Ranking Wood Preference of the subterranean termite *Reticulitermes virginicus* (Banks) using Two-, Four-, and No-Choice Experimental Designs

by

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Abstract

The preference of the subterranean termite *Reticulitermes virginicus* (Banks) was determined for four types of dimensional lumber from the following genera: *Sequoia sp* (redwood); *Quercus sp* (red oak); *Populus sp* (yellow poplar); and *Pinus sp* (southern yellow pin). Three hundred termites were placed in each of three experimental designs, no-choice, 2-choice, or 4-choice for 21 days. Wood weight loss was the measure used to obtain a preference ranking. Preference rankings were compared between and within the three designs. Results indicate that a no-choice design is sufficient if the intent of a bioassay is determination of absolute resistance (aversion). Resolution of termite wood preference at a finer scale, however, requires using all three experimental designs to verify repeatable rankings. The preference ranking for the wood types tested was; 1) Pine, 2) Red Oak, 3) Redwood, 4) Poplar.

Key words: bioassay, wood preference, subterranean termite

Introduction

Termite feeding behavior is impacted by a multitude of factors, including termite species, wood species, temperature, chemical composition of the wood samples, amount of wood, wood density, and wood moisture content to name a few (Thorne 1998, Smythe & Williams 1972, Smythe & Carter 1969). In addition to these sources of variation, comparison of results between researchers is complicated by the plethora of units used to report feeding rates, including visual rating scales, mg of wood, mg of wood/day, mg of wood/termite, percent wood loss, percent wood loss/day, percent wood loss/termite, mg of wood/ termite/day, and mg of wood/g of termite/day (Smythe & Carter 1970, Su & La Fage 1984, Thorne 1998, Indrayani et al. 2006, Hapukotuwa & Grace 2011). Experiments intended to measure termite wood preference also vary between no-choice and multiple-choice experimental designs (Smythe & Carter 1970, Su & La Fage 1984, Indrayani et al. 2006, Hapukotuwa & Grace 2011). Previous Pacific Rim Termite Research Group Proceedings contain 25 papers on resistance of lumber or other building

materials to termite feeding. Experimental designs varied, with 17 employing a no-choice test design, 2 with a choice-feeding design, and 6 did not report the method. The measurement of wood preference also varied with 1 paper reporting weight loss, 11 using percent weight loss, and 13 employed a numerical scale (9 based on percent-weight loss and 4 based on a visual estimate of feeding).

This study examined the impact of three experimental designs on ranking the preference for four types of lumber using the subterranean termite *Reticulitermes virginicus* (Banks). We hypothesized that assuming the number of termites and time in bioassay remains constant the different bioassay designs would provide the same preference ranking using the metric of wood weight loss.

Materials and Methods

Logs infested with *Reticulitermes virginicus* (Banks) were collected from various locations in Clarke County, Georgia. Logs were cut using a chain saw into bolts approximately 1-m in length and brought into the laboratory. PVC pipe (17-cm length, 10-cm diameter) containing moistened corrugated cardboard was placed beside the bolts and termites collected from the cardboard rolls were placed into a plastic container (26-cm x 19-cm x 9-cm) with moistened pinewood slats (40-mm x 120-mm x 2-mm). Termites were kept in an environmental chamber in complete darkness at 26°C for no more than 4 weeks before inclusion in bioassay.

Four types of dimensional lumber representing the following genera – *Pinus sp.* (Pine), *Quercus sp.* (Red Oak), *Sequoia sp.* (Redwood), and *Populus sp.* (Yellow Poplar) – were used in these bioassays. Wood was purchased at a local lumber retailer and cut into 1-cm^3 blocks. Wood samples were oven-dried at 64°C for approximately 24 hours, allowed to cool to room temperature inside a desiccation chamber for twenty minutes then weighed on an electronic scale to tenths of a milligram. Wood was soaked in water for approximately 24 hours prior to inclusion in bioassay.

Bioassay arenas were composed of round plastic containers (3.6-cm height, 5.2-cm diameter) connected by 7-cm lengths of Tygon tubing (2-mm internal diameter). Three containers were used for the 2-and no-choice tests while 5-containers were used to construct the 4-choice arenas. The central container in each arena was termed the introduction chamber. Introduction chambers had holes (5-mm diameter) for accepting Tygon tubing that were spaced equidistant around the circumference of the container approximately 1.7-cm above the base. A sand and vermiculite mixture (14:12) saturated with distilled water was added to a depth that reached the Tygon tubing to provide termites access to the other containers termed feeding chambers. Feeding chambers had a 5-mm hole at the base for accepting Tygon

tubing to connect with the introduction chamber. Three 1-cm^3 blocks of the same type of wood were placed in a feeding chamber.

Three hundred workers (3rd instar or higher) were added to each introduction chamber at the start of the bioassay. The Tygon tubing leading from the introduction chamber was closed using 5-cm binder clips placed within one centimeter of the introduction chamber. Binder clips were removed after a 24-hour 'acclimation period' because preliminary trials provided evidence of a variety of 'disturbance reactions' by termites placed into the arenas. Wood samples were exposed to termites for 21 days, after the 24-h acclimatization period. Arenas were dismantled at the end of the three-week exposure and wood samples cleaned and oven-dried prior to weighing and recording the final weight. Weight loss was obtained by subtracting the final weight of the three wood cubes in each feeding chamber from the initial weight.

Three bioassay designs were used in this study – no-choice, 2-choice, and 4-choice. No-choice and 2-choice designs used three containers (one introduction and two feeding chambers). The no-choice test was a force-feeding design with one empty chamber and the other with three cubes of one species of wood. The 2-choice design had a different type of wood in both feeding chambers. The 4-choice arenas had five containers; one introduction and a different wood type in each of four feeding chambers.

A replicate consisted of 11 arenas; one no-choice arena for each of the four wood types, six 2-choice arenas (representing all possible combinations of the 4 wood comparisons), and one 4-choice. A total of fifteen replicates were conducted. Data from the no-choice bioassays were compared using the mean weight loss by wood type from all 15 replicates. The no-choice mean weight loss was used to obtain a preference ranking by comparing data from all four no-choice arenas within a replicate. The highest mean weight loss was assigned the highest rank in preference (lowest numerical value). Mean wood weight loss also was used with the 2-choice and 4-choice replicates to provide a preference rank. A mean preference ranking was obtained by assigning a rank value, based on weight loss using the same ranking scale previously described, within each replicate and calculating a mean from those rankings - termed the mean replicate ranking (Table 1).

Results and Discussion

The high variability often reported from termite wood feeding bioassays (Thorne 1998) was evidenced in this study as indicated by the weight loss Standard Deviations (± SD) listed in Table 1. A simple method for ranking the preference that termites display for different types of wood would facilitate reporting and comparing results from wood preservative testing or ecological studies. This

paper describes an attempt to illuminate the impact that bioassay design has on ranking termite wood preference. The mean weight loss from the no-choice design provided a preference ranking that was the same as the 4-choice design (Table 1). The hierarchy of preference was 1) Pine, 2) Red Oak, 3) Redwood, 4) Poplar. The mean replicate preference ranking is similar when the mean values are 'rounded' to the nearest whole number (Table 1). It is interesting to note, as evidenced by the standard deviations listed in the mean replicate preference rankings, that the no-choice design consistently identified the least preferred wood while the 4-choice consistently identified the most preferred wood (Table 1).

The two-choice design showed remarkable repeatability in regard to the rankings as evidenced by the standard deviations of the mean replicate rankings (Table 1). The pine to red oak 2-choice comparison was the only pairing that did not provide a consistent 1-2 ranking within the replicates. The mean weight loss obtained in the pine/red oak 2-choice comparison is reflected in the close numerical values obtained by comparing the no-choice means (370 to 300 mg for pine and red oak, respectively) but is similar to the great (and consistent) difference in the weight loss and rankings shown in the 4-choice results (Table 1).

The most frequently stated goal of testing wood treatments is to identify aversion or complete lack of preference by termites. This comparison of experimental designs validates using the no-choice bioassay recommended in standardized protocols aimed at delimiting wood species or treatments that display complete aversion (JIS 2004, SNI 2006, AWPA 2009). All three experimental designs examined in this study demonstrated that poplar was least preferred. We therefore conclude that a no-choice bioassay is sufficient to determine if termites will not feed on a given species of wood or wood treatment. If the outcome of a wood-testing program is to determine the preference of a number of different wood species then we would recommend using a series of experimental designs and using a simple ranking system based on wood weight loss. This study showed that no-choice and multiple-choice (4-choice) bioassays provided the same ranking whether the mean weight loss data or the mean replicate rankings were used to establish a preference ranking (Table 1). The addition of the two-choice design provides further validation as well as increases the level of resolution of any final (composite) ranking (Table 1). The simultaneous use of these three bioassay designs can provide validation of the preference determination obtained using any single bioassay design in isolation… by repeating the findings.

Table 1: Mean weight loss (± SD) and preference rankings based on mean weight loss and mean ranking by replicate (± SD) for three bioassay designs.

Bioassay Design *Wood Type*	Mean Weight Loss ± SD (mg)	Mean Weight Loss Ranking	Mean Replicate Ranking* ± SD
No-Choice			
Pine	370 ± 160	1	1.4 ± 0.74
Red Oak	302 ± 170	2	1.93 ± 0.59
Redwood	160 ± 58	3	2.67 ± 0.62
Poplar	5.9 ± 7	4	4.0 ± 0
Four-Choice			
Pine	490 ± 650	1	1.0 ± 0
Red Oak	87 ± 53	2	2.07 ± 0.26
Redwood	28 ± 32	3	3.2 ± 0.56
Poplar	5.7 ± 8	4	3.53 ± 0.52
Two-Choice			
Pine, Red Oak	275 ± 153, 105 ± 87	1, 2	1.27 ± 0.5, 1.73 ± 0.5
Pine, Poplar	349 ± 161, 7.5 ± 12	1, 2	1.0 ± 0, 2.0 ± 0
Pine, Redwood	353 ± 154, 53 ± 74	1, 2	1.0 ± 0, 2.0 ± 0
Red Oak, Redwood	284 ± 97, 57 ± 56	1, 2	1.0 ± 0, 2.0 ± 0
Red Oak, Poplar	296 ± 109, 4.5 ± 7	1, 2	1.0 ± 0, 2.0 ± 0
Redwood, Poplar	181 ± 79, 4.7 ± 8	1, 2	1.0 ± 0, 2.0 ± 0

* The mean replicate preference ranking was obtained by assigning a rank to the weight loss within a replicate and the mean was taken from those rankings.

Conclusions

The preference ranking for the four woods tested against *Reticulitermes virginicus* was the same when no-choice and 4-choice experimental designs were compared providing support for the original hypothesis. The different designs afforded slight but important insights into termite wood preference in that the no-choice consistently identified the least preferred and the multiple choice (4-choice) design consistently identified the most preferred. The 2-choice design also offered consistent preference rankings with one exception, the pine/red oak comparison. It is our recommendation that a bioassay series be employed in ecological studies aimed at identifying termite preference to more than two types of wood. However, a no-choice bioassay design is sufficient to illuminate the best candidate(s) if the purpose of the test is to identify wood that termites do not prefer or avoid.

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