Selection of Tunneling Substrates for Laboratory Studies with Three Subterranean Termite Species

by

Michael I. Haverty[1]

ABSTRACT

Different tunneling substrates for *Reticulitermes flavipes* (Kollar), *R. virginicus* (Banks) and *Coptotermes formosanus* Shiraki were compared to a standard mixture of sand and water (5:1 v/v). Among these substrates were sand, vermiculite, and sand and vermiculite (1:1 v/v) at various moisture contents. For tests of short duration (4 to 8 weeks) sand was judged the most suitable substrate because termites are more easily separated from sand than from vermiculite. For long-term tests (12 weeks or more) vermiculite and a sand and vermiculite combination would appear to be best because of greater water-holding capacity.

INTRODUCTION

The suitability of substrates for laboratory studies with subterranean termites depends on varying test requirements. The capacity to retain moisture is primary as Becker (1969) emphasized. In a recent research program to determine whether termites could be controlled through disruption of their colony caste structure, the problem of moisture retention prompted a study of various substrates.

It was soon discovered that 12 weeks was the minimum amount of time required for *Coptotermes formosanus* Shiraki to reach an equilibrium caste ratio (Haverty 1979). *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks), having a slower rate of differentiation, often required more time than *C. formosanus*. Containers with termits in moist sand often dry out when left undisturbed for more than 8 weeks. Opening the container to replenish the water usually causes considerable disturbance and some termite mortality. To avoid these problems it was necessary to select a substrate which will not dry out if left undisturbed for long periods of time and encourage high survival.

[1]Research Entomologist, Forest Service, U.S. Department of Agriculture; formerly at the Southern Forest Experiment Station, Gulfport, Mississippi 39503, now at the Pacific Southwest Forest and Range Experiment Station, Berkeley, California 94701.

The standard substrate previously used in the laboratory was 35 ml of sterile, washed sand moistened with 7 ml of water for 100 termites (Smythe and Carter 1969). From substrates suggested or used in the past three were selected for evaluation: sand, vermiculite, and a combination of the two (1:1, v/v), each substrate being prepared at various moisture contents. The purpose was to select a substrate for each of the three termite species which would give results at least equivalent to the sand and water standard over a short test period of 8 weeks, and yet have the potential to provide sufficient moisture for subsequent tests taking up to 15 weeks.

In selecting the substrates for testing, account was taken of the experience of others who have worked with various materials, as follows: vermiculite (Becker and Lenz 1970; Howick and Creffield 1975; Lenz *et al.* 1976), vermiculite and sand (Fougerousse 1973; Lenz *et al.* 1976), vermiculite and sawdust (Howick 1975), soil (Sen-Sarma and Chatterjee 1970), mound material (Gay *et al.* 1955), polyurethane foam (Hinterberger 1976), and sawdust (Esenther 1977). Many authors (Becker and Lenz 1970; Fougerousse 1973; Lenz *et al.* 1976) feel that vermiculite or sand and vermiculite are superior substrates for experimenting with *Reticulitermes* and *Coptotermes* species. Sand and vermiculite were decided upon as the best candidates because they could be obtained in consistent quality and added no nutrient value to the tunneling substrate.

METHODS AND MATERIALS

All insects used in this study were undifferentiated larvae (or "workers") of at least the third stage. Two colonies each of *Reticulitermes flavipes* and *R. virginicus* were collected in sections of fallen trees in southern Mississippi, and were placed in test within 3 days of collection. *Coptotermes formosanus* were taken from colonies maintained at the laboratory in Gulfport, but were originally collected from standing dead bald cypress *(Taxodium distichum)* trees near Lake Charles, Louisiana, within a year of this study.

The 20 substrates tested are listed in Table 1. Of the five in the sand group substrate 3 is the standard, and consisted of 105 ml of sand (approx. 163 g) moistened with 21 ml of de-ionized water (12.9% moisture content w/w). The four others were variations of the standard: two were slightly drier and two slightly wetter. Substrates 6 to 13 were 12 g of vermiculite (approx. 105 ml) with moisture content ranging from 100 to 500 percent (w/w). Substrates 14 to 20 were selected combinations of sand and vermiculite. Water and sand were measured in a graduated cylinder while the vermiculite was weighed on a triple beam balance.

For testing, each substrate was packed into a ½-pint (236.6 ml) canning jar with three over-dried, sapwood blocks (2.5 × 2.5 × 1.0 cm) of southern

Table 1. Tunneling substrates examined for suitability for long-term experiments.

Substrate	Sand (ml)	Vermiculite (ml)	Water (ml)	Moisture content (%)
1	105	-	15	9.2
2	105	-	18	11.0
3	105	-	21	12.9
4	105	-	24	14.7
5	105	-	27	16.6
6	-	105	12	100.0
7	-	105	18	150.0
8	-	105	24	200.0
9	-	105	30	250.0
10	-	105	36	300.0
11	-	105	42	350.0
12	-	105	48	400.0
13	-	105	60	500.0
14	52.5	52.5	37.5	42.9
15	52.5	52.5	41.5	47.4
16	52.5	52.5	43.5	49.7
17	52.5	52.5	46.5	53.1
18	52.5	52.5	49.5	56.6
19	52.5	52.5	52.5	60.0
20	52.5	52.5	55.5	63.4

yellow pine (*Pinus* sp.) provided as food. All blocks were made from boards cut from one tree. Blocks were depressed slightly into the substrate, and 300 termites of one species were added to each container. The openings of the jars were covered by inverting the central disk of each canning jar lid, so that the rubber seal was not in contact with the glass, and then tightening the ring only slightly, thus providing some ventilation. Jars were held at optimal temperatures: 24°C for *R. flavipes* and *R. virginicus*, 28°C for *C. formosanus*.

After 8 weeks in test, the termites were removed from the jar and counted. The data for each species were subjected to analysis of variance recognizing a randomized complete-block design. Termite colony was the blocking factor. Means were derived from two replications from each of two colonies. The statistical significance of all means were compared to the control (substrate 3) at the $P = 0.05$ level by Dunnett's procedure.

RESULTS AND DISCUSSION

Survival of *R. flavipes* on all 20 substrates was essentially the same: overall mean was 78.6 percent (Table 2). Survival of *R. virginicus* was considerably lower than that of *R. flavipes:* overall mean was 66.7 percent. The vermiculite substrates appeared to be less suitable for *R. virginicus*

except at the highest moisture level. Most of the sand and all of the sand and vermiculite substrates appeared suitable. *Coptotermes* survival was similar on all substrates except on the dampest of the sand and vermiculite substrates, where survival was significantly lower than the standard. Within each substrate type all three species displayed a remarkable tolerance to a great range of moisture contents. Adverse effects on results occurred almost always at the extremes of moisture contents for any matrix.

Table 2. Survival of *Reticulitermes flavipes, R. virginicus* and *Coptotermes formosanus* after 8 weeks of feeding on wood blocks in one of 20 different substrates.[1]

Substrate	Survival		
	R. flavipes	*R. virginicus*	*C. formosanus*
Sand:			
1	81.1	73.3	88.5
2	81.7	76.6	90.3
3	78.3	65.3	89.3
4	78.3	72.3	91.6
5	81.1	71.2	91.3
Vermiculite:			
6	79.4	2/14.7*	76.1
7	77.3	46.1	83.8
8	79.3	49.6	89.8
9	79.6	52.8	89.6
10	79.0	66.0	90.0
11	75.8	57.8	85.4
12	75.9	68.7	91.1
13	74.9	77.8	90.1
Sand and vermiculite:			
14	75.8	78.8	90.8
15	77.0	74.9	88.6
16	77.9	75.4	93.2
17	76.9	77.7	90.8
18	82.3	74.5	89.4
19	81.3	77.1	90.6
20	80.1	78.8	3/66.3*
$\bar{x}$	78.6	66.7	87.8

* Significantly different from control at $\alpha = 0.05$ by Dunnett's procedure.

1/ Means are derived from two replications from each of two colonies per species; percent survival is based on the initial 300 individuals.

2/ Three of the four groups suffered total mortality.

3/ One of the four groups had 13% survival. Survival of the others ranged from 81 to 86.7%.

From this study there appears to be no advantage to using vermiculite rather than sand for short-term tests of 8 weeks or less. All three termite species were able to tolerate a wider range of moisture content in the sand substrates than was suggested by Becker and Lenz (1970). For *R. virginicus* and *C. formosanus* poorest survival was associated with the highest moisture levels in sand. Sand, in general, was associated with greater survival than was vermiculite alone. I feel that the convenience of sand should be considered. Sand has a much smaller particle size and tends to spread out when removed from the test container. Vermiculite particles, on the other hand, tend to clump. Termites are able to cling to large vermiculite particles, thus making it more difficult to separate termites for counting and, especially, weighing. Consequently, vermiculite considerably slows down the collection of data at the termination of an experiment. Delay is especially disadvantageous when data must be collected from many experimental units in a very short time. Sand has the added advantage of being obtainable in grains of a precise size range, may be washed and sterilized, and has no nutrient value.

For longer tests (12 weeks or more), such as caste differentiation studies, the disadvantages of vermiculite may be outweighed by its superior water-holding capacity. In this study, however the sand/vermiculite combinations were almost always superior to vermiculite alone over the 8-week term of the experiment. To maximize the water content without adversely affecting results, long-term experiments should probably be installed in substrates such as 17, 18, and 19, which approximate those suggested by Fougerousse (1973). Substrate 18 has been used successfully by Haverty and Howard[2] in soldier differentiation studies with average 12 week survival ranging from 60.4 to 79.0 percent. It is important to test the suitability of the vermiculite before initiating a large, long-term test. The grade and/or origin of the vermiculite may affect results (C. D. Howick, pers. commun.)

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