Size of food resource determines brood placement in *Reticulitermes flavipes (Isoptera: Rhinotermitidae)*

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ABSTRACT

Most species of subterranean termite house and care for larvae in specialised chambers or complex nurseries. In addition to these chambers, the genus *Reticulitermes* also keeps larvae at sites where foragers are feeding, a trait more commonly found in damp wood and dry wood termites. This phenomenon of holding larvae at foraging sites is quite well known among researchers who work with ***Reticditermes, yet*** it has not been investigated to any extent. Consequently, the underlying causes of larvae placement at foraging sites are only poorly understood.

During assessments of the resistance of materials to ***Reticditermes flavipes*** attack at the Harrison Experimental Forest, near Saucier, Mississippi, USA, significant numbers of 1st and 2nd stage larvae were often found in *Pinus* spp bait wood stakes.

This paper describes the results from the first of a series of ongoing experiments to test whether food resources affected termite presence and larval placement. The experiment used a variable number of pine stakes in eight plots (four in stands of pine, four in grassy forest clearings) where termites were offered a choice of food resources of diierent size (bundles of 1 to 4 pine stakes of equal size). The number of larvae recorded from feeding sites was a close reflection of the number of workers attracted to a given food source. The more substantial and suitable a food source is, the more foragers will visit it, and in turn the more likely that they will transport larvae and eggs to these feeding sites.

KEYWORDS: Subterranean termites, reproductive biology, castes, larvae, workers, soldiers

1. INTRODUCTION

In recent years major advances have been made in determining colony size and caste composition, extent of the foraging territory, seasonal patterns of foraging activity, interactions between populations and species and population genetics for subterranean termites of ***the*** genus ***Reticditermes*** in a variety of environments. (e.g. Clement 1986, Forschler 1998, Jenkins et al. 1998, 1999, Su et al. 1993, overview in Thome 1998;). Colony

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organisation in *Reticulitermes* encompasses simple families established by a pair of primary reproductives, as well as more complex systems of several interconnected breeding units headed by varying numbers of neotenic reproductives (Thorne et al. 1999). Species, locality, environmental factors and population size have been suggested to affect the pattern of colony structure. Nevertheless, only scant information is available for many aspects of the breeding biology of this group of termites.

During inspections of bait wood stakes in experiments that evaluated diierent methods for assessing the in-ground resistance of materials to subterranean termites at the Harrison SF, Mississippi, (Lenz et al. 1998, 1999) sometimes significant numbers of young larvae (L1, L2) of ***R flavipes*** were noted at these feeding sites. Locating and caring for the larvae right at the place where foragers are collecting food is an unusual strategy for subterranean termites. It is well known for dry wood and damp wood termites, termites of the one-piece life type (Abe 1987) for which feeding and nest site are identical. In species of subterranean termites, larvae are normally kept in special brood chambers or nurseries and foragers return to the nest for the provisioning of dependant castes and stages.

US termite researchers are well aware of this behaviour, which appears to be observed increasingly due to the proliferation of experiments monitoring populations of ***Reticditermes*** species over time with a variety of monitoring devices (B.T. Forschler pers. corn.). However, there are few published references to this phenomenon Howard & Haverty (1981) found no significant difference in caste composition of ***R flavipes*** populations, including the presence of larvae, in source colonies and large bait stakes positioned close to pine logs occupied by the colonies. Grace (1996) recorded larvae in traps of corrugated cardboard at sites in Ontario (in numbers not exceeding 1% of the total number of termites). Thome et al. (1999) observed that workers in laboratory colonies of ***R flavipes*** moved eggs and larvae between interconnected chambers.

Reticditermes colonies have poorly defined nests (Howard & Haverty 1980) and they are known for their mobility in response to seasonal and other environmental factors (Clement 1986, Howard et al. 1982). Lenz (1994) pointed to the importance of a key environmental factor - variations in the quantity and quality of available food resources - for the reproductive strategies and success of a termite colony. We report here the results of a study that investigated the affect of food volume on the placement of larvae by ***R flavipes***.

This paper provides only a brief summary of methods and results; a more detailed account will be published elsewhere.

2. MATERIALS AND METHODS

2.1. Study sites

2.1.1. Gulfport, Harrison Experimental Forest

The Harrison Experimental Forest (30°38'N, 89°03'W) near Gulfport, Mississippi, is located close to the coastline of the Gulf of Mexico and experiences a humid, subtropical climate. The natural forest has been largely replaced with pine plantations, the main species are ***Pinus palustris*** Mill. and ***P. elliottii*** Engelm. There ***are three*** termite species, ***all Reticulitermes, with R. flavipes*** (Kollar) ***the*** most common.

Two plots were located within mature pine forest (no. 1 and 2) and four plots were placed at two sites of open grassland and shrubs; these were 10 to 20 m from the forest edge (no. 3 to 6).

2.1.2. Laurel

Two additional plots (no. 7 and 8) were located in a small plantation of *P. taeda* L. (established 1990) near Laurel (31°70'N, 88°90'W) in northern Mississippi. The climate at the Laurel site is similar to that at the Harrison Experimental Forest[, except that it is slightly drier. *R. flavipes* is commonly encountered.

2.2. Experimental Design

Sii replicate bundles of one, two or four sapwood specimens (25 x 40 x 300mm) of New Zealand grown *P. radiata* D.Don were offered to termites in each plot using a variation of the graveyard method. The specimens in a plot were installed in random sequence in three ca. 3m long rows with a spacing of 250mm between specimens and lm between rows (for further details see Lenz et al. 1998). Bundles of two or four stakes were held together with rubber bands before inserting them into the soil.

The experiments were installed in May 1998. After 12 months all stake bundles were carefully removed from the ground, scored for the level of termite attack (see Lenz et al. 1998) and placed individually into plastic boxes. Any termites present on and inside the stakes were later extracted in the laboratory and preserved in 70% ethanol. Termites remaining in the soil immediately surroundii the stakes were not collected.

Termite samples were sorted into larvae (L1, L2), young workers (W1, W2), older workers (W3 and above), presoldiers and soldiers and nymphs, taking information from Buchli (1958), Thome (1996) and Thorne et al (1998) as a guide for characterising castes and stages. Numbers of termites for each category were recorded. The presence of eggs was noted, although the number of eggs was established in only a few cases.

2.3. Analysis of results

Plots were grouped into two categories according to vegetation cover: 'pine woods comprised plots 1, 2, 7 and 8, and plots 3 to 6 were placed in 'grassy clearings' (within the pine woods). The number of stake bundles with signs of termite attack and presence of termites was recorded for each plot. This data was analysed as the proportion of bundles attacked or occupied in each plot, with the number of stakes per bundle (1, 2 or 4 stakes) and vegetation cover (in woods or clearing) as the two factors, Proportional data were arcsine square root transformed to improve homogeneity of variance assumptions.

The level of termite attack (data not available for plot 2) was recorded as an estimate of loss of cross section (Lenz et al. 1998) of stakes. These data were analysed using the Kruskal-Wallis (non-parametric) test as the number of bundles attacked or occupied in each plot, with vegetation cover (in woods or clearings) as the factor.

The number of all termites, workers, soldiers and larvae present were tested against vegetation type and bundle sii in two way ANOVA: N was 18 stake-bundles in each plot, so 72 replicates in each vegetation type and 48 replicates in each bundle size, a total of 144 in the experiment. Numbers were log (normal) transformed to improve homogeneity of variance assumptions. The number of workers, soldiers and larvae were correlated against one another (Pearson's correlation matrix) to test whether the presence of dependents varied with worker numbers (all statistics follow Sokal and Rohlf 1995).

3. RESULTS

Termites attacked almost all stake bundles except for four in wood plots. Not all bundles that were attacked had termites feeding actively in them at the time of inspection: in the four pine wood plots 9, 4, 5 and 3 bundles respectively, all consisting of single stakes, were attacked initially but abandoned later, whereas the same occurred only with one single stake among all plots in clearings (K-W test, $p = 0.018$). Significantly fewer stake-bundles had termites in the pine woods (K-W test, $p = 0.017$). The presence of eggs did not differ significantly between stake-bundles in pine woods and grassy clearings (K-W test, $p = 0.557$), nor did the presence of larvae differ (K-W test, $p = 0.081$), but the latter was due to wide variation within plots (Tables 1 and 2).

Table 1 The number of stake bundles with presence of termites, eggs and larvae in each plot. N.b. maximum number in a plot = 18.

Vegetation	Plot	No. attacked & abandoned	No. with termites	No. with eggs	No. with larvae
Grassy clearings	3	0	18	3	13
	4	1	17	5	16
	5	0	18	6	10
	6	0	18	2	10
Pine woods	1	9	9	6	9
	2	4	14	1	11
	7	5	10	1	2
	8	3	14	5	8

Table 2. The proportion (mean ± std error) per plot of stake-bundles abandoned, with termites, eggs or larvae in each vegetation type. Numbers in columns followed by different superscripts are significantly different.

Vegetation	No. attacked & abandoned	No. with termites	No. with eggs	No. with larvae
Grass clearing	0.014 ± 0.012”	0.986 ± 0.012”	0.222 ± 0.044”	0.68 1 ± 0.069’
Pine woods	0.292 ± 0.063^{b}	0.653 ± 0.063^{b}	0.181 ± 0.063’	0.417 ± 0.093^{a}

The number of termites found depended significantly on the vegetation type and bundle size. For the total number of termites recorded, there were significantly more individuals present in grassy clearings than in pine woods, ($F_{1,138}$ = 46.2 11, $p < 0.001$). Termite numbers increased significantly with an increase in stake numbers per bundle ($F_{2,138}$ = 17.782, $p < 0.001$). These effects were independent and additive, as the interaction was not significant ($p = 0.105$) (Fig.

1). Interestingly, although the number of termites increased with stake-bundle size in grassy clearings (i.e. $1 < 2 < 4$), the number of termites in pine woods did not increase when more than 2 stakes were available (i.e. $1 < 2 = 4$) (Fig. 1).

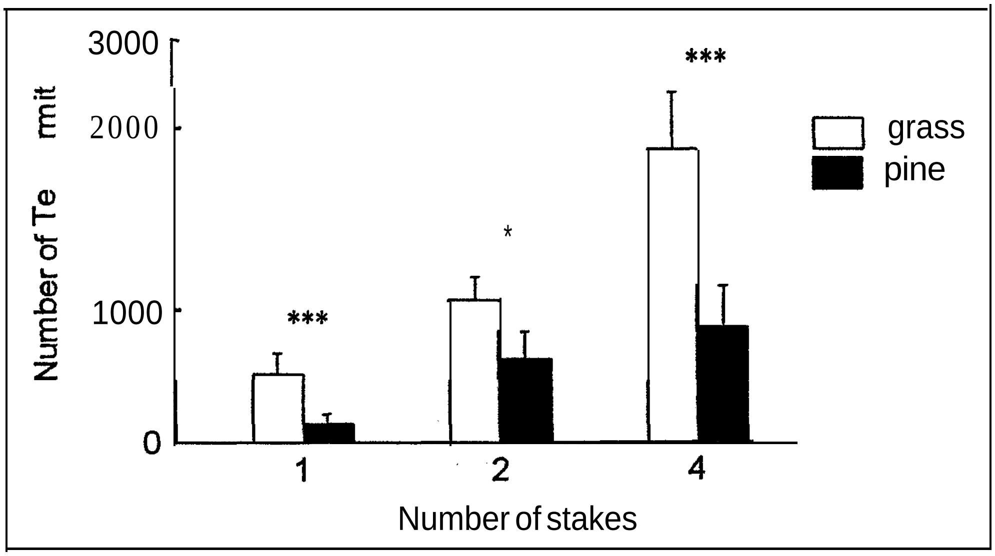
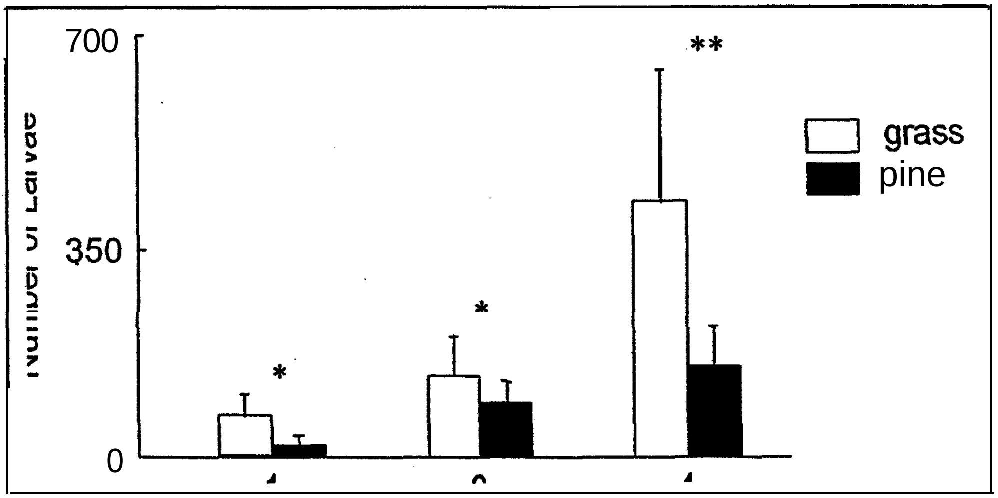


Figure 1 Number of all termites in stake-bundles in each vegetation type. Paired comparisons between grassy clearings and pine woods denoted as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, *NS*.

Figure 2. Number of larvae in stake-bundles in each vegetation type. Symbols of statistics as for Fig. 1.



Significantly larger numbers of larvae were found in grassy clearings than in pine woods ($F_{1, 138} = 4.821$, $p = 0.030$) and also in bundles of 4 than 2 than 1 ($F_{2, 138} = 7.633$, $p < 0.001$). As for all termites, effects were independent as the interaction was not significant.

The number of larvae, soldiers and workers were all strongly correlated. The tightest correlation was between workers and soldiers (Pearson's $r = 0.884$), but the correlations between workers and larvae (Pearson's *r* = 0.682) and soldiers and larvae (Pearson's *r* = 0.711) were also tight. All three correlations were significant (with Bonferroni-corrected probabilities: Bartlett ÷ 2 = 3 17.57, d.f. = 3, *p* £ 0.001) (Fig. 1). Due to the tight correlations, all types of termites, such as workers, soldiers and larvae, follow the same pattern seen for all termites added together (compare Fig. 2 with Fig. 1).

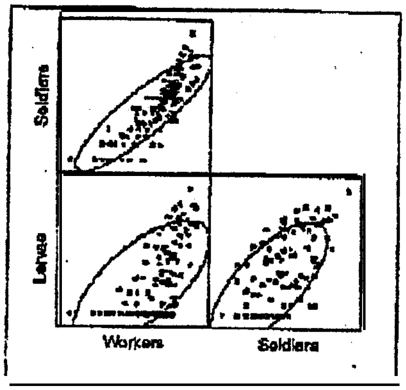


Figure 3. Correlation between numbers of workers, soldiers and larvae. Gaussian confidence ellipse drawn *p = 0.683.*

4. DISCUSSION AND CONCLUSIONS

In this study, every increase in food resource volume attracted larger numbers of termites. At this stage we cannot assign this increase in termite numbers solely to an increase in wood volume. Since the different volumes of food were not offered as single pieces of timber but rather were composed of several stakes, the concomitant increase in surface area and the availability of small gaps between stakes, rendering a food source more easily accessible for termites, may have favoured the colonisation of larger bundles.

Colonisation patterns of different volumes of food differed between the two vegetation types. In clearings with a lower preponderance of other food sources all but one stake bundle attracted termites. In pine stands, with a greater abundance of alternative food, termites entered and remained in bundles of two and four stakes, whereas they often abandoned single bait stakes, even though initial attack might have been deep. This is the clearest indication that volume of the food resourceper se mattered when termites decided which resources to exploit, to what extent and where to locate dependents. Field trials currently under way will address more specifically the question to what extent the termite response to increases in wood volumes differs when identical amounts of timber are offered as single or composed pieces.

It is also evident from the differences in termite numbers at stake bundles between clearings and pine stands how important for termites availability to alternative food sources is in determining activity patterns at any given food item. These observations have relevance to the understanding of the relative attraction, visitation rates and effectiveness of bait systems for controlling infestations of subterranean termites in different habitats.

The number of larvae recorded from feeding sites is a close reflection of the number of workers attracted to a given food source. The more substantial and suitable a food source is, the more foragers will visit it, and in turn the more likely that they will transport larvae and eggs to these feeding sites.

Thome et al.(1999) indicate that it may be a kind of insurance policy for colonies of *Reticditermes* against risk of predation and retaining reproductive capacity if parts of a colony are cut off by natural disasters (e.g. flooding) to disperse multiple neotenics over several breeding centres. Similarly, distributing larvae over several feeding sites instead of housing them in a special chamber that may be difficult to defend (due to the diffuse nest structure in this group of termites), may be the best strategy to maximise survival of the brood. At the same time this strategy could present an ergonomic advantage, in that the need for workers to communicate frequently between feeding site and brood chamber to provision the offspring is reduced.

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