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Wood Preference of *Reticulitermes virginicus* (Blattodea: Rhinotermitidae) Using No-, Two-, and Four-Choice Designs and Seven Different Measures of Wood Consumption

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Abstract

Three hundred *Reticulitermes virginicus* (Banks) workers were exposed to three 1-cm^3 wood blocks of either *Quercus* sp. (Red Oak), *Populus* sp. (Poplar), *Pinus* sp. (Pine), or *Sequoia* sp. (Redwood) placed into one of the three bioassay designs (no-, two-, and four-choice) for 21 d. Termite wood consumption was measured by wood weight loss, resistance class, and visual rating. Wood consumption rates were determined using four formulas in addition to two standardized visual rating scales (American Society for Testing and Materials [ASTM] and American Wood Protection Association [AWPA]) and a preference ranking obtained for each measure. The wood consumption formula, rating scale, and preference rankings were compared by bioassay design. The overall preference ranking of the four wood types as determined by the combination of all three designs was—1) Pine, 2) Red Oak, 3) Redwood, and 4) Poplar. Results indicate that bioassay design influenced both wood consumption and preference rankings. A no-choice design can determine aversion; a four-choice design the most preferred wood; and a two-choice design can illuminate the fine details of comparative preference. The different formulas employed for calculation of consumption rate influenced preference ranking in the no- and four-choice designs but not the two-choice design.

Key words: subterranean termite, bioassay, wood preference, consumption rate, visual rating

The preference that subterranean termites display for different food resources can provide information useful in understanding the ecology of sympatric species in addition to advice useful for management tactics (Lukamandaru and Takahashi 2008, Kadir and Hale 2012, Owoyemi et al. 2013). The ranking of subterranean termite feeding preference can be influenced by experimental conditions such as bioassay design and calculation of consumption rate (Smythe and Carter 1970, Thorne 1998). Bioassay of termite wood consumption also is affected by a number of factors attributed to experimental conditions including the vigor of the termites used in the assay, wood and termite species being tested, the number of termites per arena, wood density and age, temperature, wood and substrate moisture content, and the placement and number of food choices (Smythe and Carter 1969, 1970; Behr et al. 1972; Smythe and Williams 1972; Oi et al. 1996; Thorne 1998; Lukamandaru and Takahashi 2008; Lenz 2009).

Termite wood preference has been examined using a variety of bioassay designs including no-choice, paired-choice, and multiple-choice designs employed alone or in combination (Smythe and Carter 1970, Su and La Fage 1984, Grace and Yamamoto 1994, Oi et al. 1996, Indrayani et al. 2006, Hapukotuwa and Grace 2011). Termite wood consumption rates also have been measured using a number of units. Standardized protocols provided by wood protection organizations, such as American Wood Protection Association and American Society for Testing and Materials, use a subjective visual rating scheme based on estimated percent consumption and other characteristics of "damage" on a scale of 10 ("sound") to 0 ("failure") (ASTM 1974, Charoenkrung et al. 2007, Umphauk and Chaikuad 2008, AWPA 2009, Hapukotuwa and Grace 2011). There also are rating schemes that use a numerical scale with fewer categories (Standar Nasional Indonesia [SNI] 2006, Tsunoda et al. 2010, Eger et al. 2011, Shelton et al. 2013). Quantitative units employed in termite wood preference studies include wood weight loss (mg) and percent wood weight loss (Smythe and Carter 1970, Morales-Ramos and Rojas 2001, Indrayani et al. 2006, AWPA 2009, Hapukotuwa and Grace 2011). The potential impact of the number of termites and time in bioassay has stimulated use of units such as milligram of wood per number of termite per day and milligram of wood per gram of termite per day (Su and La Fage 1984, Thorne 1998). Su and La Fage (1984) that also factored in a "control" unit aimed at the potential for error in drying and weighing wood in addition to providing a correction for mortality over the course of bioassay.

The plethora of designs and units of measure used in bioassay of termite food preference makes comparisons difficult. This study examined the impact of three different bioassay designs (no-choice, two-choice, four-choice), using four wood genera (*Pinus* sp., *Populus* sp., *Quercus* sp., and *Sequoia* sp.) and seven different units of wood consumption (wood weight loss, percent weight loss, SNI resistance class, milligram of wood per number of termites per day, milligram of wood per gram of termite per day, ASTM visual rating, and AWPA visual rating) on ranking the feeding preference of the subterranean termite *Reticulitermes virginicus* (Banks). We hypothesized that the various bioassay designs and units of measurement would provide the same preference ranking for the four types of wood.

Materials and Methods

Termite Collection

Logs containing *R. virginicus* were collected from various sites in Clarke Co., GA, and cut into 1-m bolts using a chain saw. Bolts were brought into the laboratory and stored at room temperature in 60- by 10- by 38-cm (l:w:h) galvanized metal trays. Termites were collected from the bolts on a daily basis by placing PVC pipes (17 by 10 by 0.5 cm; l:diameter:thickness), containing moistened corrugated cardboard near shelter tubes that protruded from the bottom of the bolts (Forschler and Townsend 1996). Termites thus collected were placed into plastic boxes (26 by 19 by 9 cm^3) containing wet filter paper and moistened pine slats (12 by 4 by 0.2 cm^3) at 26°C and 78% humidity, in total darkness, for no longer than four weeks before inclusion in bioassay. Termites were identified to species using soldier and alate morphological characteristics (Lim and Forschler 2012).

Wood Preparation

Four types of dimensional lumber purchased from a local lumber store representing four genera; *Pinus* (Pine), *Quercus* (Red Oak), *Sequoia* (Redwood), and *Populus* (Yellow Poplar), were cut into 1-cm^3 cubes. The majority of the wood used in this study was a mixture of heartwood and sapwood, although all poplar cubes were chosen to represent the heartwood of this species because preliminary bioassay showed it to be resistant to termite feeding. Wood cubes were oven dried for ~24 h and allowed to cool to room temperature inside a desiccation chamber containing Drierite before weighing. Wood dry weight was measured, prior to and after bioassay, using an electronic scale (Denver Instrument APX-323) to tenths of a milligram. Wood cubes were placed in distilled water for ~24 h, and after excess surface moisture was removed using a dry paper towel, they were placed into bioassay. The termite-exposed wood cubes were collected and cleaned using a soft brush then oven dried and weighed, as previously described, to obtain a postexposure dry weight.

Bioassay Design

Three bioassay designs—no-choice, two-choice, four-choice—were used. An arena was composed of three or five round, plastic containers (3.6 by 5.2 cm; h:diameter) arranged to provide a single central chamber and two or four feeding chambers. The central chamber had 0.5-cm-diameter holes placed 1.7 cm above the base of the plastic container for the central chamber and at the base for feeding chambers. A 7-cm length of Tygon tubing (5-mm OD) was used to connect the feeding chamber to the central chamber via the aforementioned holes. The central chamber contained a water-saturated mixture of sand and vermiculite (7:6) placed to a height that reached the bottom of the Tygon tube.

The no-choice and two-choice designs were composed of one central chamber and two feeding chambers, as illustrated (Fig. 1). The no-choice design had three cubes of the same type of wood in one chamber and the other feeding chamber was empty. The two-choice design provided a choice between two types of wood, with each feeding chamber containing three cubes of the same wood type. The four-choice design had a central chamber and four feeding chambers each containing three cubes of a single wood type (Fig. 1).

Three hundred workers (third instar or higher) were added to the central chamber of each arena at the start of bioassay. The number of termites introduced was estimated by weight based on the average weight of 5 groups of 10 workers (Su and La Fage 1984), while the number of surviving termites was determined by actual count. A 5-cm binder clip was placed on the Tygon tubing connecting the central to the respective feeding chambers to prevent termites from reaching the wood choices for ~24 h. Termites were allowed access to the wood, after the 24-h acclimation period, for 21 d at which time arenas were dismantled, the wood removed, cleaned and dried, and the number of surviving termites recorded.

A replicate consisted of 11 arenas—one no-choice arena for each of the four wood types; six two-choice arenas accounting all possible paired combinations; and one four-choice arena. A series of control replicates were prepared using the same setup described for the choice tests without termites to account for change in wood weight outside of termite feeding. In total, 16 replicates were performed.

Calculation of Consumption Rate

Wood consumption was measured using Denver Instruments (Model APX-323) analytical scale to the nearest milligram and calculated using four quantitative measures—wood weight loss (g), percent wood weight loss (%), milligram of wood per number of termite per day, milligram of wood per gram of termite per day and the Indonesian "resistance index" based on percent wood weight loss (SNI 2006). Two visual rating systems also were employed the AWPA E1-09 and ASTM D335 (ASTM 1974, AWPA 2009). A rating for the standardized visual rating systems was obtained for a replicate by assigning a number, as prescribed by each system, to each of the three cubes of wood within an arena and taking an average. Wood weight loss was measured by subtracting the final dry weight from initial dry weight. Percent wood weight loss was calculated by multiplying 100 to the quotient of weight loss and initial dry weight. The milligram of wood per number of termite per day was calculated by wood weight ÷ 300 (the number of termites at the beginning of the bioassay) ÷ by the number of days (21) in bioassay (Thorne 1998). The milligram of wood per gram of termite per day was calculated according to the formula in Su and La Fage (1984).

Analysis

Analysis of the no-choice design used ANOVA to compare consumption rates of the four wood types. If the ANOVA yielded a significant difference (P-value ≤0.05), a protected least significant difference (PLSD) test was conducted. Analysis of the two-choice design involved four ANOVAs where each two-choice arena containing the same wood type was grouped together (i.e., all arenas with pine) and the consumption rate of the same wood type analyzed (i.e., all pine consumption rates were analyzed together). If the

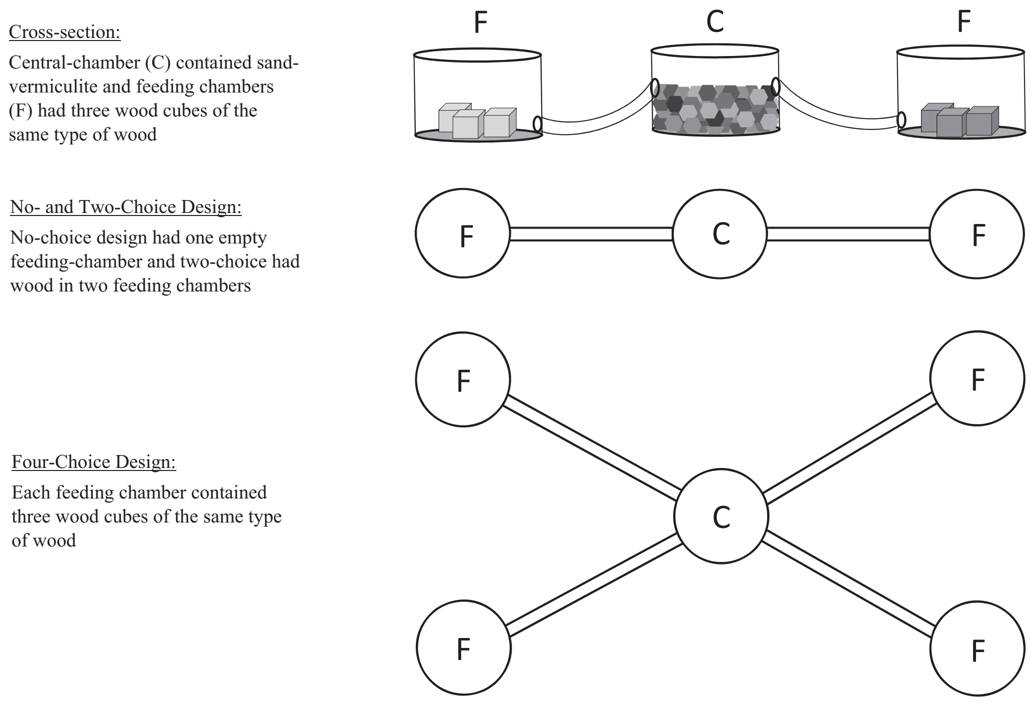

Fig. 1. Illustration of the no-, two-, and four-choice bioassay arena arrangements. Chambers (3.6 by 5.2 cm; height: diameter) were connected by Tygon tubing (5-mm OD). (Key: C—Central chamber, F—Feeding chamber).

Table 1. Mean unit of measure (avg ± SD) by wood type with the PLSD-associated preference rank for the no- and four-choice bioassay designs

Bioassay design and Wood type	Wt loss (g)		mg wood/ no. of termite/d		mg wood/ g termite/d		Percent wt. loss		Resistance class (SNI)		ASTM rating		E1-09 rating	
No-choice														
Pine	0.364 ± 0.153	A[a]	0.058 ± 0.024	A	28.27 ± 10.63	A	20.82 ± 8.960	A	4.250 ± 1.34	A	5.500 ± 2.270	A	6.810 ± 1.510	A
Red Oak	0.300 ± 0.165	A	0.048 ± 0.026	A	24.62 ± 9.900	A	9.920 ± 3.630	B	3.190 ± 0.830	B	9.000 ± 0.000	B	8.000 ± 0.760	B
Redwood	0.155 ± 0.060	B	0.025 ± 0.010	B	17.56 ± 8.940	B	9.290 ± 4.920	B	2.750 ± 1.130	B	9.000 ± 0.000	B	8.000 ± 0.530	B
Poplar	0.005 ± 0.007	C	0.001 ± 0.001	C	0.530 ± 0.950	C	0.270 ± 0.330	C	1.000 ± 0.000	C	10.00 ± 0.000	B	9.880 ± 0.230	C
Four-choice														
Pine	0.318 ± 0.117	A	0.050 ± 0.019	A	26.02 ± 5.970	A	17.98 ± 6.270	A	4.250 ± 0.860	A	5.750 ± 1.980	A	6.750 ± 0.710	A
Red Oak	0.083 ± 0.053	B	0.013 ± 0.008	B	5.340 ± 3.680	B	2.700 ± 1.240	B	1.310 ± 0.480	B	9.250 ± 0.460	B	8.810 ± 0.530	B
Redwood	0.029 ± 0.031	C	0.005 ± 0.005	C	1.520 ± 3.250	C	1.190 ± 1.080	B	1.060 ± 0.250	B	9.880 ± 0.350	B	9.190 ± 0.530	B
Poplar	0.005 ± 0.008	C	0.001 ± 0.001	D	1.230 ± 2.620	C	0.290 ± 0.430	C	1.000 ± 0.000	B	10.00 ± 0.000	B	9.630 ± 0.230	C

[a] Results of PLSD are indicated within a column and by bioassay design by capital letters with different letters signifying statistically different values ($P < 0.05$).

ANOVA yielded a significant difference (P-value $\leq$ 0.05), a PLSD test was conducted. The data from the four-choice design were analyzed using a "two-way ANOVA," with both replicate and wood type considered independent variables to examine independence between treatments (the four wood types) with replicate considered the "second factor." Data from the no- and four-choice designs were assigned a preference ranking from 1 to 4, with 1 being most preferred using the PLSD statistical separation of means (Table 1). Data from the two-choice design were assigned a preference ranking from 1 to 4 using a comparison chart of PLSD results (Table 2). The previously described analyses were repeated with all units of measure using SAS 9.3 (SAS Institute 2011).

Calculation of Numerical Rankings

We also obtained a strict numerical ranking (sans statistical tests) of wood preference. In the no- and four-choice designs, the four wood types were ranked based on the numerical hierarchy of the mean wood consumption data, with the highest numerical value receiving a ranking of 1 and lowest 4 (Table 3). In the two-choice design, wood types were assigned a 1 (preferred) or 2 (not preferred) based on the numerical hierarchy of the mean wood consumption data in each paired test (Table 4).

Results

Bioassay design and unit of measure influenced the ranking of termite wood preference, when examined by either statistical mean separation or numerical ranking of means (Tables 1, 3 and 4). The only consistent agreement with statistical and numerical mean ranking as well as displaying no effect of unit was the two-choice design (Table 4). Statistical separation of means in the no-choice and four-

Table 2. Explanation of the ranking of termite wood preference from the two-choice design data

Wood type	Rankings						
	Pairing 1		Pairing 2		Pairing 3		Sum[a]
Pine	v. Ro	A (1)	v. Rw	A (1)	v. Po	A (1)	3 (1)
Red Oak	v. Pi	B (2)	v. Rw	A (1)	v. Po	A (1)	4 (2)
Redwood	v. Pi	B (2)	v. Ro	B (2)	v. Po	A (1)	5 (3)
Poplar	v. Pi	A (2)	v. Ro	A (2)	v. Rw	A (2)	6 (4)

Key: Pi—Pine, Ro—Red Oak, Rw—Redwood, Po—Poplar.

Letters obtained from PLSD mean separation were used to assign a 1 (preferred) or 2 (not preferred) to the wood type in the first column when compared to pairing with the other three wood types listed in that same row. PLSD results and rankings listed in this Table apply to all units of measure examined in this study.

[a] The Sum Ranking (in parenthesis) was determined by adding values in each row and issuing the lowest sum with a higher ranking.

choice designs provided variable preference hierarchies depending on the unit of measure utilized (Table 1).

Examination of the data using statistical mean separation with the no-choice design showed that the quantitative units weight loss (g), milligram wood per number of termites per day, and milligram wood per gram termite per day resulted in a tie for the number 1 ranking between pine and red oak, a number 2 for redwood, and a number 3 for poplar (Table 1). The qualitative units percent weight loss, SNI, and E1-09 resulted in ranking pine number 1, a tie for number 2 between red oak and redwood, and number 3 for poplar. The no-choice design using the qualitative ASTM rating resulted in a statistically supported ranking of number 1 for pine, and a three-way tie for number 2 among red oak, redwood, and poplar. The numerical ranking of all quantitative units and the qualitative units percent weight loss and SNI resulted in ranking pine number 1, red oak number 2, redwood number 3, and poplar number 4, while the ASTM and E1-09 resulted in a number 1 for pine, a tie for number 2 between red oak and redwood, and number 3 for poplar.

Table 3. Comparison of the termite wood preference rankings by bioassay design, unit of measure, and wood type obtained from numerical ranking of means without statistical validation

Bioassay design and Wood type	Wt loss (g)	mg wood/no. of termite/d	mg wood/g termite/d	Percent wt loss	Resistance class (SNI)	ASTM Rating	E1-09 Rating
No-choice							
Pine	1[a]	1	1	1	1	1	1
Red Oak	2	2	2	2	2	2	2
Redwood	3	3	3	3	3	2	2
Poplar	4	4	4	4	4	3	3
Four-choice							
Pine	1	1	1	1	1	1	1
Red Oak	2	2	2	2	2	2	2
Redwood	3	3	3	3	3	3	3
Poplar	4	4	4	4	4	4	4
Two-choice							
Pine	1	1	1	1	1	1	1
Red Oak	2	2	2	2	2	2	2
Redwood	3	3	3	3	3	3	3
Poplar	4	4	4	4	4	4	4

[a] Rankings were given based on numerical hierarchy of mean unit of measure.

Table 4. Mean unit of measure (avg ± SD) and the preference rank for the two-choice bioassay design by wood type based on numerical ranking of means

Wood types compared	Wt loss (g)		mg wood/no. of termite/d		mg wood/g termite/d		Percent wt loss		Resistance class (SNI)		ASTM rating		AWPA E1-09 rating	
Pine vs.	0.276 ± 0.148	*1*[a]	0.044 ± 0.024	*1*	21.39 ± 9.790	*1*	15.34 ± 8.470	*1*	3.750 ± 1.440	*1*	7.130 ± 1.550	*1*	7.000 ± 0.530	*1*
Red Oak	0.101 ± 0.085	*2*	0.016 ± 0.014	*2*	7.050 ± 7.050	*2*	3.190 ± 1.810	*2*	1.440 ± 0.630	*2*	8.880 ± 0.830	*2*	8.630 ± 8.690	*2*
Pine vs.	0.352 ± 0.149	*1*	0.056 ± 0.024	*1*	28.76 ± 6.680	*1*	20.34 ± 8.390	*1*	4.310 ± 0.870	*1*	5.130 ± 1.550	*1*	6.750 ± 0.460	*1*
Redwood	0.052 ± 0.071	*2*	0.008 ± 0.011	*2*	2.510 ± 3.590	*2*	2.080 ± 2.460	*2*	1.250 ± 0.580	*2*	9.750 ± 0.460	*2*	9.130 ± 0.230	*2*
Pine vs.	0.346 ± 0.156	*1*	0.055 ± 0.025	*1*	25.41 ± 8.230	*1*	19.80 ± 9.030	*1*	4.310 ± 1.25	*1*	6.250 ± 1.390	*1*	6.750 ± 0.460	*1*
Poplar	0.007 ± 0.011	*2*	0.001 ± 0.002	*2*	1.410 ± 2.420	*2*	0.410 ± 0.650	*2*	1.000 ± 0.000	*2*	10.00 ± 0.000	*2*	9.500 ± 0.380	*2*
Red Oak vs.	0.280 ± 0.095	*1*	0.045 ± 0.015	*1*	23.54 ± 3.750	*1*	9.960 ± 3.540	*1*	3.060 ± 0.770	*1*	8.750 ± 0.710	*1*	7.630 ± 0.520	*1*
Redwood	0.045 ± 0.036	*2*	0.007 ± 0.006	*2*	2.230 ± 3.330	*2*	2.590 ± 2.160	*2*	1.190 ± 0.540	*2*	9.880 ± 0.350	*2*	9.500 ± 0.270	*2*
Red Oak vs.	0.294 ± 0.106	*1*	0.047 ± 0.017	*1*	22.22 ± 4.030		10.38 ± 3.710	*1*	3.190 ± 0.750	*1*	8.000 ± 1.070	*1*	7.500 ± 0.760	*1*
Poplar	0.004 ± 0.007	*2*	0.001 ± 0.001	*2*	0.580 ± 1.560	*2*	0.190 ± 0.320	*2*	1.000 ± 0.000	*2*	9.880 ± 0.350	*2*	9.560 ± 0.320	*2*
Redwood vs.	0.175 ± 0.080	*1*	0.028 ± 0.013	*1*	17.58 ± 7.260	*1*	11.83 ± 9.320	*1*	3.130 ± 1.310	*1*	8.500 ± 0.930	*1*	7.880 ± 0.640	*1*
Poplar	0.004 ± 0.008	*2*	0.001 ± 0.001	*2*	0.510 ± 0.980	*2*	0.230 ± 0.400	*2*	1.000 ± 0.000	*2*	10.00 ± 0.000	*2*	9.630 ± 0.230	*2*

[a] The numbers in italics represent the numerical ranking for the wood combination from the two-way arena based on the hierarchy of the mean unit of measure.

The quantitative unit milligram wood per number of termites per day provided a 1–4 ranking using mean separation with the four-choice data of pine, red oak, redwood, and poplar, respectively. The four-choice design using PLSD provided support for ranking the quantitative units weight loss and milligram wood per gram termite per day of number 1 for pine, number 2 for red oak, and a tie for number 3 between redwood and poplar. The qualitative units percent weight loss and E1-09 resulted in ranking pine number 1, a tie for number 2 between red oak and redwood, and number 3 for poplar using the four-choice design, while the SNI and ASTM resulted in a ranking of number 1 for pine, and a three-way tie for number 2 among red oak, redwood, and poplar (Table 1). The strict numerical rankings using the four-choice data resulted in the same ranking across all eight units; number 1 for pine, number 2 for red oak, number 3 for redwood, and number 4 for poplar.

In contrast, the preference ranking provided by the two-choice design whether by statistical mean separation or simple numerical ranking was the same regardless of unit (Table 4). The two-choice combinations always resulted in a statistically validated separation of preference of one wood over the other (Table 4). Pine was preferred whenever combined with any of the other three we tested (Table 4). Red oak was preferred over redwood and poplar, while redwood was always preferred over poplar (Table 4). Poplar was never the preferred wood type (Table 4). Table 2 summarizes the method we used to provide an overall ranking using the two-choice bioassay data of 1) pine, 2) red oak, 3) redwood, and 4) poplar.

Discussion

The data generated in this study of choice-feeding bioassays using a subterranean termite and four types of wood illustrated the impact of design and unit on ranking termite wood preference. The purpose was to identify a methodology that provided a level of confidence toward claiming biological relevance as evidenced by a consistent hierarchy of rank. It should be noted that Oi et al. (1996) showed termites displayed a preference when the choices were separated rather than next to one another in bioassay. In contrast, termite choice-designs most often involve presentation of food choices in the same arena (Smythe and Carter 1970, Grace and Yamamoto 1994, Quijian et al. 2006, Katsumata et al. 2008, Manzoor and Malik 2009, Malik et al. 2012, Green et al. 2014). Therefore, our physical separation of choices may have facilitated establishment of a hierarchy of preference compared with studies employing a single arena.

These experiments proved that design had a greater impact on consistent preference ranking compared with unit of measure. The design comparison clearly demonstrated that with any of the units we analyzed the no-choice bioassays identified the least preferred wood type (except the ASTM), the four-choice design the most preferred, and the two-way design a consistent hierarchy of preference (Tables 1 and 4). The choice of a bioassay design should be determined by the hypothesis of the experiment. Therefore, it is our recommendation if the purpose of the test is to identify wood aversion to employ a no-choice design. The identity of the most palatable choice can be illuminated by a multiple-choice design and if the intent is to obtain a preference rank then a two-choice bioassay design should be employed.

Determining aversion or resistance to termite feeding may arguably be the most frequent reason for performing a bioassay using termites and wood. We surveyed three peer-reviewed journals and two proceedings and found 80 papers published since 2005 that examined termite wood choice (Forest Prod. J., $n = 10$; Insects, $n = 7$; J. Econ. Entomol., $n = 6$; Proc. of International Research Group on Wood Protection, $n = 36$; and Proc. of Pacific Rim Termite Research Group, $n = 21$). The complete list of papers is available upon request from the authors (Supp Appendix A [online only]). That selected literature survey showed that the no-choice design (84%) and percent weight loss (80%) was most often employed in bioassay. The majority of those papers were related to efficacy of wood treatments (58%) and natural durability of wood (27%). We also found standards for testing resistance to termite "damage" that involve five different units of measure, including American Society for Testing and Materials (D3345-74), American Wood Protection Association (E1-09), European Standard (EN117, EN118), Japanese Industrial Standard (JIS K 1571), and Standar Nasional Indonesia (SNI 01.7207-2006). All the aforementioned standards call for using a no-choice bioassay design, with only E1-09 suggesting a concomitant two-choice bioassay. It should be noted that the European Standard includes a piece of "culture wood" and therefore does not constitute a true no-choice test, while the ASTM standard was withdrawn in 2011 in favor of the AWPA standard (EN 2005a,b; ASTM 2011). All the aforementioned standards call for reporting results as either percent weight loss (JIS 2004, SNI 2006) or outline a visual rating scale (ASTM 1974; EN 2005a,b; AWPA 2009).

Previous studies have shown that termites will feed on less preferred food in the absence of a choice (Smythe and Carter 1970, Oi et al. 1996), and our results support the use of a no-choice design for standardized testing of wood treatments (ASTM 1974; JIS 2004; EN 2005a,b; SNI 2006; AWPA 2009) because the no-choice design consistently identified the least preferred wood, ostensibly the purpose of a Standardized Testing protocol (Table 1). All the units we examined with the exception of one, the ASTM, statistically identified poplar as the least preferred wood using the no-choice data (Table 1). The E1-09 rating and ASTM yielded different rankings because the ASTM had a lower number of rating categories and lacks a "sound" or "no visual evidence of feeding" category. As a result, the ASTM data provided a three-way tie among red oak, redwood, and poplar, whereas the E1-09 separated red oak and redwood from poplar. The data, therefore, does not support the use of the ASTM for statistical determination of the least preferred wood choice. An alternative approach would be to use ASTM with the caveat that a rating of 10 be reserved for choices that show no visible evidence of feeding and a 9.5 for those that display between 10 and 9 (effectively changing it from a 5 to a 6 point scale).

Another reason for conducting bioassay of termite wood choice is to establish a hierarchy of preference (Cornelius et al. 2004, Manzoor and Malik 2009, Hapukotuwa and Grace 2011, Malik et al. 2012). The four-choice data illustrate that multiple choice tests consistently identified the most preferred wood, yet the only unit that identified a statistically validated hierarchy of preference was milligram wood per number of termites per day (Table 1). Interestingly, the four-choice design consistently, regardless of unit, identified a hierarchy of preference (pine, read oak, redwood, poplar from most to least preferred) using a simple numerical ranking—without statistical validation (Table 3). If the purpose of the bioassay is to statistically validate a preference hierarchy, our data unequivocally demonstrate through consistency of results that the two-choice bioassay design is the most appropriate approach, regardless of unit (Table 4). A wood preference hierarchy can be established from the outcome of multiple two-choice tests by using the ranking method illustrated in Table 2.

The question of the most appropriate unit to use in a termite food choice bioassay should be dictated, in part, by the research objectives. Statistical validation of results is a hallmark of the modern scientific method. Termite feeding tests aimed at obtaining statistical separation of preference also should attempt to utilize the most objective unit of measure that is biologically relevant to the test conditions. It is our opinion, for the sake of argument, that the requirement of objectivity eliminates qualitative units that rely on a subjective visual estimate of consumption such as the AWPA, and E1-09. The quantitative units we examined can be listed in order of complexity (number of "correction factors" involved) as weight loss, percent weight loss, milligram wood per number of termites per day, and milligram wood per gram of termite per day (Tables 1 and 4). The latter unit is arguably the most "accurate" measure of termite wood consumption because it accounts for a number of potential sources of error (Su and La Fage 1984). Yet, only the unit milligram wood per number of termites per day provided a statistically validated preference rank using the four-choice design that matched the rankings obtained with a series of two-choice tests (Table 1). We, however, hesitate to recommend use of any single quantitative unit to obtain a biologically relevant preference rank because the main "problem" with termite bioassay data is variability (Tables 1 and 4; Grace and Yamamoto 1994, Thorne 1998, Hapukotuwa and Grace 2011). The mean separation we obtained using milligram wood per termite per day may be an artifact related to the small numerical values generated by that unit (Tables 1 and 4).

A conundrum faced by researchers when designing a bioassay is providing a defensible conclusion based on a pragmatic number of replicates given constrains imposed by time, effort, and supplies. Variability in a data set can be addressed by increasing the number of replicates (Robertson et al. 2007). Our recommendation to use a two-choice design to validate a preference ranking of termite food choice illustrates the issue. A two-choice bioassay with four types of wood using 15 replications with 300 termites would require 90 arenas and 27,000 termites to test all possible combinations, while the same comparison conducted using a four-choice design would require 15 arenas and 4,500 termites. Large scale, industrial, screening programs could use a series of four-choice bioassays to identify the most preferred choices followed by a series of two-choice tests once the candidate substrates are narrowed down to 3 or 4. Our results indicate that ranking mean consumption rates using a numerical hierarchy determined termite aversion to poplar in the no-choice design, the preference of pine in the four-choice, and a detailed preference ranking sequence in the two-choice bioassays (Table 3). In fact, numerical ranking of means provided the same ranking sequence as the two-choice design using any unit in the no-choice and four-choice designs. The numerical ranking of means in no- and four-choice designs may serve as a quick substitute in providing a basic understanding of termite feeding preference using a large number of choices.

In summary, bioassay design had a greater impact on preference rankings compared with the units used to measure consumption. The no-choice design can identify wood treatments that deter termite feeding using any unit we examined aside from the ASTM rating scale. A four-choice design can identify the most preferred wood employing any of the units we surveyed. Paired or two-choice bioassays can provide consistent results that could be used to construct a hierarchy of preference. We recommend using the simplest quantitative measure—weight loss—for standardized testing protocols rather than a subjective visual ranking because a quantitative unit provides an objective measure easily compared between studies.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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