The Effect of Variables on Laboratory Termite Testing: Part 4—Test Block Species, Size, and Test Photoperiod

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Abstract

The objective of this study was to determine the impact on termite feeding of wood sample size and species and test photoperiod in standard tests. Native species (*Reticulitermes flavipes*) and introduced species (*Coptotermes formosanus*) were tested in an American Wood-Preservers' Association E1 standard laboratory test. For testing involving treated wood, southern yellow pine was determined to be preferable to spruce based on its treatability and availability. Test blocks of 25 by 25 by 6 mm were deemed adequate for testing, with large blocks presenting difficulty with retrieval of termites to determine mortality and smaller blocks being consumed too rapidly by the termites in the test. Photoperiod comparisons were not significantly different for *R. flavipes*; however, *C. formosanus* indicated a preference for 100 percent darkness. Therefore, the recommendation is to maintain tests using each species in a 100 percent dark environment.

In an article to the International Research Group on Wood Preservation, LaFage and Jones (1986) analyzed the original American Wood-Preservers' Association (AWPA 1972) laboratory termite testing protocol and pointed out several flaws. This is the last in a series of articles addressing some of these issues. The first article (Lindsey et al. 2021a) investigated the worker-to-soldier ratio in laboratory tests using *Reticulitermes flavipes* and *Coptotermes formosanus*. The second (Lindsey et al. 2021b) examined the effect of grain direction on termite feeding. The third evaluated density (or ring count) and moisture content (Lindsey et al. 2021c). This article examines the effect of wood species, sample size, and photoperiod on termite feeding.

Species Comparison

In our previous study (Lindsey et al. 2020c), it was determined that initially termites had a small preference for wood blocks with higher densities. Southern pine is the most common species of wood used in laboratory testing with subterranean termites in North America because it is easy to mill, has a broad sapwood band, is easily treated with various preservatives, and is easy to obtain. AWPA guidelines specify that wood without prior anti-sapstain or other treatments be allowed in testing. In the Southeast, with warm, moist conditions suitable for the growth of all types of fungi, many mills use anti-sapstain treatments in their process. Special provisions can be made to obtain wood prior to treatment. The southern yellow pine (SYP) in these tests was not treated.

Another species of wood commonly used in conjunction with subterranean termites is spruce (*Picea* spp.). Spruce is commonly found in commercial termite baiting systems (Su and Tamashiro 1986). One of the reasons spruce does well in termite tests is because it contains from 74 to 98 percent sapwood, but it is not readily treatable with wood preservatives (Wang and DeGroot 1996). This study phase was established to determine if termites prefer SYP versus spruce or weathered versus nonweathered wood in AWPA E1-97 (AWPA 1997). This study could help in deciding a more desirable wood species or combination to use in commercial baiting systems if future use of nonrepellant

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preservatives or termiticide-impregnated solid wood were used in the baiting program.

Block Size

According to Lenz (2005), termites will feed at rather low, constant rates when confronted with small volumes of food. He also stated that termites increase their wood consumption rate with an increase in volume of available food source. Cornelius and Osbrink (2001) found that consumption rates were higher when *R. flavipes* were confronted with larger pieces of wood (11 by 3.5 by 1 cm vs. 4 by 3.5 by 1 cm). Waller (1988) and Lenz (1994) also found that consumption rates increased as block size increased. Consumption rates of *C. formosanus* were not affected by the size of the test block in the Cornelius and Osbrink (2001) study. AWPA E1-97 requires a sample to be approximately 25 by 25 mm (cross section) by 6 mm. Our objective was to determine if the rate of *C. formosanus* termite feeding in AWPA E1-97 tests would be influenced by changing the size of the wood samples specified in the standard.

Effect of Photoperiod

The effect of photoperiod on termite foraging has not been studied extensively. Termite workers do not have functional eyes but do show an ability to sense light (Kofoid et al. 1946). Tests done by Park and Raina (2005) found that *C. formosanus* termites avoided light and fed more in areas of darkness than of light. *C. formosanus* alates swarm at night and are attracted to light, while *R. flavipes* alates swarm during daylight hours. Because of this trait, this test was conducted to determine what effect photoperiod had on wood consumption by workers of both *R. flavipes* and *C. formosanus* in a laboratory setting.

Methods and Materials

Termite collection

Collection techniques and assembly of test bottles have been described previously (Lindsey et al. 2020a). Of the approximately 30 g of termites (1 g per bottle) used for this test, 2 g were counted to determine total termites and percent soldiers. The first gram had 401 total termites with approximately 5 percent soldiers. The second gram had 419 total termites with approximately 3.6 percent soldiers. All termites were collected from a single colony in AWPA Hazard Zone 5 (Little and Riggins 2011).

Comparison of wood species

According to AWPA E1-97, alternate species of wood can be used during testing as long as there are five untreated SYP samples included in the testing for comparison among other tests. Experimental groups of approximately 1 g of *C. formosanus* were used in testing to determine the effects of two wood species on test results. Ten replicates of each variable were tested. All test blocks were selected according to AWPA E1-97, with the exception of species. Parameters included nonweathered SYP versus weathered SYP and nonweathered SYP versus nonweathered spruce (*Picea* spp.). Nonweathered spruce alone was tested in a no-choice test. SYP alone was not tested at the same time in this study due to the large amount of no-choice data with SYP already collected in other phases of this research (Lindsey et al. 2020a, 2002b, 2002c). Weathered stock was obtained from SYP stock measuring 25 by 25 mm in cross section that had been allowed to weather outside in open-air conditions for 6 months prior to testing. Variables used for this experiment are summarized in Table 1.

The samples were then cut to size as specified in AWPA E1-97. All samples were conditioned at room temperature to 10 ± 4 percent moisture content prior to testing. AWPA E1-97 choice tests were conducted to provide the termites with a choice of the type of wood to consume. Data were analyzed using Fisher's least significant difference at $\alpha = 0.05$ ($LSD_{0.05}$).

Block size

In this experiment, test samples of different sizes were exposed to *C. formosanus*. Variables used for this experiment are summarized in Table 2. Block sizes are illustrated in Figure 1. The 19-mm^3 sample size was chosen in part due to the possible comparison between the weight loss results of AWPA E1-97 and the results of AWPA E10-06 (AWPA 2020), the standard method for testing wood preservatives and treated products against fungal decay. No samples larger than 25 mm^3 were chosen due to the size of the test bottles used for testing.

Table 1.—Parameters used in tests to determine the effect of wood blocks on feeding by Coptotermes formosanus.

Species	Test type	Replicates
Nonweathered southern yellow pine vs. weathered southern yellow pine	Choice	10
Nonweathered southern yellow pine vs. spruce	Choice	10
Spruce only (control)	No choice	10

Table 2.—Test parameters for experiment to determine the effects of wood sample size on wood consumption rates for Coptotermes formosanus.

Sample dimensions (radial by tangential by longitudinal), mm by mm by mm)	Volume (mm^3)	Replicates
25 by 25 by 6[a]	3,750	10
18 by 18 by 18[b]	5,832	10
25 by 25 by 25	15,625	10

[a] Sample size required by AWPA E1-97.
[b] Sample size required by AWPA E10-06.

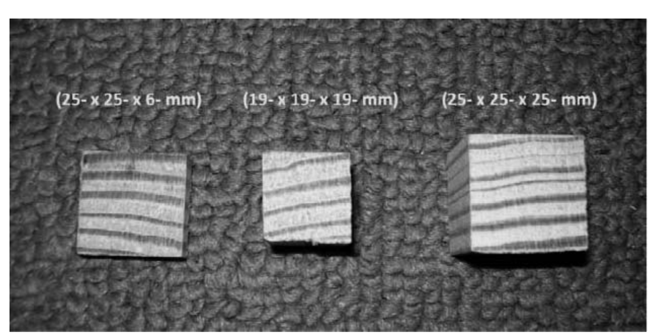

Figure 1.—Examples of samples for testing various sample volumes.

Photoperiod

Experimental groups of 1 g of both *R. flavipes* and *C. formosanus* termites were exposed to photoperiods of 100 percent light, 100 percent dark, and a natural photoperiod of approximately 50 percent light. Groups of 10 replicates using 25 by 25 by 6-mm (radial by tangential by longitudinal) samples were used for each of the three variables for each species of termite. Exposures were at room temperature (~22°C).

To achieve a 100 percent light photoperiod, the samples in these sets were placed inside enclosed containers that were lighted continuously with fluorescent lights (wavelength = 400 to 700 nm) to provide light without the heat associated with incandescent bulbs (Fig. 2). For a 100 percent dark environment, the bottles were simply placed inside a box that was completed covered allowing no light to enter. Natural photoperiod samples were placed next to windows and blocked from any artificial light sources. The natural photoperiod averaged approximately 11 hours of darkness in a 24-hour period. Light intensities are shown in Figure 3. Light intensity for *C. formosanus* testing was measured with a HOBO (Onset, USA) data logger in lumens per area.

Results and Discussion

Wood species comparison

In the choice tests, the average weight loss for weathered SYP was 15.9 and 18.5 percent for nonweathered SYP. The weathered blocks were not intentionally inoculated with decay fungi but may have been naturally inoculated with mold or decay fungi during the weathering process. Studies have shown that species of wood have been found attractive to termites when inoculated with some species of decay fungi (Amburgey and Smythe 1977). Weight loss (%) data were analyzed using $LSD_{0.05}$, and no significant difference was found.

In the choice test using SYP and spruce, the average weight loss for SYP was 13.8 percent, and that for spruce was 32.0 percent. Termites rarely ingest the hard summerwood bands in SYP, but spruce does not have such dense components of growth rings; termites can feed across the growth rings, causing a higher weight loss (Fig. 4).

$LSD_{0.05}$ comparisons showed a significant difference between spruce and nonweathered SYP. While spruce weight loss was greater than RECAST due largely to the fact that spruce has less summerwood, SYP is much easier to impregnate and could very easily be used in commercial baiting systems if impregnated solid wood is used. Hadi et al. (2014, 2015) have shown higher weight losses for less dense wood.

(a) (b)

Figure 2.—Layout for 100 percent light photoperiod for (a) Reticulitermes flavipes *and (b)* Coptotermes formosanus.

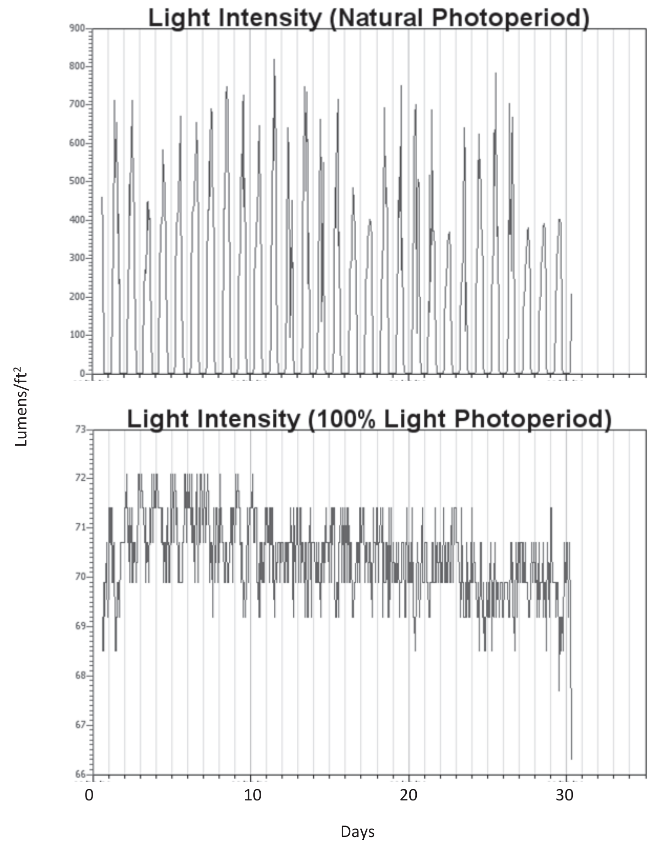

Figure 3.—Light intensities for the natural and100 percent light photoperiods.

Effect of block size

The greatest weight loss obtained was in test units containing the block size required by AWPA E1-97 (3.75 cm^3). The average weight loss for this group was 33.5 percent. The average weight loss for 19-mm cubes (6.86 cm^3) was 29.6 percent, and the average weight loss for 25-mm cubes (15.63 cm^3) was 15.2 percent (Fig. 5).

Similar to what Cornelius and Osbrink (2001) found, these results indicated that the larger block sizes used did not signal an increased feeding response from *C. formosanus* termites based on the percent weight loss values. The feeding rate (% weight loss/sample volume) is linear, as shown by the line graph in Figure 5. This confirms Cornelius and Osbrink's (2001) observations. Results also indicate that the standard size required by AWPA E1-97 is adequate to obtain quality results with the bottle size specified. Test samples smaller than the standard size would likely be fully consumed prior to the end of the 4-week period required for testing. An unfavorable outcome of using larger block sizes was that termites were difficult to remove from the specimens larger than the E1-97 standard size when obtaining survival rates. The termites formed galleries inside the 19- and 25-mm cubes and were difficult to remove at the end of the test. When calculating mortality, this would add considerable time and would not be accurate

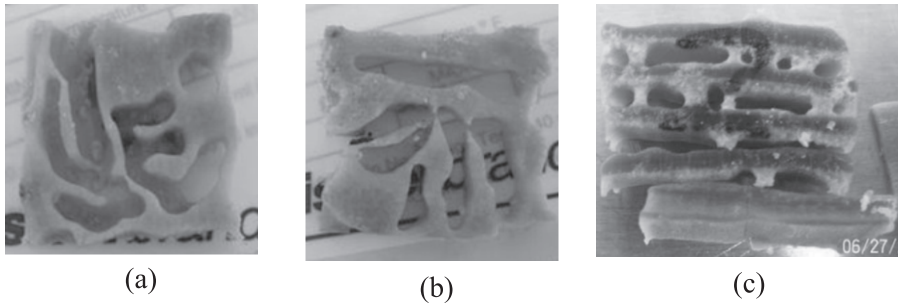

Figure 4.—C. formosanus damage on spruce blocks (a and b) after choice testing against southern yellow pine (c).

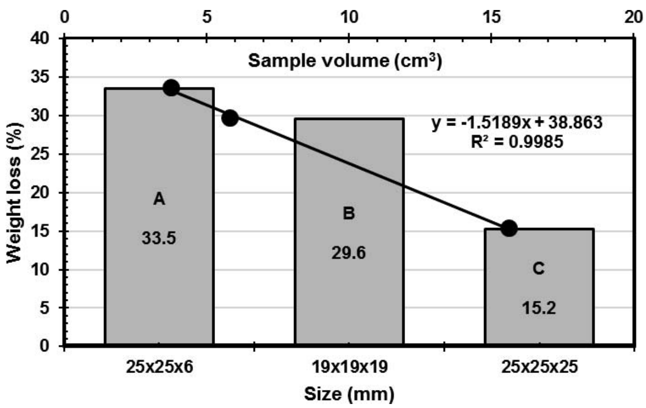

Figure 5.—Percent weight loss for different sample volumes (means with the same letter are not significantly different at $P = 0.05$).

since there would be uncertainties as to whether all termites had been counted.

Effect of photoperiod

Average wood consumption values for *C. formosanus* determined by weight loss indicated that the 100 percent dark photoperiod showed the greatest percentage of weight loss (39.6%) and was significantly different from the other photoperiods (Fig. 6a). Average weight losses for photoperiods of 100 percent light (34.4%) and the natural photoperiod (26.4%) varied and were significantly different according to Fisher's test. The natural photoperiod averaged approximately 11 hours of darkness in a 24-hour period.

Average wood consumption values for *R. flavipes* determined by weight loss indicated that the 100 percent light photoperiod showed the greatest percentage of weight loss (19.4%) but was not significantly different from the other photoperiods. Weight losses for photoperiods of 100 percent dark (18.9%) and the natural photoperiods (15.7%) for *R. flavipes* varied and were not significantly different (Fig. 6b).

Both species of termites produced more shelter tubes when subjected to a 100 percent light source. An example of *C. formosanus* tubing when subjected to 100 percent light can be found in Figure 7. Termites subjected to the natural photoperiod had a higher mortality than those subjected to the other photoperiods. This may be attributed to mold growing in the test bottles subjected to natural photoperiod. This mold was likely a result of the constant fluctuation of temperature, the location next to a window causing excessive condensation in bottles.

The percent weight loss values obtained would indicate that it would be advantageous to test *C. formosanus* in a 100 percent dark environment and *R. flavipes* in a 100 percent light environment. However, since there was no significant difference in the results for *R. flavipes*, it would be a simpler method to test all species in a 100 percent dark environment

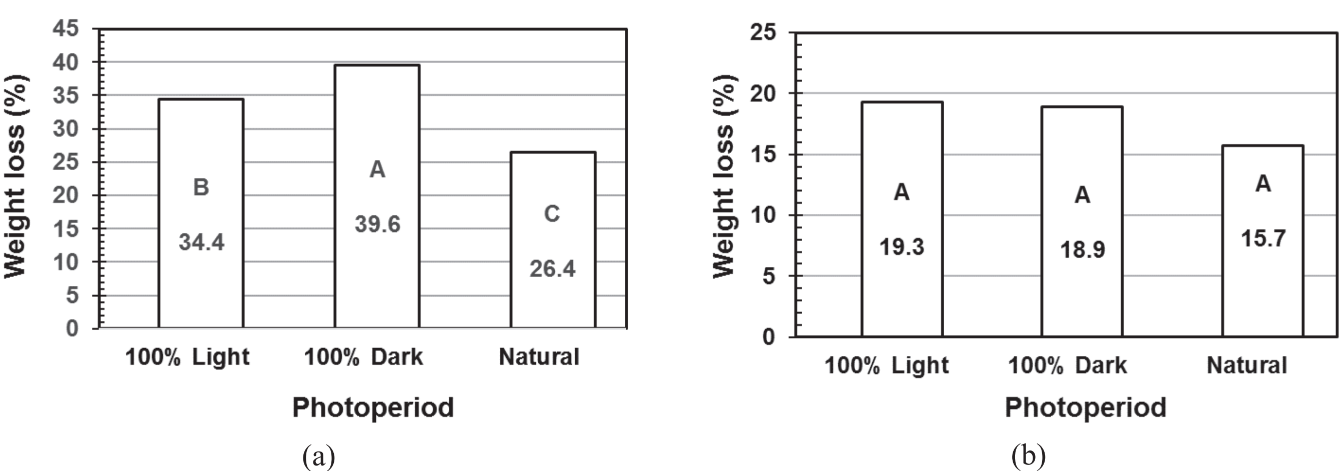

Figure 6.—Weight loss for different photoperiods for (a) Coptotermes formosanus and (b) Reticulitermes flavipes (means with the same letter are not significantly different at $P = 0.05$).

Figure 7.—Increased amount of tubing found after testing C. formosanus *with 100 percent light exposure when compared to those tested under a 100 percent dark environment.*

since the temperature can be regulated in the dark environment of a chamber.

Summary and Conclusions

Spruce wood was evaluated to determine if this would make an adequate alternative species to use as a control. Spruce is known to be favorable to termites, as it is used mainly in commercial baiting stations for termite control. Spruce should be used in evaluations only if the remaining test blocks are spruce as well. Very favorable results were obtained with spruce when compared to SYP. SYP should still be the preferred choice when doing E1 tests because it is easy to obtain and is readily treatable with wood preservatives. Spruce is not readily treatable. Weathered SYP was also compared to nonweathered SYP but did not have a significant effect on the outcome of test results.

The investigation of sample size effects indicated that the specified sample size should remain unchanged in future updates to the standard, as this produced adequate results. Increasing the sample size made termite collection difficult for determining mortality and for obtaining weight to calculate mass loss percentages. Decreasing the sample size likely would cause the sample to be consumed prior to the end of the 28-day period required for testing.

Photoperiods of 100 percent light, 100 percent dark, and the natural photoperiod (11 hours light, 13 hours dark) were tested to determine tendencies for both *R. flavipes* and *C. formosanus*. The results from this test indicated a higher average test block weight loss percentage in the 100 percent light period for native *R. flavipes* species and a higher average test block weight loss percentage for the 100 percent dark period for *C. formosanus*. These results correspond with what is known about each termite's biology. Alates of *R. flavipes* swarm during daylight hours, while alates of *C. formosanus* swarm during nighttime hours and are attracted to light. Even though weight loss data indicated that *R. flavipes* slightly favored 100 percent light conditions, it was not significantly different from the other photoperiods. However, the tendency shown by *C. formosanus* for dark conditions was significantly different from the other photoperiods tested. Based on these data, it is recommended that a statement be put into the standard to require testing in a 100 percent dark environment. This would be easy to accomplish since most testing is likely done in dark environmental chambers with controlled temperatures.

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