 and 2000 for the presence of subterranean termites in *S. invicta* mounds occurring within 2 m of the forest edge. Examinations were made between 0900 and 1100 h CST, approximately once every 30 days. Temperature data (described below) were recorded for every mound within the sampling area. The destructive nature of mound examinations (see below) could induce mound movement by the ants (Hays *et al.* 1981, JTV personal observations), therefore no attempt was made to keep track of individual mounds. Following temperature determinations, mounds were dissected using a shovel; presence of ants, wood, termites and their location (either in mound soil or in wood within the mound) were noted for each dissected mound. For each mound containing termites, a sample (~200 ml) of mound soil containing the termites was collected in 400 ml of 70% ethanol in a 0.95-L glass jar.

Temperature data were recorded at three locations for each mound: 5 cm into the top of the highest part of the mound, soil temperature 0.3 m away from the mound (5 cm depth), and air temperature 5 cm above the soil temperature sampling location (Fig. 1). Temperature data were collected using a digital thermometer [BAT-12; Sensortek (Bailey) Instruments, Clifton, NJ], with a copper-constantan thermocouple (PT-6, Type T, Physitemp, Clifton, NJ). Temperature data were recorded for all mounds (within 2 m of forest edge) encountered during each sampling period. T-tests (PROC TTEST, SAS Institute 1985) were used to test for differences between mound temperatures of termite-infested mounds and termite-free mounds. To account for differences in mean temperatures over the course of the year, comparisons were made between the differences of mound and soil temperatures for termite-infested and termite-free mounds (PROC TTEST, SAS Institute 1985). The pattern of mean air, mound, and soil temperatures at each sampling interval was modeled using nonlinear regression with day number (starting from 01 January 1999) as the dependent variable (Tablecurve 2D, AISN Software Inc. 2000). Waveform functions were examined since our temperature data followed a cyclic pattern. Among significant models only the most parsimonious (*i.e.*, simplest) are reported here.

Samples were returned to the laboratory for counting and caste proportion determination. Samples were poured into metal trays and examined carefully for termites. Termites were counted into caste

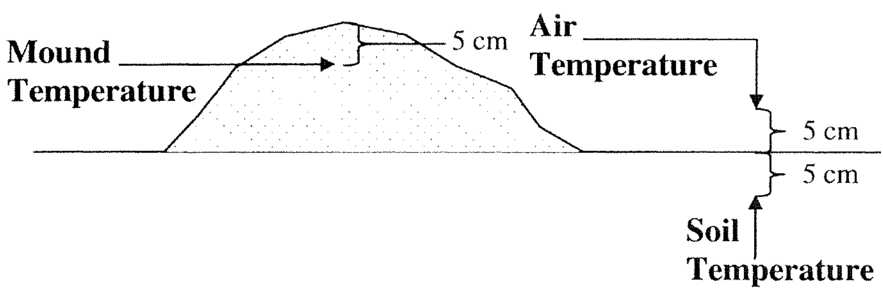

Fig. 1. Temperature sampling locations on *S. invicta* mounds.

categories and retained in alcohol for collection. Caste categories were: workers (3rd or greater instar as determined by size and morphology), young workers (<3rd instar), soldiers, nymphs (wing-pad bearing pre-alates), and alates, no supplementary reproductives (physogastric nymphs) or primary reproductives were recovered from the mounds. When soldiers were obtained, the genus of the sample members was determined using Su & Scheffrahn's (1994) key.

RESULTS

Infestation rates

Proportions of ant mounds infested with termites ranged from 0 to a maximum of 0.11 in both August and November 1999 (Fig. 2). All mounds containing termites also contained wood, however, in only 60% of samples were termites found within the wood, the remainder were found in the mound soil. Only 14.3% of mounds containing wood also contained termites.

Temperatures

Mean mound, soil, and air temperatures over the course of the study are presented in Fig. 3. Mound temperatures exceeded air (exceptions: April 1999 and January 2000, when mean air temperatures were 2.6

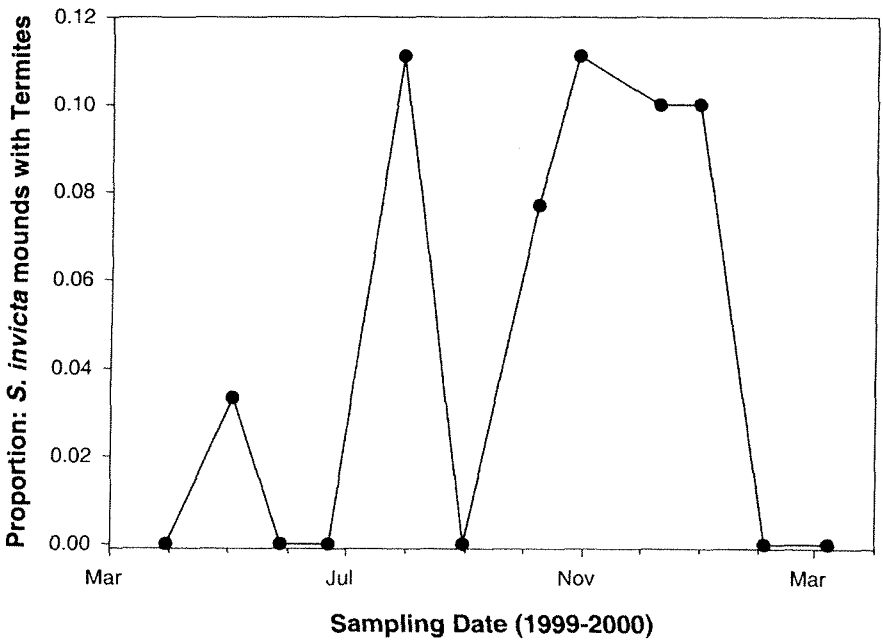

Fig. 2. Proportion of *S. invicta* mounds infested with *Reticulitermes* spp. termites by sampling date.

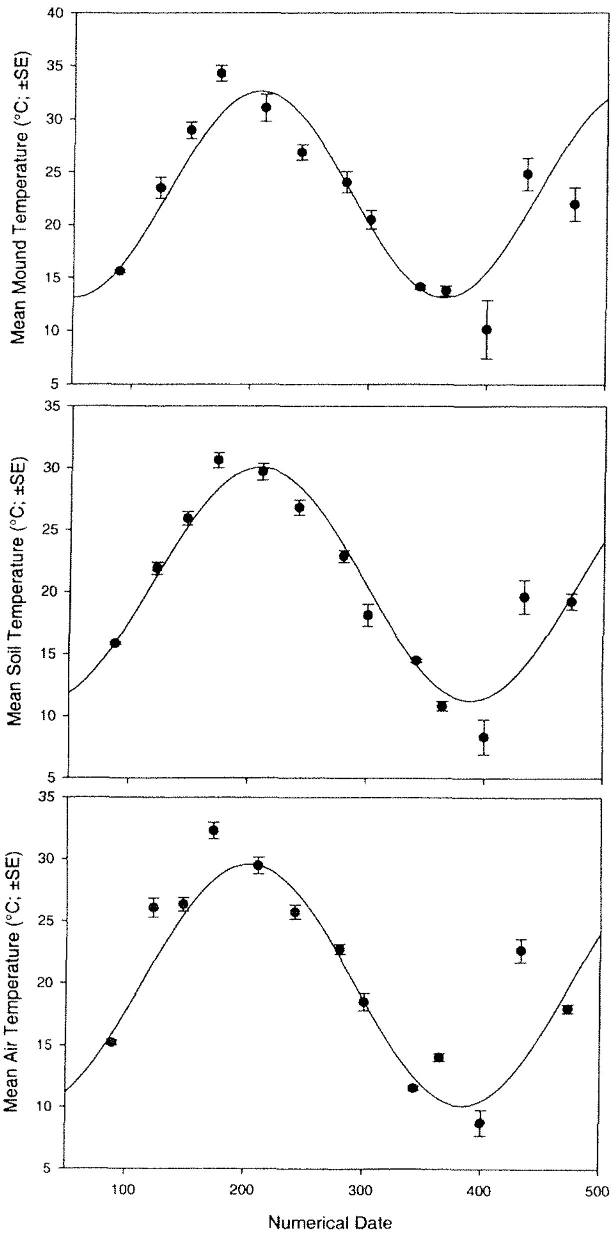

Fig. 3. Mean (± SE) mound, soil, and air temperatures by sampling date from March 1999 to May 2000. Curves denote models detailed in Table 1.

and 0.3°C higher than mound temperatures, respectively) and soil temperatures (exceptions: March 1999 and December 1999, when mean soil temperatures were 0.2 and 0.4°C greater than mound temperatures, respectively) in 65.3% and 65.9% of the samples (n=176) respectively. Mean soil temperatures ranged from 0.6 (September 1999) to 5.2°C (March 2000) below mound temperatures. Mean air temperatures ranged from 0.4 (March 1999) to 3.9°C (April 2000) below mound temperatures. Mean mound temperatures ranged from 10.1±2.8°C (February 2000) to 34.2±0.8°C (June 1999). Mean soil temperatures ranged from 8.34±1.4°C (February 2000) to 30.6 ±0.6°C (June 1999). Mean air temperatures ranged from 8.74±1.04°C (February 2000) to 32.3±0.7°C (June 1999). Mean temperatures of mounds containing termites were not significantly different from those containing only ants (T = 1.18; df = 138; $P = 0.24$). When differences between mound and soil temperatures were compared, mounds containing termites did not have significantly different temperature differences than did mounds without termites (T = 0.945; df = 138; $P = 0.35$). Thus, in terms of both absolute and relative temperatures, mounds containing termites did not differ significantly from those without termites. Nonlinear regression models indicated that a sine wave equation of the form:

$$\text{Temperature (°C)} = a + b\sin\left\{\frac{2\pi \times day}{d} + c\right\}$$

described each of the temperature patterns throughout the year. In this equation, *day* is the numerical value of the date starting from 01 January 1999, *a* is the offset from zero (or mean) temperature, *b* is the amplitude or difference between the extreme and the mean temperatures, *c* is the phase or correction factor for *day* and *wavelength*, and *d* is the wavelength or number of days between successive iterations of the same part of the wave (or season, Fig. 3). Table 1 provides the statistics and values for *a-d* in mound, soil, and air temperature equations.

Table 1. Variable list for sine model describing the relationship of mean air, soil, or mound temperatures during 1999-2000.

Area	*a*	*b*	*c*	*d*	dF	F	*P*	Adj. r^2
Air	19.84±0.83	9.73±1.3	4.32±0.19	361.3±14.5	3, 12	21.2	0.0002	0.81
Soil	20.6±0.48	9.40±0.64	4.20±0.11	359.3±10.9	3, 12	87.5	<0.00001	0.95
Mound	22.86±0.80	9.74±0.88	3.70±0.20	312.9±16.1	3, 12	45.3	0.00001	0.91

Caste proportions

All subterranean termites collected from fire ant mounds in this study belonged to the genus *Reticulitermes*. Plotting caste proportions over time (Fig. 4) indicates that worker proportions are greatest at all dates as expected. However, soldier proportions increase during fall and winter, as does the proportion of young workers in the samples. Interestingly, the greatest change in caste proportion comes at the beginning of winter (December 1999), with worker proportions declining to 0.33 and young workers increasing to 0.67. Soldier proportions reached their zenith (0.33) just prior to the increase in young worker proportion. Nymph proportions never reached above 0.03 during the course of this study, with a maximum at the end of fall 1999. Fig. 4 indicates that during the late fall to early winter months a change in the caste proportions of fire ant mound-inhabiting *Reticulitermes* spp. takes place: in late fall nymph proportions increase briefly, followed by an increase in soldier proportion, with a final peak of young workers at the start of winter. No termites were detected in fire ant mounds from early 2000 to the end of the study (March 2000, Fig. 2).

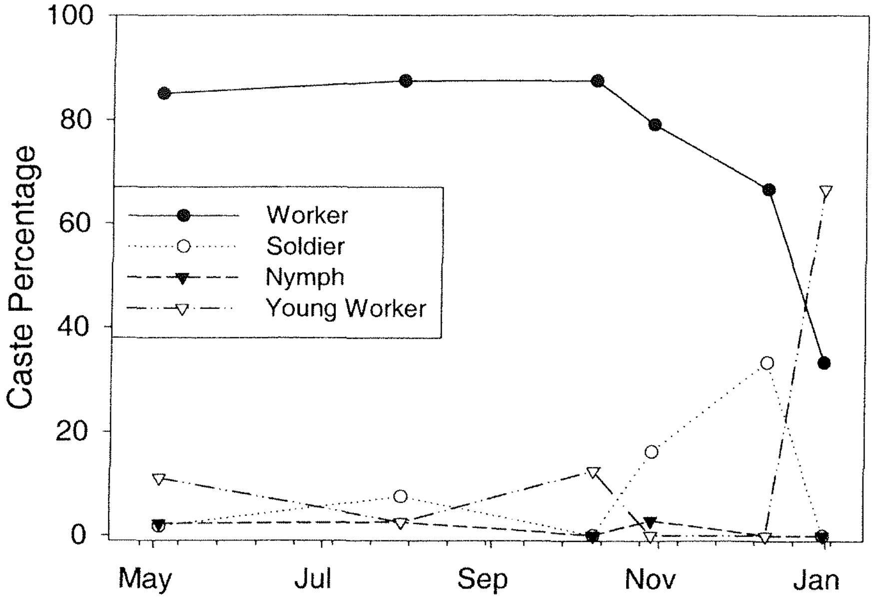

Fig. 4. Caste proportions (as %) by sampling date.

DISCUSSION

Both caste proportions and overall mound infestations varied substantially from literature values (Shelton *et al.* 1999). Even during the spring months, nymph proportions did not rise above 3%. Simultaneously, mound infestation rates were down throughout the year, never rising above 10%, peaking during fall and winter months of 1999. Shelton *et al.* (1999) found an overall infestation rate of 19% from 5 field sites, including the site examined in this study. That study did not examine differences in mound infestation between sites, but site infestations in that study ranged from 0 to 42.9%. Due to differences in sampling dates among sites direct comparisons of site infestations were not presented in that paper. The site used in the current study had been 23.1% infested previously (March 1997, Shelton *et al.* 1999), while in March of 1999 and 2000 the infestation rate was 0% (current study). These data suggest considerable variability in site infestation percentages over time.

There were no differences in the temperatures of mounds that would predict infestation by termites. These data suggest that termites do not seek out particular *S. invicta* mounds (or even ant mounds in particular) having much higher temperatures, but instead encounter and remain in areas of greater soil temperature (which also happen to be unused sections of fire ant mounds). Other authors have reported the use of termite mounds (Termitidae) by ants, including *Solenopsis* spp. (Whitcomb 1974, Redford 1984, Wojcik 1986). Wojcik (1986) observed that ant infestation of termite mounds and galleries in Brazil was not likely the result of movement away from flooded areas. The current study as well as Shelton *et al.* (1999), indicate that flooding is probably not responsible for the occurrence of termites in ant mounds. Of the five sites used by Shelton *et al.* (1999), only the Tuskegee site (used in the current study) had areas that might be flooded. However, only part of this site (~ 40%) was close enough to the pond for flooding, and flood conditions were not observed during the study.

To demonstrate the importance of temperature on development of termites, we can develop a model based on previously published data. Buchli (1958) examined instar duration of *R. lucifugus santonensis* de Feytaud workers for a variety of instars. There were differences between durations based on the nestmate caste composition in the groups measured. Unfortunately, developmental durations at lower temperatures were not examined for the various caste compositions (Buchli 1958). For our model we have used data from his experiment using single individuals, since only it includes data for multiple temperatures. From his data sets for 19.5 and 25°C, we can develop the following equation relating duration of the 3rd instar to environmental temperature: Duration of 3rd instar (in days) = -10.0xTemperature (in °C) + 265.0

The increased proportion of young workers in the December 1999 sample needs explanation. While at other times, the proportion of young workers was elevated above 0, at no other sample was the proportion as high as the 0.667 from late December 1999. Comparing the January 2000 sample with that of December 1999, the temperatures are similar, yet in January 2000 the large increase in young worker proportions is not observed. These data are not consistent with the hypothesis that a minimum soil temperature is necessary to induce movement of young workers into *S. invicta* mounds. The answer to this contradiction may lie in Fig. 3. Only during the late December 1999 sample do the standard errors in mean soil and mound temperatures not overlap. It may be that while there is a difference in the mean values of soil and mound temperatures in January 2000, this difference is not biologically significant, and perhaps does not justify the movement of young *R. flavipes* into *S. invicta* mounds.

For all termites captured in *S. invicta* mounds during this study, wood was found in the mound. This is in contrast to the findings reported by Shelton *et al.* (1999) who did not always find wood in termite infested ant mounds. However in 40% of the samples taken, the termites were not found in or on the wood in the mound. Wood in a *S. invicta* mound is a poor predictor of termite presence, as only 14.3% of the mounds containing wood also contained termites. Of course in the dynamic relationship between termites and ants, it may be that termites had formerly infested all wood found in ant mounds, but had been evicted or eaten by the ants prior to our sampling. Termites may also abandon and revisit foraging areas over time as well, as seen in baiting and monitoring devices (Grace *et al.* 1996, Tsunoda *et al.* 1998).

Caste proportions in this study show a different picture of the *Reticulitermes* spp. infestation populations in *S. invicta* mounds compared with previous studies. Our previous work has shown that during the spring, nymph proportions were greater than worker proportions within the mounds (Shelton *et al.* 1999). Our data (present study) indicate that while termite colonies may use *S. invicta* mounds, hypothetically for their increased temperature regimes, different colonies may have different needs for increased temperature. In the fall and early winter of 1999, the proportion of young workers in the samples increased in relation to other caste proportions in ant mounds. These increases in young workers may indicate a change in temperature requirements of colonies, moving from emphasis on reproduction to emphasis on worker capacity.

This paper has presented a more elaborate investigation of the interactions of *S. invicta* and subterranean termites in the Southeastern U.S. Important questions remain regarding other risks possibly taken by termites to alter their thermal environments, as well as methods by which termites may avoid their natural enemies in such environments. We encourage other researchers to consider the physiological ecology of ant-termite interactions in future studies.

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