Nutritive Supplement Method to Evaluate Resistance of Natural or Preservative-Treated Wood to Subterranean Termites[1,2]

GLENN R. ESENTHER

U.S. Forest Products Laboratory: Madison, WI 53705

ABSTRACT

A bioassay for determining resistance of wood samples to *Reticulitermes flavipes* (Kollar) and *R. virginicus* Banks uses a small, measured amount of aspen wood sawdust as a nutritional supplement. Trials of this sawdust supplement method resulted in generally high termite survival (>75%) which, in turn, produced greater attack on resistance samples than normally occurs in termite assays. Block weight loss data thus could be used as the sole criterion for comparison of the relative resistance of different wood samples. Antitermitic properties were assessed with the supplement method for 2 oilborne and 2 waterborne preservatives and 21 different timbers. Except for a toxic effect by one of the oilborne preservatives, antitermitic properties of the preservatives and the natural woods were due primarily to repellency or feeding deterrency factors. The sawdust supplement method appears adaptable to any subterranean termite for assessment of wood resistance.

Bioassays which use *Reticulitermes* to determine either natural resistance of wood or efficacy of wood preservatives have been primarily compulsory feeding tests (Becker 1969, Behr et al. 1972, Fougerrouse 1973, Mannesmann 1973, Carter and Smythe 1974). With such tests it is difficult to determine whether termite mortality is due to toxicity or to nontoxic resistance factors, i.e., any interference with feeding results in starvation, which produces high mortality whether or not samples are toxic. Highly varying mortality in compulsory feeding tests makes it difficult to apply any single criterion by which to ascertain sample resistance. Criteria chosen to evaluate sample resistance in such tests include mortality of termites, amount of attack on the wood sample, or both.

Inclusion of a small, measured amount of sawdust as a nutritive supplement can eliminate starvation in a bioassay, making it more possible to distinguish between nontoxic resistance factors and actual toxicity. Consistently high levels of termite survival with wood samples having nontoxic types of resistance make possible the use of sample weight loss as the sole criterion for evaluating sample resistance. This report demonstrates the advantages of the nutritive supplement method in assays of 4 wood preservatives and 21 different woods.

MATERIALS AND METHODS.—*Reticulitermes flavipes* (Kollar) was collected at Janesville, Wis., during July 1967, and *R. virginicus* Banks near Gulfport, Miss., Mar. 1969. The colonies were stored in 32-gal metal garbage cans up to and through the periods that the termites were used—beginning Feb. 1968 for *R. flavipes* and July 1969 for *R. virginicus.*

A cylindrical plastic box (ca. 5.0 × 3.8 cm) was used in a 4-wk exposure of a wood block (0.4 × 2.5 × 2.5 cm) to 1 g termites (undifferentiated, nymphal and soldier forms, 4th instar and older). A 4-wk exposure was used because at > 4 wk there is a risk that development of sexuals and reproduction in some units will make results incomparable. The block was set on 0.5 g (ovendried at 105°C) aspen sawdust (Wiley milled with 1-mm screen; washed overnight with hot tapwater) to which 2 ml distilled water had been added. The sawdust alone was a 3–4 wk food supply for 1 g termites incubated at 26°C, 70% RH.

Recording procedure included biweekly notes on microbial contamination and behavior and mortality of termites. Weight loss of each block (based on moisture equilibrium at 26°C, 70% RH) was determined at the end of the exposure period. Initial and final number of termites and their final group weight also were recorded.

Two groups of wood blocks were assayed with the sawdust supplement method, the 1st group treated with 2 oilborne and 2 waterborne preservatives, the 2nd consisting of untreated blocks of 21 wood species.

Blocks of the 1st group were prepared by preservative-treating heartwood of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, ca. 2 annual rings/cm. All samples were cut from a single board. Four sets of blocks, one for each of the 4 preservatives, were vacuum-impregnated. Each preservative was used at 6 retention levels from 0-ca. 5.5 kg/m^3, yielding 24 combinations of preservative and retention level. The 2 oilborne preservatives were pentachlorophenol (PCP) and copper naphthenate (CN, retention as percentage copper). The 2 waterborne preservatives were acid copper chromate (ACC, 50% $CuSO_4 \cdot 5H_2O$: 48·3% $Na_2Cr_2O_7 \cdot 2H_2O$:1.7% H_2CrO_4) and copper chrome arsenate (CCA-type 1, 56% $K_2Cr_2O_7$:33% $CuSO_4$:11% $As_2O_5 \cdot 2H_2O$). *R. flavipes* trials were made on 3 replicates and *R. virginicus* trials on 2 replicates of all treatment combinations.

A matching assay of one set of the 6 CCA samples was made with *R. flavipes* only, using 100 cc sand rather than 0.5 g sawdust. Trials with sand were to obtain force test results for comparison with the sawdust test results.

The 2nd group of bioassays evaluated the *R. flavipes* resistance of 2 series of untreated blocks; both series were cut from single boards of 14 native

[1] Isoptera: Rhinotermitidae.

[2] Received for publication Sept. 15, 1976.

[3] Maintained by the USDA Forest Service in cooperation with University of Wisconsin Madison.

Table 1.—Average results:[a] exposure to *R. flavipes* of Douglas-fir heartwood blocks treated with a graded series of 4 preservatives using sawdust supplement assay (trial 1).

Preservative	Retention kg/m³	Wt loss of blocks mg	Termites: Initial no. 1 g	Termites: Survival[b] %	Termites: Final wt g
PCP	0.00	401	240.7	96	0.96
	0.33	179		61	0.60
	0.67	68		44	0.41
	1.31	5		0 (3-4 wk)	-
	2.62	7		0 (3 wk)	-
	5.29	13		0 (2 wk)	-
CN	0.00	473	255.8	95	0.96
	0.34	373		87	0.85
	0.68	341		86	0.81
	1.36	148		85	0.74
	2.52	2		77	0.65
	4.34	0		79	0.67
CCA[c,d]	0.00	313	235.0	96	0.97
	0.41	92		71	0.63
	0.77	52		54	0.43
	1.40	65		44	0.36
	3.12	60		76	0.69
	5.95	78		84	0.77
ACC	0.00	457	257.5	96	1.02
	0.43	415		95	1.02
	0.84	446		95	1.00
	1.65	315		95	0.98
	3.05	299		94	0.96
	6.14	254		94	0.96

[a] For 3 replicates with very similar quantitative results except where noted.
[b] Duration to complete mortality in parentheses.
[c] Termites for this trial had been maintained as a subgroup in a plastic shoe box prior to use.
[d] A retrial of CCA with retentions of 3–26 kg/m³ and with termites directly from the garbage can colony averaged 53 mg weight loss of wood blocks and 81% survival (0.76 g) of termites.

woods and 7 exotic woods. The aim was to include material with a broad range of natural resistance. Also, as with the CCA trials, 4 woods (aspen, western redcedar, redwood, and Osage-orange) were evaluated by the sand method. Table 3 lists all of the woods.

Exposures to test conditions, but without termites, of preservative-treated and natural wood reference blocks indicated that weight changes due to volatile or water-soluble chemicals in the woods were small (avg 3.9 mg weight loss) and would not affect assessment of a wood's antitermitic properties. Thus, the weight loss datum was not adjusted.

RESULTS.—In all 3 trials, termites tunneled under the wood block; due to this tunneling the attack on the block was always greatest on the bottom; and visible estimates of attack agreed with the weight loss datum.

Tables 1–3 summarize survival of termites and weight loss data for blocks. Only the 3 highest retentions of PCP were completely toxic to the termites. High termite mortality in the 1st trial of CCA and the 2nd trial of ACC was associated with a poorer initial condition of the termites and with microbial and mite contamination during the trials; however, alternate trials of these preservatives were free of these problems. In the sawdust trial of 21 woods, no contamination or toxic effects were noticeable. However, in the matching force (sand) test of 4 of these woods, some initial mortality, apparently from starvation, was followed by microbial growth on the dead termites that contributed to additional mortality.

Survival data show that the 3-4 wk supply of supplementary nutrient resulted in generally high survival of 1-g groups of termites (>750/, survival: >0.65 g live weight). Lower survivals occurred only with PCP, which was toxic, and only in the 2 trials of waterborne salts (1st trial of CCA and 2nd trial of ACC) involving contamination problems. Repetition of the 1st trial of CCA at retentions up to ca. 4 times higher than the previous highest level (Table 1, footnoted) did not have high termite mortality. The attempt to obtain compulsory feeding test data (using sand rather than supplement) for CCA in the 1st trial also failed because of the contamination problem.

Except for previously noted cases of toxicity or

Table 2.—Average results? exposure to *R. virginicus* of Douglas-fir heartwood blocks treated with a graded series of 4 preservatives using sawdust supplement assay (trial 2).

Preservatives	Retention kg/m³	Wt loss of blocks mg	Final wt[b] of termites g
PCP	0.00	370	1.02
	0.33	260	0.92
	0.67	127	0.75
	1.13	36	0.00 (4 wk)
	2.62	1	0.00 (3 wk)
	5.29	*0*	*0.00* (2 wk)
CN	0.00	311	0.96
	0.33	261	0.93
	0.68	107	0.87
	1.36	34	0.82
	2.52	11	0.77
	4.34	2	0.74
CCA[c]	0.00	275	0.95
	0.41	243	0.92
	0.77	205	0.94
	1.40	193	0.92
	3.12	131	0.91
	5.95	48	0.89
ACC[d]	0.00	299	0.93
	0.43	44	0.00 (3 plus wk)
	0.84	31	0.00 (3 wk)
	1.64	37	0.00 (4 wk)
	3.04	19	0.00 (3 plus wk)
	6.14	30	0.66

[a] For 2 replicates with generally very similar quantitative results.
[b] Initially the termites averaged 400.5/1 g: final numbers of termites were not recorded; parentheses indicate time to complete mortality.
[c] A matching trial with 10 cc sand rather than 0.5 g sawdust had microbial and mite contamination, complete mortality in all boxes by 12 days after setup, and only 8–12 mg weight losses of wood blocks, including the controls.
[d] Termites for this trial were the dregs of those isolated for the entire set of 4 trials.

Table 3.—Average results:[a] evaluation of natural resistance in 21 woods exposed to *R. flavipes* using sawdust supplement assay.

wood	Wt loss[b] of blocks mg	Termites[e] Survival %	Final wt g
Native			
Bigtooth aspen, *Populus grandidentata* Michx. (sapwood)[d]	363(381)	89 (78)	0.98 (0.83)
Loblolly pine, *Pinus taeda* L. (sapwood)	340	89	0.95
Shortleaf pine, *P. echinata* Mill. (sapwood)	320	86	0.95
Longleaf pine, *P. palustris* Mill. (sapwood)	318	83	0.91
White oak, *Quercus alba* L. (heartwood)	229	89	0.98
Western redcedar, *Thuja plicata* Donn (heartwood)[d]	215(43)	86 (7)	0.89 (0.09)
Northern red oak, *Quercus rubra* L. (heartwood)	209	85	0.93
Slash pine:, *P. elliotzz* Englm. (sapwood)	200	85	0.91
Redwood, *Sequoia sempervirens* (D. Don) Endl. (heartwood)[d]	169(72)	90 (31)	0.95 (0.31)
Port-Orford-cedar, *Chamaecyparis lawsoniana* (A. Murr.) Parl. (heartwood)	118	81	0.85
Black locust, *Robinia pseudoacacia* L. (heartwood)	54	79	0.87
Eastern redcedar, *Juniperus virginiana* L. (heartwood)	24	88	0.89
Osage-orange, *Maclura pomifera* (Raf.) Schneid. (heartwood)[d]	19(0)	83 (14)	0.91 (0.19)
Baldcypress, *Taxodium distichum* (L.) Rich. (heartwood)	9	86	0.87
Exotic			
Teak, *Tectona grandis* L.F. (heartwood)	67	90	0.96
Iroko, *Chlorophora excelsa* (Welw.) Benth. et Hook. (heartwood)	33	89	0.96
Mahogany, *Swietenia mahagoni* (L.) Jacq. (heartwood)	8	89	0.95
Australian cypress-pine *Callitris columellaris* F. Muell. (heartwood)	5	89	0.96
Niove, *Staudtia stipitata* Warb. (heartwood)	1	87	0.90

Table 3 (continued)

Wood	Wt lossb of blocks mg	Termites[c] Survival %	Final wt g
Greenheart, *Ocotea rodiaei* (Schomb.) Mez (heartwood)	0	80	0.88
Holywood lignumvitae, *Guaiacum sanctum* L. (heartwood)	0	75	0.80

[a] For 2 replicates.
[b] Losses less than 20 mg were primarily or entirely attributable to the loss of volatile or leachable extractive chemicals.
[c] Initially the termites averaged 248.8/1 g.
[d] Results in matching trial with 10 cc sand rather than 0.5 g sawdust are given in parentheses.

contamination, deaths of termites were due to cannibalism. Termites were occasionally observed chewing on live termites but active termites with partly or completely chewed-off abdomens were observed frequently. At the conclusion of sawdust trials, all surviving termites appeared vigorous and healthy.

Factors of toxicity, repellency, and unpalatability were noted in the trials. Except for toxicity with PCP preservative, the preservatives and 17 of the 21 woods had repellency or unpalatability as the principal resistance factor. The remaining woods (aspen, loblolly pine, shortleaf pine, and longleaf pine) were heavily attacked, indicating high susceptibility.

Abnormally greater fecal plastering of sample blocks by termites indicated repellent factors rather than unpalatability as being primarily responsible for resistance of these blocks. Fig. 1 shows CN-treated series of blocks after 4-wk exposure and illustrates their repellency. The untreated blocks and those with the lowest retention of CN had normal amounts of fecal plastering such as occurred even on susceptible wood blocks (Fig. 1, A, and B). However, increasing CN retentions in blocks were accompanied by noticeably stronger fumes, increased fecal plastering, and decreasing amounts of attack (Fig. 1, C, D, E, and F). Untreated blocks of 3 cedar species also were notable for fumes and excessive fecal plastering. However, the termites did not avoid any blocks, except for a few of the untreated blocks during the 1st h. The termites, therefore, became somewhat acclimatized to even repellent fumes except for the increased fecal plastering and reduced attack.

Wood blocks with unpalatability as the primary resistance factor had normal fecal plastering but were less attacked than susceptible woods. This included CCA-, and ACC-treated wood, the 2 oaks, black locust, Osage-orange, redwood, and the 7 exotic woods. Slash pine and baldcypress also seemed to have some repellent properties, but unpalatability seemed to be their principal source of resistance. (Slash pine is usually highly susceptible to termite attack; therefore, samples of this wood must have contained an abnormally high amount of antitermitic

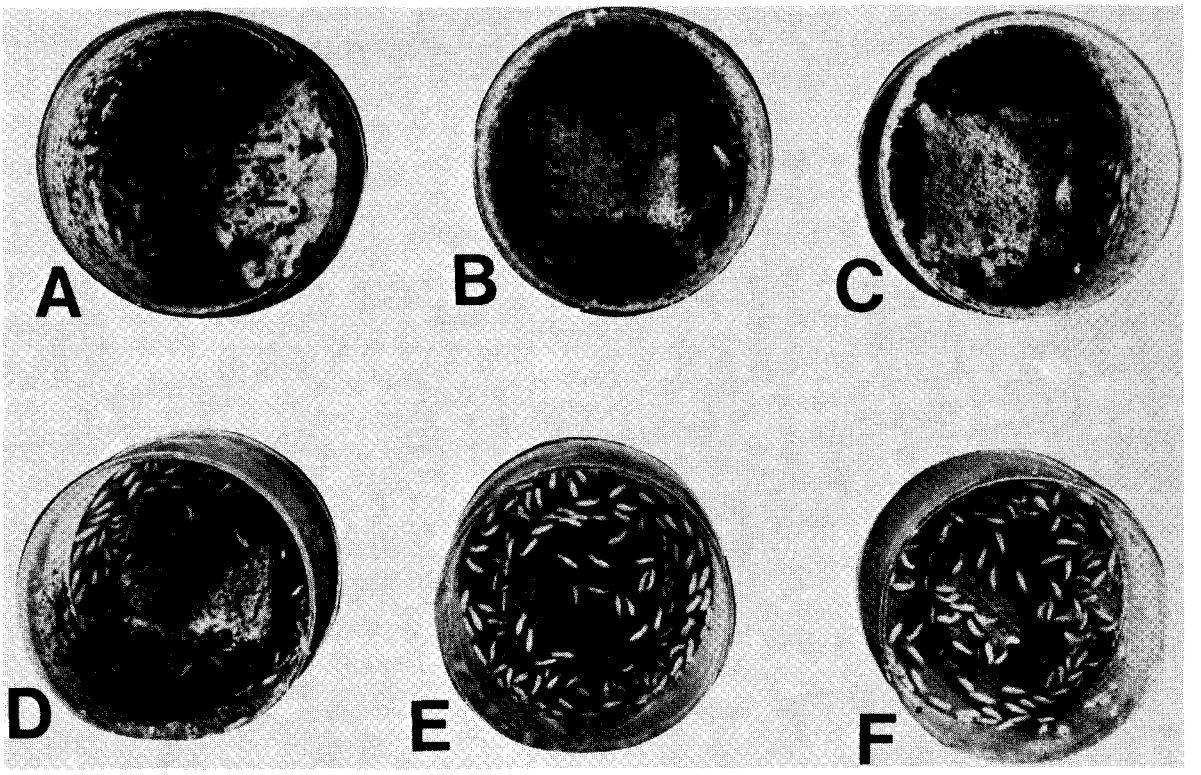

FIG. 1.—Final appearance of test units in copper napthenate series. Preservative concentration increases from A through F. Note increasing fecal plastering by termites as concentration—and repellency—becomes greater.

substances such as Becker (1966) reported in Scots pine.)

Behr et al. (1972) demonstrated that denser wood has a hardness factor which can reduce the amount of attack on that wood in a bioassay. The hardness factor may have been a primary factor in the resistance of the 2 oaks. It could account for the complete resistance of the very dense greenheart and holywood lignum vitae; however, these woods contain highly effective antitermitic chemicals in addition to hardness. The relative importance of hardness and antitermitic chemicals in termite resistance is difficult to assess because no solvent systems are available to completely extract antitermitic compounds.

DISCUSSION.—The sawdust supplement method appears to fulfill the basic cultural and behavioral requirements of subterranean termites during the 4-wk test period. Considerable emphasis has been placed on the quantity, type, or arrangement of moist tunneling medium in termite assays (see discussions in Becker 1969, Sen Sarma and Chatterjee 1970, and Fougerrouse 1973), and yet the simple conditions available to termites in the sawdust method seem biologically adequate. This method has taken into account the preference of termites to tunnel under solid wood and to congregate there. Becker (1969) indicated that the addition of a supplement markedly improves a compulsory feeding test. The sawdust method extends that idea to maintenance of termite survival and vigor for the duration of an exposure period, and to channeling as much termite energy as possible into attack on a wood sample.

The sawdust method, by alleviating starvation with termite-resistant wood samples, permits block weight loss/1 g termites/4 wk to serve as the sole criterion for assessing resistance of natural or preservative-treated wood. Haverty and Nutting (1974) and Howick and Creffield (1975) discuss the advantages of such block weight loss notation in termite assays when comparing results obtained at different laboratories, including results obtained by different assay methods. Indeed, block weight loss data are the only appropriate measure for termite attack because the attack does not alter the specific gravity of uneaten wood components. Visual, volumetric, and percentage weight loss notations ignore specific gravity differences of wood species and annual ring components. These differences can be significant: the 21 woods (Table 3) have specific gravities between ca. 0.3–1.2, and the springwood-summerwood specific gravity ratio of southern yellow pine is ca. 1:3.

Final group weight of termites seems to be a more sensitive indicator of the final condition of the termites than is percentage survival. Values based on final group weight were similar to values for percent survival (Table 1 and 3), but with slight differences. Final group weight values slightly higher than percentage survival values suggest regular feeding and

slight growth of the surviving termites after 4 wk. Weight values lower than percentage values show a lower avg weight per termite and imply a lower standard of health.

Specifically, comparisons of the matching group weight and percent survival values show that weights are slightly higher for CCA (feeding deterrent) and the 21 woods (mostly nonresistant or feeding deterrents), and slightly lower for PCP (toxic), CN (repellent), and CCA (contamination problem). Moreover, lower termite weight values for CN become progressively lower with increasing retentions, and similar weight values for the 3 cedars (repellents) are below the avg termite weight values of the 21 woods. A hypothesis can explain these differences between final group weight and percent survival values, i.e., feeding deterrents in the sample blocks do not affect termites feeding on the sawdust, However, toxicants, repellents, or contamination affect the entire test container, interfere with sawdust feeding, and thus decrease overall termite nutrition. This hypothesis for feeding effects of resistance factors has been consistently supported by my other similar trials.

Compulsory feeding (sand) test and sawdust test results in matching trials of 4 woods (Table 3) were qualitatively similar but quantitatively dissimilar. The results with the termite-susceptible aspen were similar, but in my other similar trials using sawdust, high but variable weight losses resulted for similar blocks of a susceptible wood because the termites fed primarily on either the sample block or the sawdust. Replicate samples of resistant woods had generally very similar weight losses by either assay method, but the force test and the sawdust test gave greatly different weight losses for cedar, redwood, and Osage-orange. Lower weight losses for these 3 resistant woods after the force test were due to poor survival of termites as compared with good survival by the sawdust test. Other recent examples of the diverse and generally low survival of *Reticulitermes* in force tests were given by Smythe and Carter (1970), Behr et al. (1972), Carter et al. (1972), Mannesmann (1973), Beal et al. (1974), and Carter and Smythe (1974).

As preservative retentions of samples increase, a threshold is reached beyond which there is no further improvement in protective effect. Data in Table 1 and 2 suggest approximate threshold retentions for the 4 preservatives as follows (in kg/m^3): PCP, 1.2; CN, 2.5; CCA, 3.0; and ACC, above 6.14. CCA even at 26 kg/m^3 did not have sufficient deterrency to prevent ca. 50 mg weight loss of sample blocks. Yet this slight weight loss still suggests good protection.

Comparable threshold estimates from published compulsory feeding tests, using *Reticulitermes*, are as follows (in kg/m^3): PCP, 1.28 (Martinez 1963); CCA-type 1, 1.28 (Fougerrouse 1973); and ACC, 4.0 (Lund 1959).

Weight loss data for the 21 woods are inadequate to supply trustworthy resistance ratings because only single boards of each species were used. However, the data do suggest 4 general categories of natural resistance: nonresistant (>300 mg weight loss), slightly to moderately resistant (near 200 mg weight loss); highly resistant (<100 mg weight loss); and completely resistant (<20 mg weight loss). These resistance categories for many of the 21 individual woods generally agree with the findings of Smythe and Carter (1970), Carter et al. (1972), Mannesmann (1973), Beal et al. (1974), and Carter and Smythe (1974).

The sawdust supplement method seems adaptable to bioassays with any wood-eating subterranean termite species. However, aspen wood sawdust may not readily be available in other countries, or may not be favorable for all termite species. Therefore, a medium such as the fortified cellulose diets of Mauldin and Rich (1975) may be better. But these authors reported microbial contamination of cellulose diets containing nutritional supplements. I have indicated the unfavorable impact of such contamination on bioassay results. Therefore the use of purified cellulose alone may be the most satisfactory.

REFERENCES CITED

Beal, R. H., F. L. Carter, and C. R. Southwell. 1974. Survival and feeding of subterranean termites on tropical woods. For. Prod. J. 24: 44–8.

Becker, G. 1966. Termiten-abschreckende Wirkung von Kiefernholz. Holz Roh- Werkst. 24: 429–32.

Becker, G. 1969. Rearing of termites and testing methods used in the laboratory, p. 351–85. *In* K. Krishna and F. Weesner [eds.] Biology of Termites. Vol. I. Academic Press, New York and London.

Behr, E. A., C. T. Behr, and L. F. Wilson. 1972. Influence of wood hardness on feeding by the eastern subterranean termite, *Reticulitermes flavipes* (Isoptera; Rhinotermitidae). Ann. Entomol. Soc. Am. 65: 457–60.

Carter, F. L., and R. V. Smythe. 1974. Feeding and survival responses of *Reticulitermes flavipes* (Kollar) to extractives of wood from 11 coniferous genera. Holzforsch. 28: 41–5.

Carter, F. L., C. A. Springer, and R. V. Smythe. 1972. Survival of six colonies of *Reticulitermes flavipes* on unfavorable woods. Ann. Entomol. Soc. Am. 65: 984–5.

Fougerrouse, M. 1973. Some aspects of laboratory and field testing methods of antitermitic wood preservatives. Holzforsch. 27: 134–42.

Haverty, J. K., and W. L. Nutting. 1974. Natural wood consumption rates and survival of a dry-wood and a subterranean termite at constant temperatures. Ann. Entomol. Soc. Am. 67: 153–7.

Howick, C. R., and J. W. Creffield. 1975. The development of a standard laboratory bioassay technique with *Mastotermes darwiniensis* Froggatt. Z. Ang. Entomol. 78: 126–38.

Lund, A. E. 1959. Douglas-fir plywood: its treated and natural resistance to termites. For. Prod. J. 9: 220–3.

Mannesmann, R. 1973. Comparison of 21 commercial wood species from North America in relation to feeding rates of the Formosan Termite, *Coptotermes formosanus* Shiraki. Mater. Org. 8: 107–20.

Martinez, J. B. 1963. Investigaciones sobre termiticidas y maderas resistentes a los termitos. Inst. For. Invest. Exper., Min. Agric., Madrid. 34 (81): 1–119.

Mauldin, J. K., and N. M. Rich. 1975. Rearing subterranean termites, *Reticulitermes flavipes* and *Coptotermes formosanus,* on artificial diets. Ann. Entomol. Soc. Am. 68: 454–6.

Sen Sarma, P. K., and P. N. Chatterjee. 1970. Recent trends in laboratory evaluations of termite resistance of wood and wood products. J. Indian Acad. Wood Sci. 1: 220–3.

Smythe, R. V., and F. L. Carter. 1970. Feeding responses to sound wood by *Coptotermes formosanus, Reticulitermes flavipes,* and *R. virginicus* (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 63: 841–7.

Reprinted from the
JOURNAL OF ECONOMIC ENTOMOLOGY