# THE EFFECT OF VARIABLES ON LABORATORY TERMITE TESTING: PART 3-TEST BLOCK DENSITY AND MOISTURE CONTENT[1]

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**Abstract.** The objective of this study was to determine the impact on termite feeding of wood ring count and MC in standard tests. Introduced species (*Coptotermes formosanus*) were tested in an American Wood Protection Association (AWPA) E1-97 standard laboratory test. Samples with a ring count in the range of 6-10 rings per inch (RPI) had a higher, but not significantly different, weight loss than the 4-6 RPI specified by E1-97. With respect to the MC, it was determined that a lower level of moisture than that currently specified by AWPA E1 would be preferred over the higher MC achieved in ASTM D3345. Sample placement on top of the sand was recommended over the burial procedure in ASTM D3345.

***Keywords:*** *Coptotermes* Formosan termites, test methods, AWPA E1 ASTM, D3345 density, moisture content, ring count.

## INTRODUCTION

Waller et al (1990) found that *Coptotermes formosanus* (Formosan subterranean termite [*FST*]) consumed comparable volumes of biomass but larger percentages of wood volume than compressed pine or higher density pine vs non-compressed or lower density pine. AWPA (1997) Standard E1-97 requires four to six growth rings per 25 mm (RPI) when using solid wood. Termites are known to prefer the less dense earlywood portion of wood (Behr et al 1972) as seen in Fig 1.

One of the most important parameters for conducting a termite laboratory test is ensuring an adequate supply of moisture. Termites are delicate insects and are very prone to desiccation if enough moisture is not maintained in their environment (Kofoid et al 1946). Too little water can cause desiccation in termites, but excessive

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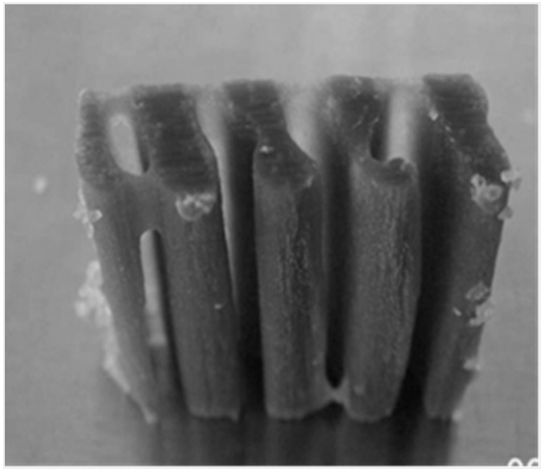

Figure 1. Example of southern pine block after exposure in an E1 test showing termite preference for earlywood.

moisture can have adverse effects on termite foraging and survival. Haverty (1979) found that *Reticulitermes virginicus* had its worst survival rate in sand with high MC levels. In their natural environment, subterranean termites have many avenues to prevent desiccation or to maintain their desired level of MC. Subterranean termites build shelter or mud tubes made of mud and feces to maintain a humid environment while traveling across exposed areas. They also tend to move higher or lower in food sources, such as dead standing timber, to their desired level of MC via galleries they have formed. *FST* frequently build carton material nests to provide a constant and consistent supply of moisture. According to Forschler and Henderson (1995), when termites are subjected to large quantities of water, they do not try to escape but enter a "state of quiescence." This indicates that if termites were subjected to a higher than normal MC, feeding rates could be affected.

This is the third in a series of four articles investigating parameters affecting termite feeding in AWPA (1997) Standard E1-97. The first article investigated the worker to soldier ratio in laboratory tests using *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) and *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) (Lindsey et al 2021a). The second article (Lindsey et al 2021b) examined the effect of grain direction on termite feeding. Other wood sample properties to consider when evaluating possible variations to the standard include the 1) ring count and MC (this manuscript), 2) wood species, 3) test block size, and 4) photoperiod (discussed in Lindsey et al 2021c).

The objective of the first phase of this study was to determine if *FST* had an actual preference for wood samples of different ring count. Two tests were conducted to determine what effects moisture would have in the AWPA (1997) Standard E1-97. The first test was to determine if the moisture required by the standard was the proper amount for testing and, if not, to provide alternate levels that would accelerate feeding when compared with standard levels. The second test was to determine the moisture contents of blocks exposed to ASTM (2020) D 3345 conditions (block below sand) and the MC of test blocks exposed to AWPA (1997) E1-97 conditions (block above sand).

## METHODS AND MATERIALS

### Termite Collection and Sample Preparation

Experimental groups of approximately 1 g of *FST* were used in testing to determine the effects of sample ring count on test results. Ten replicates of each variable were tested. All test blocks were 100% southern pine (SYP) sapwood selected according to the E1-97 standard (AWPA 1997) apart from ring count requirements. Samples of the same ring count were taken from the same board. All samples measured 25- × 25-mm in cross section (r × t) × 6-mm longitudinally. Collection and exposure procedures are described

Table 1. Test parameters for the no-choice experiment to determine the effects of sample density on wood consumption by *C. formosanus.*

| Average specific gravity | Growth rings (RPI) (number/25 mm) |
|---|---|
| 0.42 | 2-3 |
| 0.52 | 5[a] |
| 0.51 | 7[b] |
| 0.72 | 13-15 |

[a] In range of the growth ring count required by AWPA Standard E1 (1997).
[b] In range of the growth ring count required by AWPA (1997) Standards E7 and E10.

in detail elsewhere (Lindsey et al 2021a). Results were compared using a Tukey's test at $p = 0.05$.

## Effect of Ring Count (RPI)

***No-choice test.*** Variables used for this experiment are summarized in Table 1. In a no-choice test, only one block per bottle is used.

***Choice test.*** For this phase, a choice test was conducted. A block of low specific gravity (2-3 RPI, SG = 0.43) was placed in the test bottles along with a high specific gravity block (13-15 RPI; SG = 0.72), allowing for choice feeding by the termites.

## Effect of MC (MC)

***Experiment #1.*** In this first test, experimental groups of approximately 1 g of *FST* were used in testing to determine the effects sand MC had on test results. Ten replicates of each variable were tested. All test blocks were placed on top of the sand. Screened, washed, and dried silica sand (120 g) was added to each test bottle. Variables used for this experiment are summarized in Table 2. Distilled water was added to each test bottle 2 h before the introduction of termites as required by the standard. Amounts of water used were 10 mL (8% sand MC), 20 mL (17% sand MC), 30 mL (AWPA E1-97 standard volume, 25% sand MC), 40 mL (33% sand MC), and 60 mL (50% sand MC).

***Experiment #2.*** In a second test, various volumes of distilled water were added to each test container (provide details on the media, without termites). The objective was to determine the final percent MC of blocks using the ASTM D3345 (buried in sand) and AWPA E1 (top of sand) methods for block placement. Amounts of water used for this test were 5, 10, 20, 30, and 40 mL.

# RESULTS AND DISCUSSION

## Effect of RPI

Some standards, such as AWPA Standards E7-15 (AWPA 2020a) and E10-16 (AWPA 2020b), have higher ring count requirements (6-10 RPI) than those found in AWPA E1 (4-6 RPI). The highest weight loss group for this test was the seven RPI samples (higher than specified in E1-97, but within the specifications of E7 and E10), with an average weight loss percentage of 33.3% (Table 3). The lowest weight loss group was 13-15 RPI, with an average weight loss of 20.5%. The average weight loss for the lowest ring count group (2-3 RPI) was 29.5%. This would indicate that at both the highest and lowest RPI, termites did not consume as much of the wood when compared with wood consumption at 5-7 RPI groups. The reason for the low ring count result is not clear. Low weight loss in the high ring count may be due to the lower volume of earlywood.

Results of the choice test when comparing high and low RPI show a significantly lower weight loss of 9.9% for the low 2-3 RPI samples and 16.5% for the 13-15 RPI samples. This would indicate that termites preferred the higher specific gravity samples. It was also evident, visually, during this testing that termites, initially, had a greater attraction to the higher ring count samples. As seen in Fig 2, 2 h after being introduced into the test bottles, approximately 100% of

Table 2. Test parameters for the no-choice experiment to determine moisture preferences for *C. formosanus*.

| Moisture volumes/120 mL of washed sand (mL) | Sand MC (%) |
|---|---|
| 10 | 8 |
| 20 | 17 |
| 30[a] | 25 |
| 40 | 33 |
| 60 | 50 |

[a] Specified by AWPA E1-97.

Table 3. Percent weight loss in a no-choice test for various wood sample ring counts.

| Rings per inch | Weight loss (%)[a] |
|---|---|
| 2-3 | 29.5 A |
| 7 | 32.8 B |
| 5 | 33.3 B |
| 13-15 | 20.5 C |

[a] Means with the same letter are not significantly different at $p = 0.05$.


Figure 2. Example of workers aggregating on a higher specific gravity sample 2 h after introduction to a choice test (Note how FST are attracted to the high SG sample, while avoiding the low SG sample).

termites were attacking the higher ring count samples.

At the high growth ring counts, more of the wood volume consists of latewood bands when compared with the lower ring count samples. The low growth ring samples used in this study may be juvenile wood that is characterized as fast growth timber found near the center of trees. Juvenile wood generally contains more lignin and thus less cellulose than does slower growth mature wood (Haygreen and Bowyer 1989). This could be the reason why the termites consuming more of the mature growth, higher ring count samples.

Using a range within those specified in AWPA (2020) Standards E7-01 and E10-01 (6-10 RPI) could be advantageous, opposed to having different ring count specifications in the AWPA evaluation standards. Higher weight losses and no significant difference were found when comparing 6-10 RPI vs the standard count of 4-6 RPI. Using the same ring count in E1, E7, and E10 could possibly allow later comparison if samples for both laboratory and field testing came from the same parent board or boards of the same ring count. The RPI could be changed much as the grading scales in all AWPA standards have been changed most recently to correspond with each other so further comparison could be achieved. This would also make sense since some of the 4-6 RPI samples may contain some undetected juvenile wood, thus less cellulose that could prove to be unfavorable initially to termites.

## Effect of MC

***Experiment #1.*** Average weight loss values (Fig 3) indicated the greatest weight loss occurred in the lowest moisture volume test. The average weight loss for this group (10 mL) was 33.2%. The average weight losses obtained from the remaining groups were 26.8% for 20 mL, 26.7% for 30 mL, 24.8% for 40 mL, and 20.1% for 60 mL. As seen in Fig 3, as the volume of moisture increased the weight loss decreased. This would indicate that the termites might have been in a more relaxed state with the higher volumes of water as shown by Forschler and Henderson (1995). According to Tukey's test, the 10-mL addition resulted in a significantly higher weight loss than all other addition rates. Sixty milliliter was also significantly different from the 10, 20, and 30 mL groups, but not the 40 mL group. The 20, 30, and 40 mL groups were not significantly different from one another. These data differ from those of LaFage and Jones (1986) with *R. virginicus* (Banks) in a review of M12-72 where they found no difference between the 9-17% MC groups. It is possible that species of *Reticulitermes* spp. have MC requirements different from *FST*. This should be determined in future research.

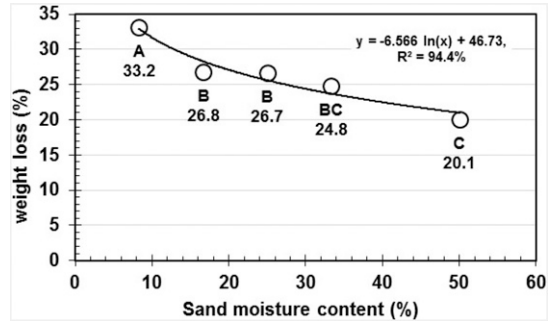


Figure 3. Average weight loss data for various moisture volumes tested against *Coptotermes formosanus* in an E1 test (means with the same letter are not significantly different at $p = 0.05$).

Lowering the volume of water in each test container could have further advantages such as decreasing the leaching that is typically found with some wood preservatives. Decreasing the amount of water per test container from 30 mL to 10 mL could reduce the risk of mold contaminations caused from excessive moisture. The mortality was visually observed at the end of the test and was found to be only slight for all groups tested with no major differences detected.

***Experiment #2.*** MC values obtained when placing the sample on the bottom of the jar such as specified in ASTM D3345 when compared with placing the test sample on the top of the sand as specified in AWPA E1 are shown in Fig 4. Taken with the results of the first test, this indicates the best placement for the sample is on top of the sand.

## SUMMARY AND CONCLUSIONS

Testing showed that termites were initially attracted to the higher ring count samples. This fact suggests a need for more research to determine the exact cause. These higher density samples are not recommended for the AWPA E1 test because these samples are more difficult to obtain, being from slower and older growth pine trees. However, a slight increase in the ring count from the 4 to 6 RPI specified in the standard could be advantageous. Using test blocks with a ring count in the range of 6-10 RPI, weight losses were not significantly different from the standard count and had a higher average weight loss. This would allow researchers to take samples from the same parent board for other laboratory tests such as AWPA E10 and some portions of field tests such as AWPA E7. If samples were taken from the same parent board, then further comparisons among these tests would be possible.

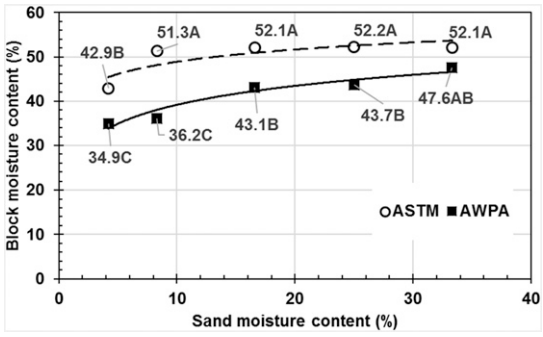


Figure 4. Average moisture contents of blocks after 28 d of equilibrium in termite test bottles for the ASTM D3345 (block placement at bottom) and AWPA E1 (block on top of sand) methods (means with the same letter are not significantly different at $p = 0.05$).

The MC of test samples and containers plays a very important role when testing subterranean termites as they are very delicate and prone to desiccation. When various moisture levels and two block locations were tested, it was determined that a lower level of moisture than that required by AWPA E1 would be beneficial. The lower level tested, 10 mL, compared with the standard amount, 30 mL, yielded significantly higher weight loss. Using a lower volume of moisture could have many benefits, such as a reduced chance of mold contamination and possible reduced leaching effects from wood preservatives. Future tests should be based on these results to determine if a lower level of moisture could reduce the amount of chemical leaching from preservative impregnated samples. Leaching is an issue because sometimes the chemicals leach into the sand before termites have a chance to feed on the test specimen. In comparing the ASTM D3345 and AWPA E1 methods of sample placement, it was clear that test blocks should be placed on the surface of the sand, as in standard AWPA E1, as opposed to the bottom placement in ASTM D3345.

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