Effects of Termite Procurement Variables on the Test Results Based on the AWPA E1-06 Standard Method for Laboratory Testing to Determine Resistance to Subterranean Termites

Todd Shupe
Qinglin Wu
Jay P. Curole
Dennis Ring
Louisiana State University Agricultural Center
Baton Rouge, Louisiana

ABSTRACT

The AWPA E1-06 test (Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites) is an important test to determine the resistance of a preservative treatment, heat treatment, chemical modification, etc. of cellulosic material to subterranean termites. This test is of particular interest to companies developing new wood preservatives or protection methods for various market sectors. The Formosan subterranean termites (FSTs; *Coptotermes Formosanus*, Shiraki) were introduced in the United States following World War II. The FSTs have led to renewed interest in wood durability, particularly for housing applications in the U.S. South, which is in an inherently high deterioration risk zone. The E1 test may be affected by numerous factors that are not currently accounted for and perhaps may not need to be in the current test protocol. Particular factors of interest that may influence the FST vigor include geographic location, collection method, collection season, laboratory storage time, and average termite size. This study was therefore initiated to determine effects of these variables on termite mortality, sample weight loss and sample visual rating (0-10) after 28 days of testing. To measure these effects, untreated southern yellow pine solid wood samples (controls) were used.

INTRODUCTION

A pest that mostly goes unseen is causing a massive amount of damage to the U.S. Destruction caused by the Formosan subterranean termites (FSTs) is a growing problem in the United States, particularly in southern areas. The cost of treatment and damage in the U.S. is in the billions for pre-treatment, treatment of infestations, structural damages, and reconstruction. *C. formosanus* has its greatest impact in North America. Lax and Osbrink (2003) states that, "C. formosanus Shiraki is currently one of the most destructive pests in the USA. It is estimated to cost consumers over US $1 billion annually for preventative and remedial treatment and to repair damage caused by this insect." Termites have evolved for millions of years, thus making it very difficult to control their spread. The Formosan subterranean termite *Coptotermes formosanus* Shiraki, was first found in Lake Charles, Louisiana, in 1966 and in New Orleans, Louisiana, in 1967 (Spink 1967). Areas located near the ports where it is believed the Formosan subterranean termite was initially brought to the U. S. are hardest hit by this pest. *C. formosanus* is the most economically serious pest in Hawaii, costing residents $100 million a year (Tulane 2002). Historic structures in Hawaii have been threatened, such as Iolani Palace in Honolulu (Grace et al. 2002). *C. formosanus* are believed to have been brought into the U.S. on ships returning from Asia after WWII. These ships returning from the war contained infested wood materials which were later transported into Louisiana. Termites can be dispersed by humans in many different materials ranging from railroad ties, utility poles, wood structures, lumber, pallets, firewood, trees, mulch, potted plants, paper, etc. Later, once established, *C. formosanus* began to spread alate flight dispersal (Messenger 2002). This flight dispersal usually begins around Mother's Day and can last up to two months. There are several ways to identify Formosan termites from native termites. Formosan termites fly at night and native termites fly during the day. Also Formosan alates are an amber/brown/white color while native alates are dark brown/black in color. Formosan subterranean termites have hairs on the wings and native subterranean termites do not have hairs on the wings. Louisiana especially has the ideal climate for Formosan termites. The largest swarms in Louisiana occur between New Orleans and Lake Charles, the two locations of this study. In New Orleans, 30-50% of the city's 4,000 historic live oak trees are believed to be infected with total damage costing the city $300 million a year (Tulane 2002). These termites are more aggressive than our native termite colonies. Raloff (2003) states that in North America *C. formosanus*, "create significantly bigger colonies, and therefore more damage, than do their native U.S. cousins, which reside underground and enter buildings only to forage." The major problem with the Formosan subterranean termite is that they can number up to ten million or more individuals per colony. A native colony will only number up to three-hundred thousand to two and a half million individuals.

The FSTs have resulted in a heightened awareness of wood durability by home owners (Vlosky and Shupe 2002). New treatments are needed to help develop wood building components that resist the attack of the Formosan Subterranean Termite (FST) (Coptotermes formosans) in the Southern United States. Leading companies are putting lots of money into new products and formulations to find new ways to control this pest. Companies are also working on finding more user and environmentally friendly pesticides for controlling FST damage.

Previous research has been conducted to determine the effect of selected variables on the AWPA E1 test. Lindsey (2010) evaluated selected parameters specified in the AWPA E1-97 standard for the laboratory evaluation to determine resistance to subterranean termites. The research determined the effects of variables such as moisture of sand substrate; size, type, and location of test material within a test bottle; and termite number, and ratios of workers to soldiers per test bottle. This research was used to improve the comprehension of termite biology in a laboratory setting to help researchers better develop new control methods. The study found that the worker to soldier ratio has been changed to not exceeding a ten percent soldier proportion in the current standard. It was determined that the test block grain orientation should be specified or required as either 6 mm in the longitudinal or tangential direction, but no changes were suggested for the testing block size. Density of the test block was tested, however it was suggested that more testing needs to be conducted to see how density of the test blocks will affect results. Spruce wood was evaluated as an alternative to pine wood, but because of the ready availableness of pine no change to the standard was made. Based on photoperiod data it was recommended that a statement be put in the standard to require testing in a 100% dark environment. Soil moisture content was tested and it was suggested that future tests need to be conducted to see how moisture content plays a role in mold growth and chemical leaching from wood blocks and lowering the moisture content of the AWPA E1 test yielded a significantly higher weight loss of the testing blocks.

This research addresses the idea that better handling and collection methods could result in termites with a greater vigor. By eliminating or reducing as much human related variability as possible, perhaps termite vigor and attack on untreated control samples in the E1 test can be improved. This ongoing research might lead to modification to the test standard.

OBJECTIVES

The objective of this study was to determine the effect of termite collection method, geographic location, average termite size, laboratory storage time, and season collected on termite mortality, sample weight loss, and sample rating (0-10) using untreated southern yellow pine solid wood samples (controls) after 28 days of testing in the AWPA E-1 test (AWPA 2006). Numerous tests have been performed by the Wood Durability Laboratory (WDL) over the years for industry clients using these methods. Each industry test must be accompanied by 5 WDL control jars to determine the health and vigor of the termites. This paper will report on the control samples from these studies, our improved methods of collecting termites, and how we are enhancing our laboratory testing methods.

MATERIAL AND METHODS

Jars were set up to compare the dependent variables of termite mortality, sample weight loss, and sample rating to the independent variables of collection method, collection season, geographic location, laboratory storage time, and average termite size for wood consumed by termites on control wood blocks. The two collection methods were wood blocks and veneer sheets, the seasons were spring (March-20 to June-20), summer (June-21 to September-21), autumn (September-22 to December-20), and winter (December-21 to March-19), the geographic locations were Lake Charles, Louisiana and New Orleans, Louisiana, and the laboratory storage times were 1 week, 2 weeks, 3 weeks and greater. Data were analyzed for statistical differences. Data for sample weight loss were analyzed using Proc GLM and LSmeans with the Tukey/Kramer multiple comparison adjustment. Data for termite mortality and sample ratings were analyzed using Proc Logistic with an optimization technique of the Fisher's scoring.

Termite Collection

Standard plastic milk crates were buried in the soil near or around an area that was found to be infested with Formosan subterranean termites. The crates were filled with either untreated southern yellow pine (*Pinus sp*.) wood blocks or southern yellow pine veneer sheets. The wood block material is typically kiln dried and has a moisture content of approximately 4-8% at the time of installation and the veneer sheets used for this study are considered dry. The original method of collecting termites was to use crates filled with (44) 1 x 1 x 11 in. pieces of southern yellow pine solid wood blocks. The preferred method was later devised by following some European and Australian methods of using southern yellow pine veneer cut to 11 x 11 in. and placed inside the plastic milk crates.

Laboratory Storage

Once the collection crates are determined to be infested with termites, the crates are collected in plastic garbage cans and returned to the Wood Durability Laboratory (WDL) in Baton Rouge, La. A brick is laid on the bottom of a metal tub, the garbage can is placed on top the brick, and the tub is filled with water to the bottom of the garbage can. The water keeps any ants from getting inside the garbage can. Also the water keeps the termites inside the garbage cans, because *C. formosanus* can and will eat through the plastic garbage cans. The termites are then stored in the laboratory and extracted for used in accordance with the American Wood-Preservers' Association Standard E1-06 (AWPA 2006) for single choice testing.

Termite Extraction

Progress has been made to decrease the amount of stress applied to the termites and increase the ease of extracting the termites from the collection material. The wood block extraction method can be very stressful to the termites because each individual termite is delicate. A hammer is used to strike the wood blocks to extract the termites. This is also a time consuming process. Through this process, the termites are exposed to dry air for several hours. A new method was considered to collect termites to lessen the stress applied to the termites. Therefore, the current method of collecting termites was developed. This process uses dry pine veneer. The veneer extraction seems to be less stressful on the termites and is a faster means of getting the termites into our laboratory testing jars for the AWPA E1-06 (AWPA 2006) test. This extraction method is much cleaner and faster than extraction out of the wood blocks. Veneers are placed in the milk crates in a similar manner as the wood blocks. The termites are extracted by simply gently shaking a piece of veneer.

Weight per Termite

In order to get 400 termites determined by weight in each testing jar, samples of termites are taken, weighed, and an average weight per termite determined. One hundred termites are counted out and weighed (done five times). These weights are added up and divided by 5 to get an average weight per one hundred termites. This weight is then divided by 100 to get an average weight per termite. The weight per termite is multiplied by 400 to get an average weight of 400 termites. This weight is then used to measure out approximately 400 termites to go in each testing jar.

Standard E1-06 Laboratory Jar Test

Each E1-06 test includes five replications of untreated southern yellow pine controls (AWPA 2006). Two different options are available with the E1-06 standards, (1) a no-choice test and (2) a choice test. In the no-choice test, each jar contains one sample for the termites to feed. This test is good for determining the toxicity of material to termites. The other test is a choice test where there are two samples, which the termites are to choose from. This test is consistent with most field tests in that it offers the termites a choice of another available material. All data presented here are from the no-choice AWPA E1-06 (AWPA 2006) jar test control samples from 2003 – 2007. Each testing jar was autoclaved and contained 150 g of autoclaved sand and 30 ml of distilled water. A sample was placed in each jar on top of the sand with two sides of the sample contacting the jar wall. Four hundred termites were introduced to each jar on the side opposite the sample.

Sample Preparation

Sample preparation include includes five southern yellow pine samples plus five matched moisture content (MC) samples milled from the same board into 25 mm (1 inch) by 25mm (1 inch) by 6mm (1/4 inch). These samples must be southern yellow pine sapwood clear of knots and defects. Each sample must have a paired sample, which is used to determine moisture content (MC). MC values for each specimen are calculated by weighing each sample along with its paired MC sample. The MC sample is placed in a drying oven to dry then reweighed. This oven-dry weight is used to determine the MC value of the MC sample and its matched test specimen at the time they were first weighed. This MC value is then used to calculate the oven-dry value of the test specimen. This keeps us from having to actually dry the test specimen prior to putting it in the testing jar. This procedure is used for all samples that are tested, (the control samples contain no treatment or preservative) so that no volatile chemicals are dried off before they are exposed to termites.

Termite Location

Termites were obtained from Brechtel State Park (New Orleans, La.) and Sam Houston Jones State Park (Lake Charles, La.). The New Orleans site is in a lower lying area that is moist for most of the year. Lake Charles is in extreme southwest Louisiana, and this site is located on higher/well drained land when compared to the New Orleans location.

Sample Weight Loss

Percent weight loss is based on the original oven dry weight using this formula: (calculated ODWt – final ODWt)/calculated ODWt. The test sample oven dry weight is determined by measuring the moisture content of the matched sample and using it to calculate the test sample oven dry weight. It is assume that the termite test samples had the same MC as the matched MC samples. The final oven dry weight of the test sample is determined by oven drying the sample after the test. After 28 days of exposure, the samples were removed and cleaned with distilled water to remove termites and sand. Sample weight loss was determined by oven drying each sample, then weighing each sample to get an oven dry weight.

Termite Mortality

All live termites are counted after the 28 day exposure period. Percent mortality was obtained with this calculation: ((initial termites – live termites) / initial termites)*100. Samples of termites were taken and weighed and an average weight per termite was determined. Termite compositions consisted of no more than 10% soldiers and the rest workers. Termite mortality is determined by using a vacuum pump to count the live termites remaining in the test jars after the 28 days of testing. The initial numbers of termites are subtracted for the live number of termites. That number is then divided by the initial number of termites resulting in percent mortality.

Sample Rating

Each block is examined and visually rated using the following rating system:

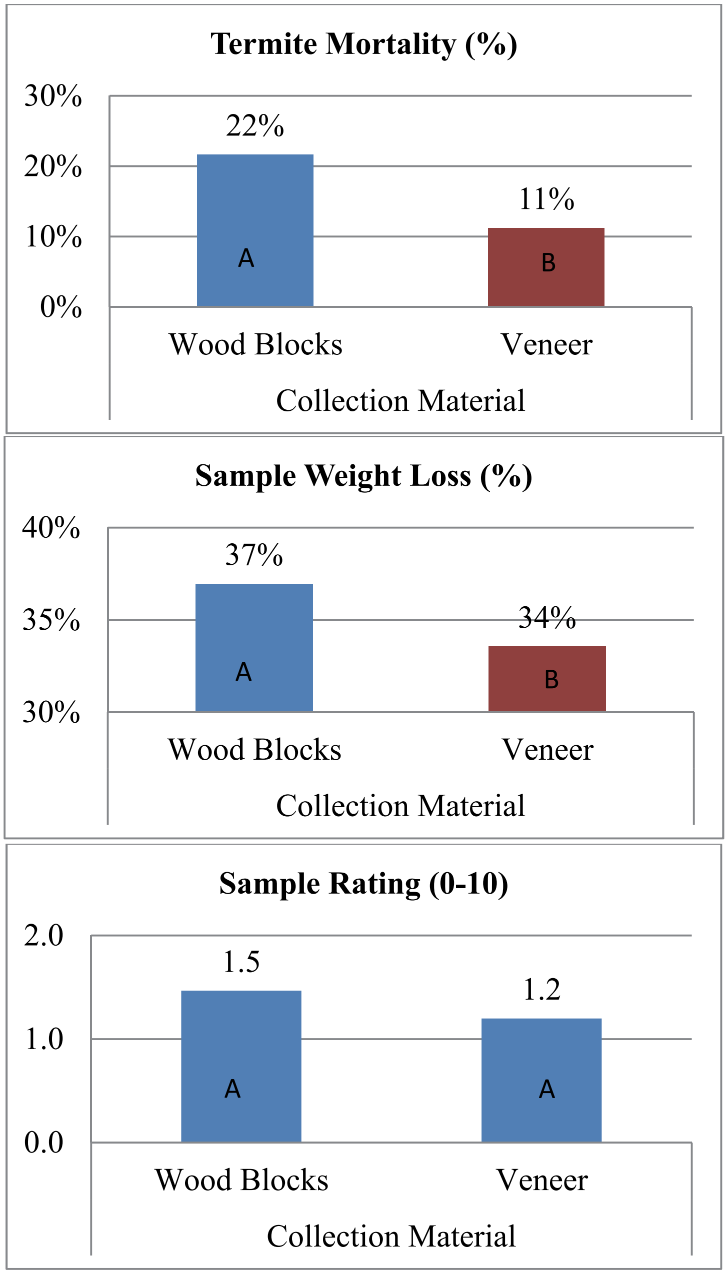
10	Sound
9.5	Trace, surface nibbles permitted
9	Slight attack, up to 3% of cross sectional area affected
8	Moderate attack, 3-10% of cross sectional area affected
7	Moderate/severe attack, penetration, 10-30% of cross sectional area affected
6	Severe attack, 30-50% of cross sectional area affected
4	Very severe attack, 50-75% of cross sectional area affected
0	Failure

Data Analysis

Data were analyzed for statistical differences (Steel and Torie 1980). Data for sample weight loss were analyzed using Proc GLM and LSmeans with the Tukey/Kramer multiple comparison adjustment. Data for termite mortality and sample ratings were analyzed using Proc Logistic with an optimization technique of Fisher's scoring (SPSS 2006).

RESULTS

Figures 1-5 show the sample rating, weight loss and mortality, respectively, as affected by collection location, collection season, collection method, laboratory storage, and average termite size.

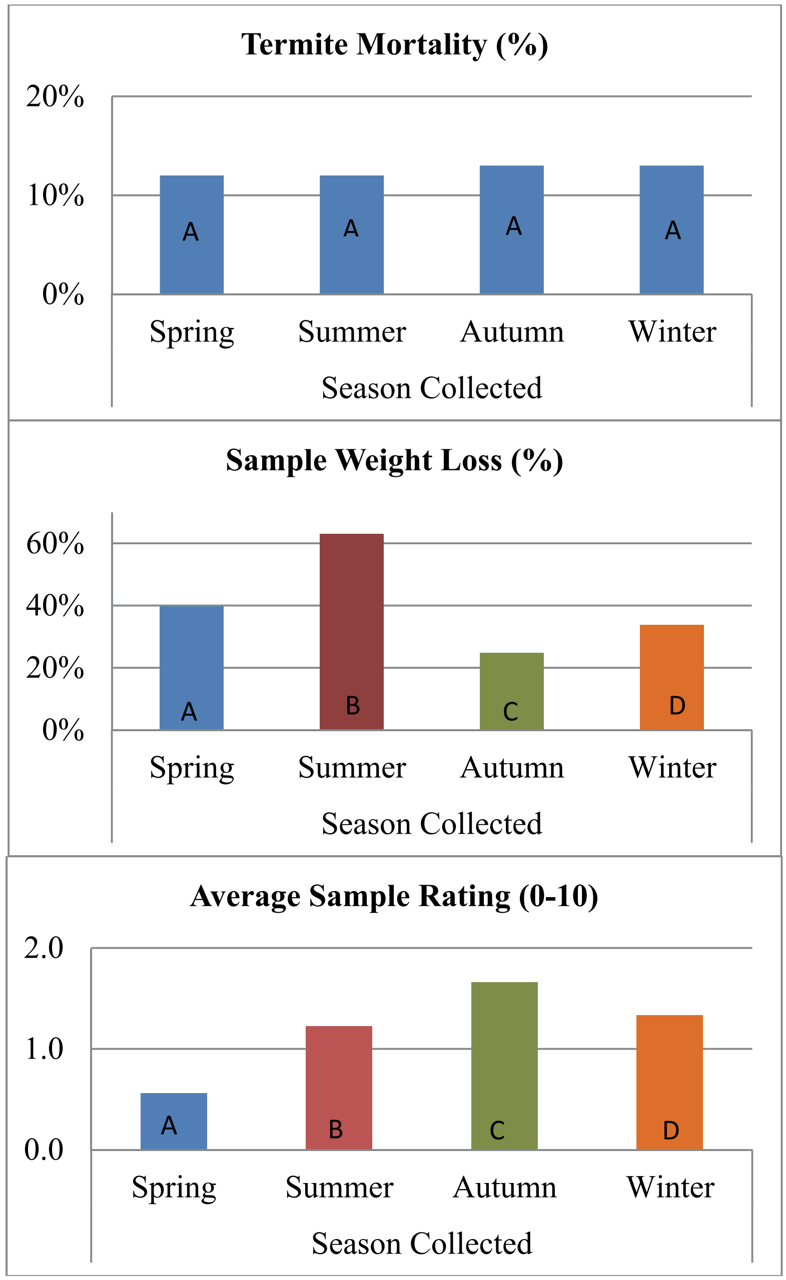


Note: Groups with the same letters indicate no significance at alpha = 0.05.

Figure 1: Termite mortality, sample weight loss, and sample rating based on termite collection material

Statistical Results for Collection Material

Significant differences were observed between collecting material (Chi square = 59.664, 1df, P>Chi square = 0.001) for termite mortality (%). Significant differences were observed between collecting material (F = 13.36, df = 1,270, P< 0.001) for sample weight loss (%). Significant differences were not observed between collecting material (Chi square = 0.777, 1df, P > Chi square = 0.378) for sample rating (0-10).

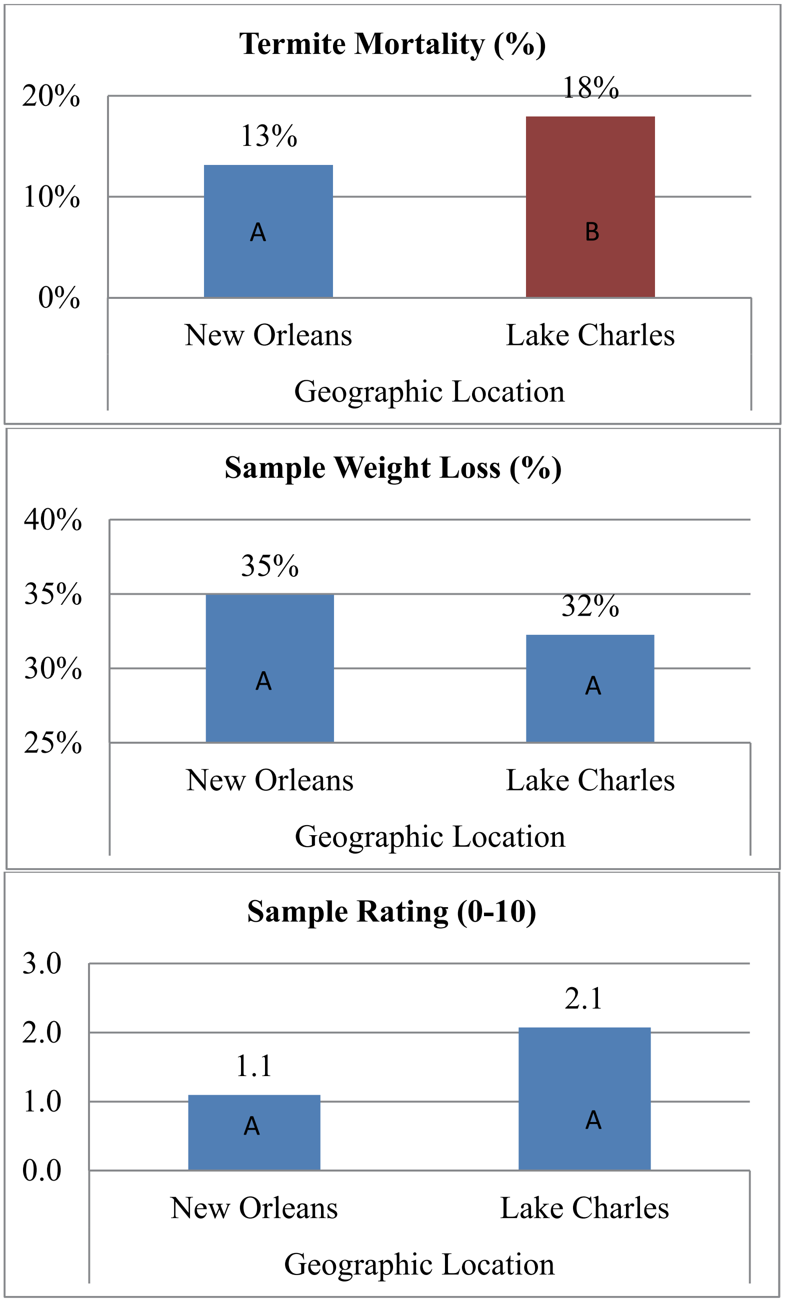


Note: Groups with the same letters indicate no significance at alpha = 0.05.

Figure 2: Termite mortality, sample weight loss, and sample rating based on collection season

Statistical Results for Collection Season

Significant differences were not observed among collecting seasons (Chi square = 5.694, 1df, P>Chi square = 0.128) for termite mortality (%). Significant differences were observed among collecting seasons (F = 17.66, df = 3,270, P < 0.001) for sample weight loss (%). Significant differences were observed among collecting seasons (Chi square = 31.434, 3df, P > Chi square 0.001) for sample rating (0-10).

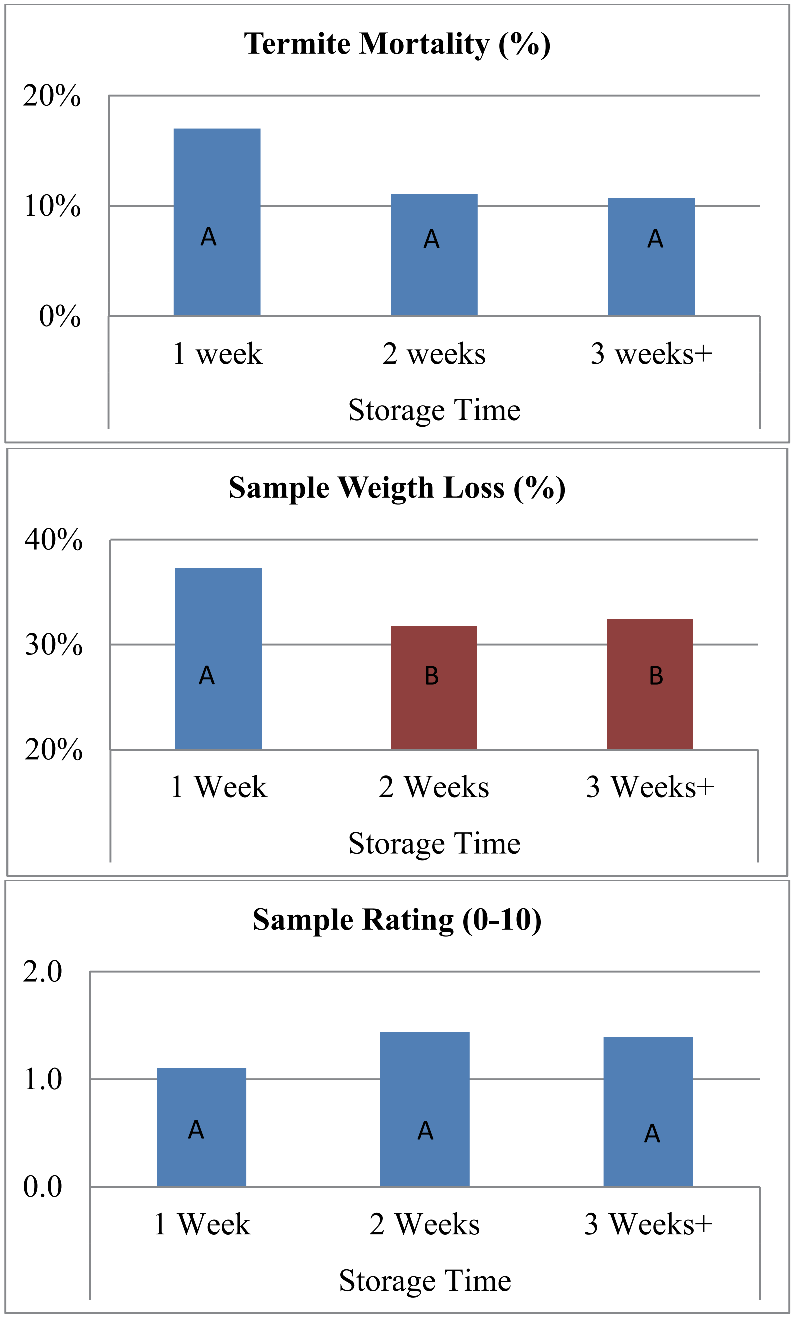


Note: Groups with the same letters indicate no significance at alpha = 0.05.

Figure 3: Termite mortality, sample weight loss, and sample rating based on geographic location

Statistical Results for Collection Location

Significant differences were observed between geographic locations (Chi square = 17.351, 1df, P>Chi square = 0.001) for termite mortality (%). Significant differences were not observed between geographic locations (F = 1.54, df = 1,270, P > 0.216) for sample weight loss (%). Significant differences were not observed between geographic locations (Chi square = 0.568, 1 df, P > Chi square = 0.451) for sample rating (%).

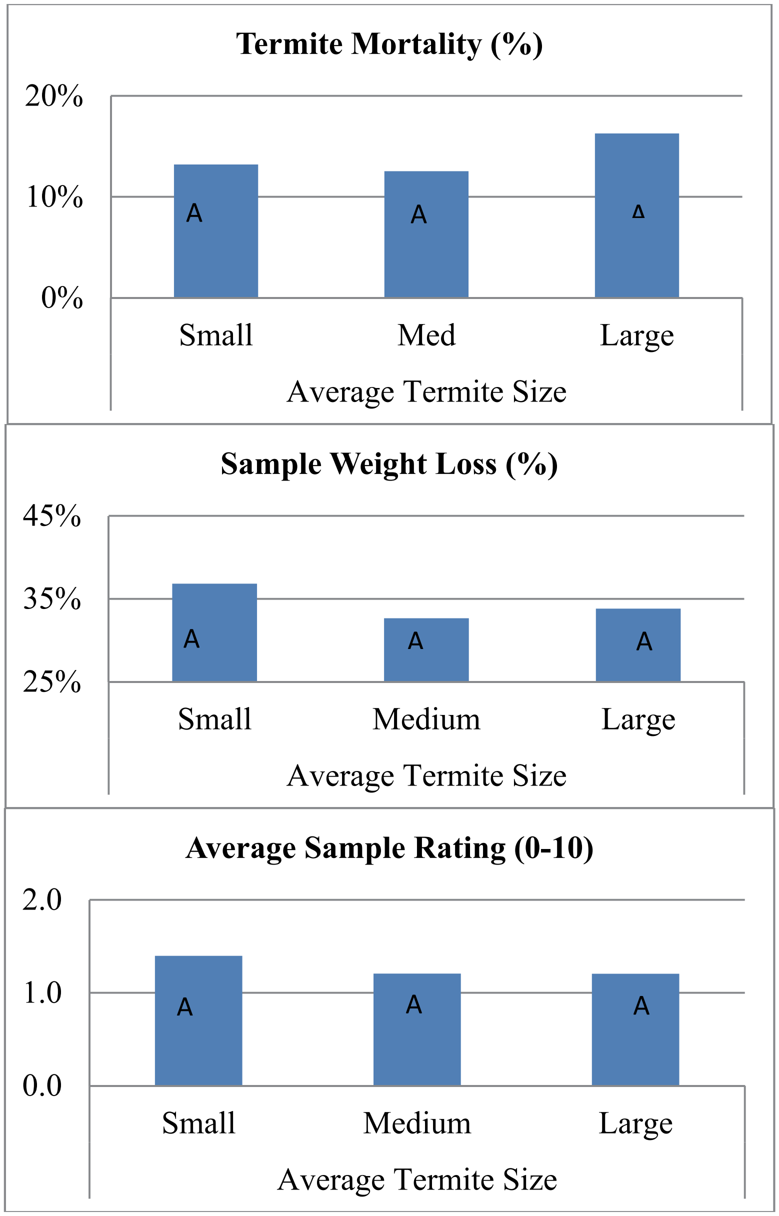


Note: Groups with the same letters indicate no significance at alpha = 0.05.

Figure 4: Termite mortality, sample weight loss, and sample rating based on storage time

Statistical Results for Storage Time

Significant differences were not observed among storage times (Chi square = 1.269, 2df, P>Chi square = 0.530) for termite mortality (%). Significant differences were observed among storage times (F = 13.05, df = 2,270, P < 0.001) for sample weight loss (%). Significant differences were not observed among storage times (Chi square = 4.878, 2 df, P > Chi square = 0.087) for sample rating (0-10).



Note: Groups with the same letters indicate no significance at alpha = 0.05.

Figure 5: Termite mortality, sample weight loss, and sample rating based on average termite size

Statistical Results for Average Termite Size

Significant differences were not observed among average termite size (Chi square = 4.915, 2df, P>Chi square = 0.856) for termite mortality (%). Significant differences were not observed among average termite size (F = 0.17, df = 2,279, P > 0.843) for sample weight loss (%). Significant differences were not observed among average termite size (Chi square = 0.311, 2 df, P > Chi square = 0.856) for sample rating (0-10).

CONCLUSIONS

Termite procurement is necessary for laboratories to conduct the AWPA E1 standard method for laboratory testing to determine resistance to subterranean termites. This study was developed to see what, if any, of the selected procurement variables have an effect of termite mortality, sample weight loss, and sample rating (0-10) in this standard method. Based on this research, we were able to draw the following conclusions. Termite extraction using the veneer crate collection method is a simple and better process. Veneer sheets are simply peeled apart from one another and the termites are shaken off of the wood. This extraction method is much cleaner and faster than extracting out of the wood blocks. It applies less stress to the termites during extraction when compared to the wood block method. With the wood block method, the termites are hammered out of the galleys created in the wood and this surely results in increased stress on the insects and a decrease in vigor. Termite collection using the veneer sheets is the optimal method of collection. The termites showed the highest vigor when collected in this manner.

Significant differences in termite mortality were found between collection methods and geographic location. This was expected for the collection methods due to the nature of removing the termites from the wood blocks by hammering the termites out of the wood. Geographic location differences could be due to biological and genetic differences in termites between the locations. Collection season, storage time, and average termite size had no significant effect on termite mortality.

Significant differences in sample weight loss were found between collection methods, among collection seasons, and among storage times. Sample weight loss for collection method was significantly higher with termites collected using wood blocks as opposed to veneer sheets. Weight loss using veneer sheets was an interesting result for collection materials because the opposite result was expected to occur. Differences can be expected among collection seasons as termite feeding habits change during the swarming season and as temperatures change. Sample weight loss slightly decreased from 1 week to 2 & 3 weeks+ storage time. Geographic location & average termite size had no significant effect on sample weight loss.

Significant differences in sample ratings were found among collection seasons. All four collection seasons (spring, summer, autumn, and fall) were significantly different from one another. Many factors can contribute to this behavior; however we know that termite swarming season (May/June and alate production) and temperature play a major role in termite behavior. As night time temperatures start increasing termites begin to move up and out of the ground via mud tubes. They become much more active in the spring and summer months. Collection method, geographic location, storage time, and average termite size and no significant effect for sample rating.

Our laboratory has switched to only using the veneer sheet method of termite collection. Although significant differences were found among seasons in which the termites were collected; termites still significantly consumed sufficient untreated wood to consider the test data valid. Due to convenience and slightly higher vigor, we now exclusively used termites from the New Orleans area. Termites are also used as soon as possible. Termites are not used in our laboratory testing after 4 weeks of storage time. Although some significant differences were observed, our minimum acceptance criteria of less than 20% mortality, greater than 25% sample weight loss, and visual rating of 4 or less were met for these tests. Therefore, all data and test had valid results. It is acknowledged that other factors in addition to those addressed in this study may be influential in determining termite mortality, sample weight loss, and sample rating (0-10) in the E1-06 test.

BIBLIOGRAPHY

1. American Wood Protection Association (AWPA). 2006. Standard method for laboratory evaluation to determine resistance to subterranean termites (E1-06). 2006 book of standards. Birmingham, AL.
2. Grace, J. K., R. J. Woodrow, and J. R. Yates. 2002. Distribution and management of termites in Hawaii. Sociobiology 40: 87-93.
3. Lax, A. R., and W. L. A. Osbrink. 2003. United States Department of Agriculture-Agriculture Research Service research on targeted management of the Formosan subterranean termite *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). Pest Management Science. 59(6-7):788-800.
4. Lindsey, G. B. 2010. Evaluation of parameters specified in AWPA Standard E1-07, “Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites.” Master of Science Thesis. Mississippi State University. Mississippi State, MS. 70 pp.
5. Messenger, M.T. 2002. Current distribution of the Formosan subterranean termite and other termite species (Isoperea: Rhinotermitidae, Kalotermitidae) in Louisiana. Florida Entomol. 85(4): 580-587.
6. Raloff, J. 2003. Munching Along - Warning: Aggressive alien termites could be headed your way. Science News 164(22):344
7. SAS Institute Inc. 1999. SAS/STAT® User’s Guide, Version 8. SAS Institute Inc. Cary, NC. 3884 pp.
8. Spink, W.T. 1967. The Formosan subterranean termite in Louisiana. Circular No. 89. Louisiana Agricultural Experiment Station. Baton Rouge,La. 12 p.
9. SPSS For Windows. 2006. Chicago, IL.
10. Steel, R.G.D. and J.H. Torrie. 1980. Principle and procedures of statistics – A biometrical approach. 2nd edition. McGraw Hill. New York. 633 p.
11. Tulane University. 2002. Formosan Subterranean Termite. URL http://www.tulane.edu/~mrbc/2001/MRB%20Project/termite.htm (10 Oct 2002).
12. Vlosky, R.P. and T.F. Shupe. 2002. Homeowner Attitudes and Preferences for Building Materials With an Emphasis on Treated Wood Products. Forest Prod. J. 52(7/8):90-95.