Laboratory Bioassay on Termite Wood Consumption Using Two Different Substrates (Isoptera)

by

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ABSTRACT

In Australia, laboratory bioassays with subterranean termites typically contain groups of workers and soldiers in a substrate of mound material, and/or vermiculite and sawdust. However, the termite substrate used may affect termite consumption. This hypothesis was tested using five different colonies of *Coptotermes acinaciformis* (Froggatt). Blocks of *Pinus radiata* (D. Don) 20 x 20 x 5 mm were exposed in two different substrates to one gram of termites from five colonies for 56 days, and the wood consumption rate was calculated. Termites did not always behave similarly on the different substrates. The amount of wood consumed in the sand/mound material substrate was significantly higher than in mound material alone. The use of termite mound material as a major substrate did not encourage maximum termite feeding of the wood blocks.

Key words: workers, soldiers, subterranean termites, *Coptotermes acinaciformis*, consumption, *Pinus radiata*, colonies.

INTRODUCTION

The standard Australian technique for laboratory bioassays with subterranean termites (Gay *et al.* 1955) typically employs orphaned groups of termites on substrates of termite mound material and/or vermiculite and sawdust (Creffield *et al.* 1985; Lenz *et al.* 1987). Laboratory studies have been undertaken to evaluate variations in consumption within and between mound colonies of *Coptotermes acinaciformis* (Froggatt) (Creffield *et al.* 1985), and the variability in vigor between colonies of these termites has been recorded (Lenz 1985). Similar studies have been reported overseas with respect to

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measuring wood consumption and wood preferences in termites (Su and La Fage 1984, Waller *et al.* 1990, Getty & Haverty 1998, Su & Messenger 2000, Cornelius & Osbrink 2001, Fei & Henderson 2002, Ripa *et al.* 2002, Nakayama *et al.* 2004, Uchima & Grace 2003, Morales-Ramos & Rojas 2003, 2005, Katsumata *et al.*, 2007).

Laboratory bioassays provide a first step in testing potential termiticides, and assist in formulating recommendations for regulatory agencies and users (Cornelius & Lax 2005, Perrott *et al.* 2004, Peters & Fitzgerald 2004). However, such bioassays are not designed to predict effects on natural populations or on ecosystems at large (French 1988).

Furthermore, many investigators have related wood consumption to the initial termite biomass introduced into test chambers (Su and La Fage 1984, French *et al.* 1996, Morales-Ramos & Rojas 2005). Most of these studies have examined termite wood consumption using a single substrate. Information is scarce on comparisons of the amount of wood consumed (and termite survival) by termites from various colonies on different substrates. One would expect to observe variations in wood consumption when comparing termite feeding using two nutritionally different substrates. In toxicological terms, the aim should be to observe how the test termites react to the candidate active ingredient, rather than to the nutritional substrate. In other words, such bioassays require a substrate with minimum nutritional components in order to obtain the most efficacious physiological effects. Bioassayists need to design for maximum termite feeding of the candidate termiticide, and the emphasis of termite feeding on a substrate needs to be minimised, as it is not the major focus of such toxicological bioassays. With this in mind, we ask the question, "does termite mound material encourage maximum termite feeding of the wood blocks impregnated with candidate termiticides?" It would seem somewhat counterproductive to have the termites feed more vigorously on the substrate over the test period than on the wood blocks impregnated with various concentrations of the candidate termiticide.

To test this hypothesis the following bioassay was undertaken. Our approach was to observe the amount of wood consumed by various colonies of *C. acinaciformis* when contained in mound material alone or in sand and mound material. The proportion of the original material in the wood block

consumed was the index of interest and statistical analysis involved carrying out analysis of variance on the arcsine transformation of this index. For the purposes of this paper, the term "termite" refers to "subterranean termite".

MATERIALS AND METHODS

Termite species

Workers and soldiers of *C. acinaciformis* were baited from active, mound colonies near Townsville, north Queensland. After collection the orphaned groups were transported by air to our laboratory within 24 hrs and maintained in the insectary rooms (27°C, 75% R.H.) to acclimatise for two days before being used in the bioassay evaluation.

Timber

All the wood specimens were prepared from radiata pine (*Pinus radiata* D. Don) and cut to 20 x 20 x 5 mm, with the grain in the 5 mm direction. The wood blocks were vacuum oven-dried for four days at 40°C and weighed prior to use.

Bioassay condition

Clear styrene cylindrical vials (No. 16; 35 mm wide, 70 mm high) were used in the bioassay. One gram (comprising workers and soldiers in collected proportion) of the test termites were placed in each plastic vial. Half the vials contained mound material (10 g) from a *C. acinaciformis* mound and the remainder contained a similar amount of 50/50 by volume of mound material and washed river sand (>2.0 mm diameter). The sand was thoroughly washed further with acetone, mixed and allowed to dry in a fume cupboard. Each vial contained a single wood block, with eight replicate vials per colony. Five colonies of *C. acinaciformis* were used in the bioassay. All vials were capped with a vented lid.

C. acinaciformis bioassays were conducted in a conditioned room of 27°C and 75% relative humidity (R.H.) for a period of 56 days. Inspections were undertaken on a weekly basis and the vials were rotated 180° at the inspection in order to reduce any effects of light on foraging termite behavior and test results.

Test criteria

After completion of the bioassay, the blocks were again vacuum oven dried and weighed.

RESULTS

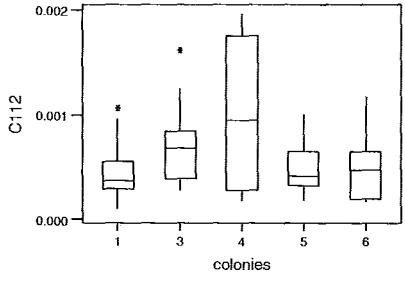
The number of termites in one gram from the different colonies varied (Table 1). While the degree of variation is not large, it is characteristic of the variation to be expected within random groups of individuals randomly selected from the colonies.

Table 1: Three groups of one gram of each colony were collected. Mean number (Standard deviation) of *C. acinaciformis* individuals in one gram groups from the five colonies measured prior to the bioassay.

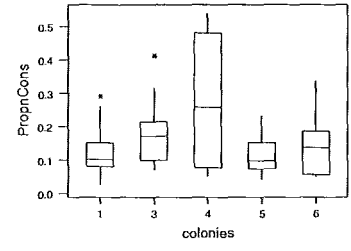
	Number of termites per gram			
Colony #	Group 1	Group 2	Group 3	Mean
1	276	273	275	275(1.5)
3	250	257	255	254(3.6)
4	271	278	273	274(3.6)
5	232	235	232	233(1.7)
6	292	290	285	289(3.6)

Key: Colony # = colony number, Group = individual termites in one gram

Notice that colony 4 is poorly represented compared to the other colonies but it is the colony for which proportion consumption has been the highest. The boxplots for the data are presented in Fig. 1



A



B

Fig. 1: proportion of specimens consumed per termite of each colony (A), and proportion of specimens consumed by each colony (B).

To enhance some statistical properties it is not uncommon to transform proportions by the arcsine of the square root of the proportion.

Treating the two groups of colony 1 as just one colony:

If the two groups of colony 1 are treated as if they are one colony, then analysis based on the arcsine transformation of proportion finds a significant difference between colonies ($p=0.001$) and between substrates ($p=0.006$) and there is no colony BY substrate interaction ($p=0.257$).

Treating the two groups of colony 1 as two different colonies:

Recall that colony 1 differs between its group A form and its group B form (see page 4) so it is probably judicious to analyze the data as if colony 1 was really two (pseudo) different colonies however if this is done then not surprisingly we get exactly the same result as above albeit with different levels of significance. In particular, a significant difference between colonies ($p=0.002$) and between substrates ($p=0.005$) and there is no colony BY substrate interaction ($p=0.363$).

Colony differences within substrates:

Table 2: Mean, maximum, and minimum feeding rates of five colonies of *C. acinaciformis* when placed on mound material and sand/mound material substrates. Eight replicates per treatment with the test period of 56 days.

Termite colony No. & rate of consumption	Mound material µg wood/ termite/day	Sand/mound material µg wood/ termite/day
1 Mean	6.3	9.6
Max.	9.9	18.9
Min.	4.8	1.9
3 Mean	6.1	13.9
Max.	8.4	22.3
Min.	3.8	5.2
4 Mean	12.4	20.4
Max.	17.2	34.6
Min.	7.2	4.8
5 Mean	8.8	9.5
Max.	14.7	20.5
Min.	4.4	3.6
6 Mean	6.9	10.9
Max.	12.9	22.7
Min.	3.1	3.2

KEY: Max. = Maximum amount consumed, Min. = Minimum amount consumed.

(A) On mound material substrate all colonies are not significantly different and all colonies consume about the same although colony 4 seems to consume marginally, but not statistically, significantly more. Colony 4 (in this part of the analysis) only has 3 specimens in the mound material substrate case and perhaps it should be excluded from the analysis. If this is done there is still no significant difference between colonies with respect to the amounts consumed on mound material substrate.

(B) On sand and mound material substrate there is a significant difference with respect to the amount consumed ($p=0.015$); colonies 3 and 4 consume more than the other colonies. Basically the difference occurs because colonies 1 (group 1) and 5 consume significantly less than the other colonies on sand and mound material.

Substrate differences within colonies:

The amount consumed on each substrate differs significantly for colony 3 ($p=0.0003$); colony 1 (Group B) is borderline ($p=0.063$).

DISCUSSION & CONCLUSION

Colonies did not behave the same on similar or different substrates. Also, the amount of wood consumed was not the same in the substrates tested over the 56 days. There was a significant difference between colonies ($p=0.002$) and between substrates ($p=0.005$). However, consumption was significantly ($p=0.015$) higher in sand/mound material substrate compared with the mound material alone. The termites consumed about the same amount of wood on the mound material (ranging from 6.1 to 12.4 μg wood /termite/ day), with colony 4 registering the highest amount of feeding at 17.2 μg wood/termite/day.

Termites from colonies 4 and 6 generally consumed significantly ($p=0.015$) more wood than termites from the other colonies on the sand/mound material substrate (up to 34.6 and 22.7 μg wood/termite/day respectively). These findings suggest that "substrate" is an important factor in laboratory bioassays conducted over a 56 day period in which candidate termites are screened. Toxicologically speaking, it would seem somewhat counterproductive to have the termites feed more vigorously on the substrate over the test period rather than on the wood blocks impregnated with various concentrations of the candidate termiticide.

Thus, we would recommend that there is a need to repeat termite feeding consumption rates using various proportions of the sand/mound material mix (say, 10, 20, 40, 80 % sand). Furthermore, there will need to be a sand only substrate in any future laboratory comparison. However, the data gathered so far strongly suggest that the emphasis needs to be placed on bioassay conditions that enhance termite feeding of the treated wood blocks containing a candidate termiticide, rather than on the termites consuming an introduced substrate. Particularly, a substrate that has nutritional value and can allow the termites to survive over the test period reduces the need for termites to feed on wood blocks impregnated with a termiticide. For some bioassays it may be better to prevent the termites from having a choice, when the aim is to evaluate an active ingredient, rather than to prolong termite survival. Laboratory bioassays need to answer the "big question" unequivocally, and not leave the evaluator with doubt as to precisely what the results indicate, as was shown in the international collaborative laboratory of two wood preservatives against subterranean termites (French *et al.* 1996).

Basically, this present experiment highlights the need to be ever critical and objective with bioassay schedules and the need to effect procedural changes when inconsistencies or statistically significant findings are confirmed that suggest the bioassay system needs refining to achieve maximum validity. To do otherwise is to continue to make a Type I error in termite experimentation (= bioassays), that is, to reject the hypothesis when it is true.

We propose that in future research on the estimation of termite wood consumption rates as affected by sub-lethal and lethal doses of termiticides in wood blocks that consideration of the bioenergetic relationships of the termites be taken into account. Termites must be considered highly efficient detritovores. Such high production efficiency appears to be characteristic of poikilotherms in contrast to homeotherms (Schowalter *et al.* 1977). Furthermore, it has been concluded that ecologists should directly determine the energy content of ecological materials when studying energy flow through natural systems (Golley 1961). Thus, if termite researchers estimated the energy budget of test termite's reared on artificial diets under constant conditions and at various seasons, the termite research establishment would be able

to compute the net production energy efficiency of the most economically important termite pest species. As Schowalter *et al.* (1977) have shown the energy budget of an individual or population can be expressed by the equation I = P + R, where I = ingestion,. R = respiration and E = egestion, and assimilation (a) = (I-E).

This approach would indicate termite production under the conditions of test. Now, introduce various concentrations of termiticides into this equation, and compare the difference in energy flow between treated and untreated regimes of treatments. This bioenergetic approach would hopefully deduce the real effects of termiticides on termite vitality, which has not been previously included in standard termite bioassays.

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