Effect of Year and Season on the Variability of Control Samples Used in the AWPA E1-06 Standard Method for Laboratory Testing to Determine Resistance to Subterranean Termites

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ABSTRACT

The AWPA E-1 06 test (Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites) is an important test to determine the resistance of cellulosic material to subterranean termites. This test is of particular interest to companies developing new wood preservatives for various market sectors in the U.S. South which is an inherently high deterioration risk zone. The Formosan subterranean termite (*Coptotermes Formosanus*, Shiraki) has been introduced in the Gulf South and has led to renewed interest in wood durability, particularly for housing applications. The E-1 test can be affected by numerous factors that are not accounted for, and perhaps may or may not need to be, in the current test protocol. Particular factors of interest that may influence Formosan subterranean termite vigor include the year and season in which the termites were collected. This study was therefore initiated to determine effects of these variables on termite performance as measured using untreated southern yellow pine solid wood samples to determine mortality, sample weight loss, and sample rating (0-10) after 28 days of testing.

INTRODUCTION

Destruction caused by the Formosan subterranean termite (FST) is a growing problem in the United States, particularly in southern areas. Formosan subterranean termites are thought to be native to mainland China. 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). *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 parts of Louisiana. Later, once established, C. formosanus began to spread via alate flight dispersal (Messenger 2002). These termites are more aggressive than our native termite colonies. 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 in the thousands. By the year 2001, the Formosan subterranean termite had spread to 95 countries in 11 states including Alabama, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, and Texas (Woodson et al. 2001). FST are social insects that build colonies in trees, underground, and in wood structures here in the United States. Most colonies consist of a main nests with other satellite nest connected with a maze of underground tunnels and mud tubes.

The FST has resulted in a heightened awareness of wood durability by home owners (Vlosky and Shupe 2002). Su and Scheffrahn (1990) stated that since it was initially discovered, *C. formosanus* has spread to several southern states and causes over $1 billion a year in damage and control cost. Lemaster et al. (1997) estimated that $2 billion dollars in damage is caused annually by termites in the United States. More recent estimates by the National Pest Management Association (NPMA) suggest this figure to be closer to $2.5 billion (NPMA 2003). Worldwide damage caused by termites may account for over $20 billion annually (Su 2002). The AWPA (2000) E1-06 test (Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites) is an important test to determine the resistance of new wood preservatives to subterranean termites. This test is of particular interest to companies developing new wood preservatives for various market sectors in the U.S. South which is an inherently high deterioration risk zone.

As part of this test, five untreated southern pine (Pinus sp.) control samples are tested. The E-1 test can be affected by numerous factors that are not currently accounted for, and perhaps may or may not need to be, in the current test protocol. In addition, some factors of interest that may influence Formosan subterranean termite vigor include the geographic region in which the termites were collected, the method used to collect the termites, termite storage time in the laboratory, exposure time in the laboratory, soil type, termite size and weather conditions may influence termite health and vigor, but were not analyzed statistically for this study. The results for those factors will be discussed separately. For this study two factors were analyzed. The two factors are year and season. This research spans over a four year period starting in the beginning of 2003 and continuing through the end of 2006. The four seasons are 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). Although collection method was not an objective in this study, some interesting results for collection method were observed.

MATERIAL AND METHODS

Standard plastic milk crates were buried in the soil near or around an area that has been found to be infested with Formosan subterranean termites. The crates were buried to a depth such that the top one inch of the crates is clearly visible to aid with crate location and retrieval. The crates were filled with either untreated southern yellow pine (*Pinus sp*.) wood blocks, rolled corrugated cardboard or southern yellow pine veneer sheets. The wood material is typically kiln dried and has a moisture content of approximately 4-8% at the time of installation. The original method of collecting termites was to use plastic milk crates containing forty-four pieces of southern yellow pine blocks measuring 1 in. by 1 in. by 11 in. Our second collection method was to use rolls of corrugated cardboard placed into a plastic milk crate. The cardboard is rolled up so several rolls can be place into the crate. For the most current method, we followed some European and Australian methods of using southern yellow pine veneer pieces cut 11 in.^{2} by thickness to fit the plastic milk crate. The American Wood-Preservers' Association (AWPA) Standard E1-06 for single choice testing was used. Southern yellow pine controls were tested using 5 replications. 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. Four hundred termites were introduced to each jar on the side opposite the sample. Termites were obtained from Brechtel State Park (Algiers, La), Segnette State Park (Westwego, La), and Sam Houston Jones State Park (Lake Charles, La) and added to the E1-97 test. Samples of termites were taken, weighed and an average weight per termite determined. Termite composition consisted of approximately 20 soldiers and 380 workers.

Once the collection crates are determined to be infested with termites, the crates are collected in plastic garbage cans and returned to the Louisiana Forest Products Development Center (LFPDC) 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 eat through plastic garbage cans. The termites are then stored in the laboratory and extracted for used in accordance with the American Wood-Preservers' Association (AWPA 2006) Standard E1-06 for single choice testing.

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 was 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. Also the wood blocks tend to be more of a messy extraction process. It was possible to extract fifty thousand termites out of two wood crates in about two hours. We decided to try different methods to collect termites to lessen the stress applied to the termites. Therefore, cardboard was then used in place of the wood blocks. We found this method was good for drawing termites into the crate. The termites would get up into the cardboard very fast, but due to the wet conditions in the field the cardboard would collapse. Therefore, the cardboard method was soon abandoned. The current method of collecting termites is to use sheet of 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. With the veneer crates one-hundred thousand termites could be collected out of one veneer crate in twenty minutes. This extraction method is much cleaner and faster than extraction out of the wood blocks or cardboard.

Each E-1 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. This data is comprised of the control samples from 54 no-choice AWPA E1 jar test (AWPA 2006) from 2003 – 2006. 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. Four hundred termites were introduced to each jar on the side opposite the sample.

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. Percent sample weight loss is based on the original oven dry weight. The test sample oven dry weight is determined by measuring the moisture content of the matched sample and using it to calculate the sample oven dry weight. The final oven dry weight is determined by oven drying the sample after the test. The sample weight loss was determined through the following five steps:

1. Determining moisture content of the matched moisture content samples by oven-drying;
2. Assuming that the termite test samples had the same MC as the matched MC samples;
3. Determining oven dry weight of each termite sample using measured weight at room conditions and estimated MC;
4. Determining oven dry weight of each termite sample after termite testing and a through washing to remove all sand, and oven drying;
5. Determining sample weight loss using two oven dry weights determining from steps 4 and 3.

The rating of each sample was done visually by estimating the extent of damage. The rating scale used was 0 to 10 with 0 being complete failure and 10 being sound with nibbles allowed. Each sample was rated based on the following AWPA rating system:

10	Sound, surface nibbles permitted
9	Light attack
7	Moderate attack, penetration
4	Heavy attack
0	Failure

Samples of termites were taken and weighed and an average weight per termite was determined. Termite compositions consisted of approximately 20 soldiers and 380 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 is subtracted for the live number of termites. That number is then divided by the initial number of termites resulting in percent mortality. The data obtained were analyzed for resistance with means and standard deviations determined (SAS 1999).

RESULTS AND DISCUSSION

The year 2005 had the best sample rating and weight loss, but results for all years' indicate sufficient termite vigor and performance in the AWPA E1-06 jar test (Table 1). Seasonal results indicate that during the spring and summer months termites seem to be more active and vigorous, and seem to slow down during the winter months.

Table 1A. Test setup for year.

Year Collected	Number of Test	Total Number of Observations
2003	8	40
2004	13	65
2005	17	85
2006	16	80
Totals	54	270

Table 1B. Test setup for season.

Season Collected	Number of Test	Total Number of Observations
Spring (3-20 to 6-20)	14	70
Summer (6-21 to 9-21)	12	60
Autumn (9-22 to 12-20)	24	120
Winter (12-21 to 3-19)	4	20
Totals	54	270

Termite extraction using the veneer crate collection method is a simple process. You simply peel the veneer sheets apart from one another and shake the termites off of the wood. This extraction method is much cleaner and faster than extracting out of the wood blocks or the cardboard rolls. With the wood block method the termites are hammered out of the galleys created in the wood. Extracting termites from the cardboard rolls was nearly impossible because the cardboard rolls would easily collapse due to environmental pressures. The veneer method applies less stress to the termites during extraction when compared to the wood block method. Termite mortality has decreased from 2003 to 2006. These findings are largely attributed to the reduced level of stress that the veneer method places on the termites as compared to the other collection methods.

Table 2 contains a summary of the test data including the means and standard deviations for the primary data of interest (i.e., percent termite mortality, percent sample weight loss, and sample ratings). In addition, it provides information on significant differences determined between treatments for these variables using the Duncan test procedure. If a treatment within a column contains the same letter as another, there is no significant difference at a 95% confidence interval. All data and records collected during the tests are maintained at the Louisiana Forest Products Development Center.

Table 2A. Mean and standard deviation values and significance between treatments at alpha = 0.05 using the Duncan mean separation test for year.

Year Collected	Mean Mortality (%)	Standard Deviation Mortality	Mean Weight Loss (%)	Standard Deviation Wt. Loss	Mean Rating (1-10)	Standard Deviation Mortality
2003	22.92 C	0.106	37.39 B	0.093	1.2 B	0.9
2004	21.49 C	0.112	30.66 A	0.144	3.0 C	2.4
2005	15.32 B	0.103	43.13 C	0.094	0.2 A	0.6
2006	9.59 A	0.079	29.20 A	0.071	1.4 B	1.1

Note: Means with the same letter denote no significant difference at alpha = 0.05.

Table 2B. Mean and standard deviation values and significance between treatments at alpha = 0.05 using the Duncan mean separation test for season.

Season Collected	Mean Mortality (%)	Standard Deviation Mortality	Mean Weight Loss (%)	Standard Deviation Wt. Loss	Mean Rating (1-10)	Standard Deviation Mortality
Spring	14.25 A	0.116	42.95 C	0.104	0.4 A	0.8
Summer	12.57 A	0.050	33.88 B	0.089	0.8 A	1.2
Autumn	19.28 B	0.120	29.13 B	0.118	2.5 B	1.9
Winter	17.05 AB	0.118	36.98 A	0.062	1.0 A	0.9

Note: Means with the same letter denote no significant difference at alpha = 0.05.

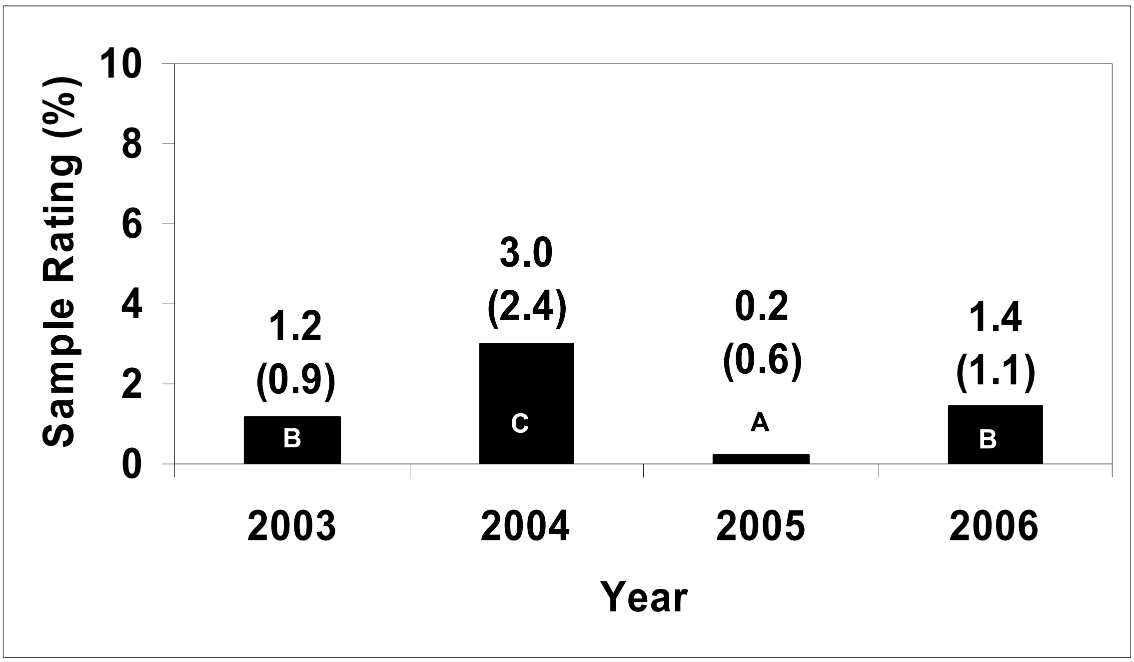
Figure 1 shows sample rating, sample weight loss, and termite mortality graphs for the data collected over four years 2003, 2004, 2005 and 2006. The number in percent represents the mean of all values collected during that year and the number in parentheses represents the standard deviation of all values collected during that year.

Sample Rating (0-10) for Years. For year the termites collected in 2005 had the lowest mean sample rating, followed by 2003, 2006, and then by 2004, as shown in Figure 1A.

Sample Weight Loss (%) for Years. Figure 1B indicates that the termites collected in 2005 had the highest mean sample weight loss, followed by 2003, 2004 then by 2006.

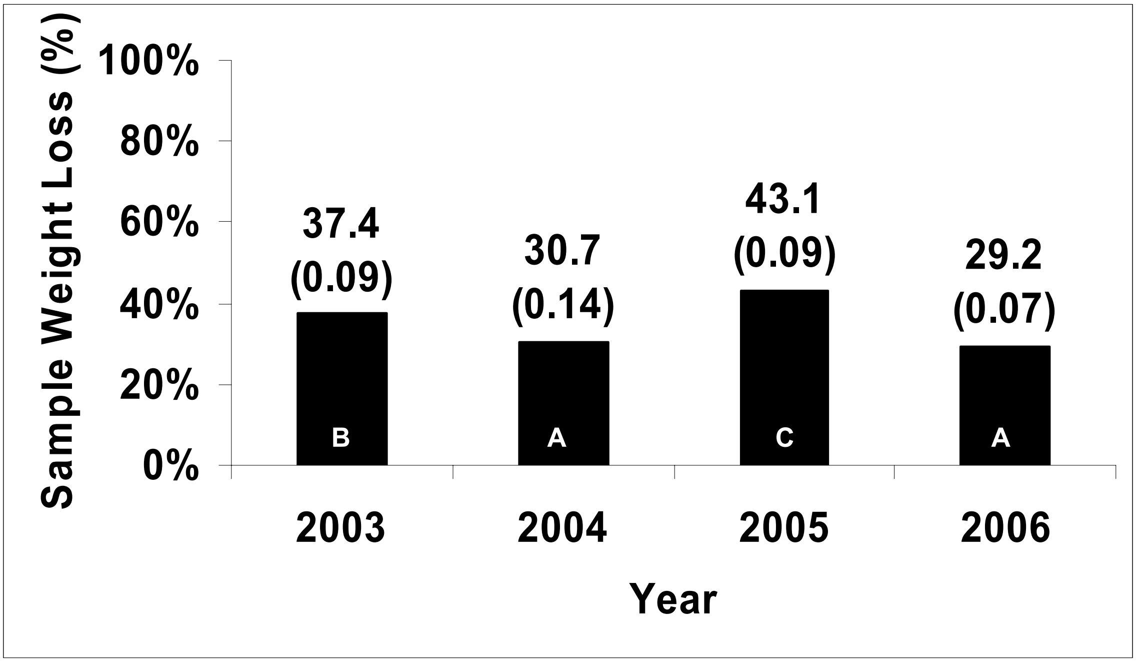
Termite Mortality (%) for Years. Termite mortality was highest in the control jar that used termites collected in 2003, followed by 2004, 2005 and 2006. Figure 1C illustrates the decrease in termite mortality over the years; we feel this is because of our changed collection method going from using solid wood blocks to veneer sheets to collect termites.

Figure 1A. Sample rating graph for the data collected over four years 2003, 2004, 2005, and 2006.



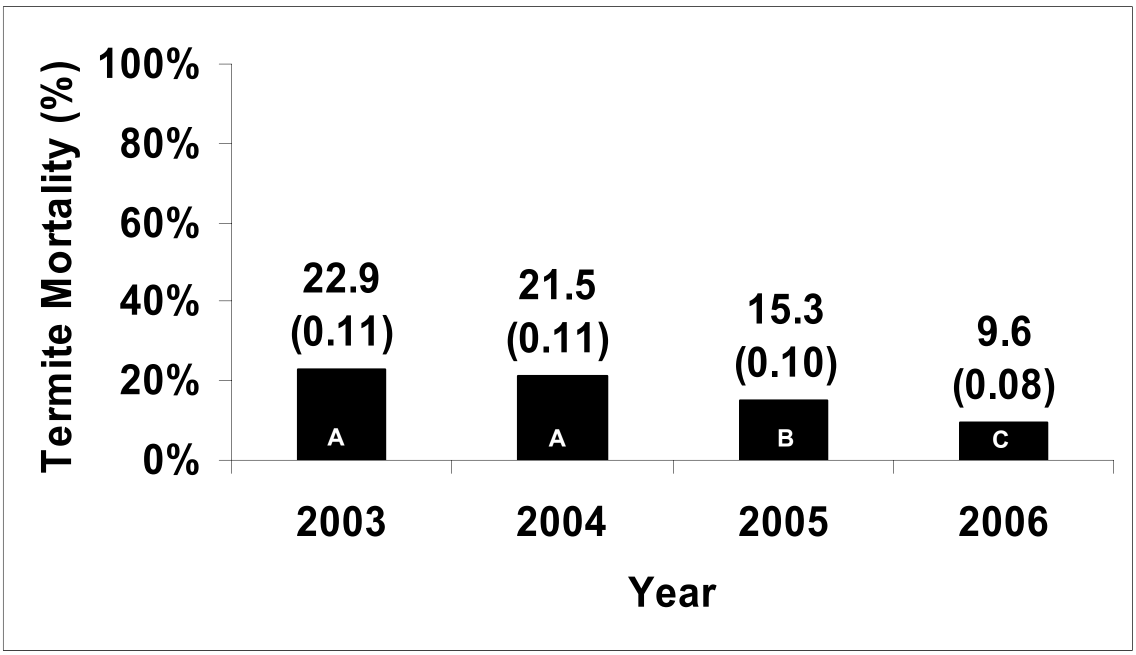
Note: Groups with same letters indicate no significance. Values in parentheses are standard deviations.

Figure 1B. Sample weight loss graph for the data collected over four years 2003, 2004, 2005, and 2006.



Note: Groups with same letters indicate no significance. Values in parentheses are standard deviations.

Figure 1C. Termite mortality graph for the data collected over four years 2003, 2004, 2005, and 2006.



Note: Groups with same letters indicate no significance. Values in parentheses are standard deviations.

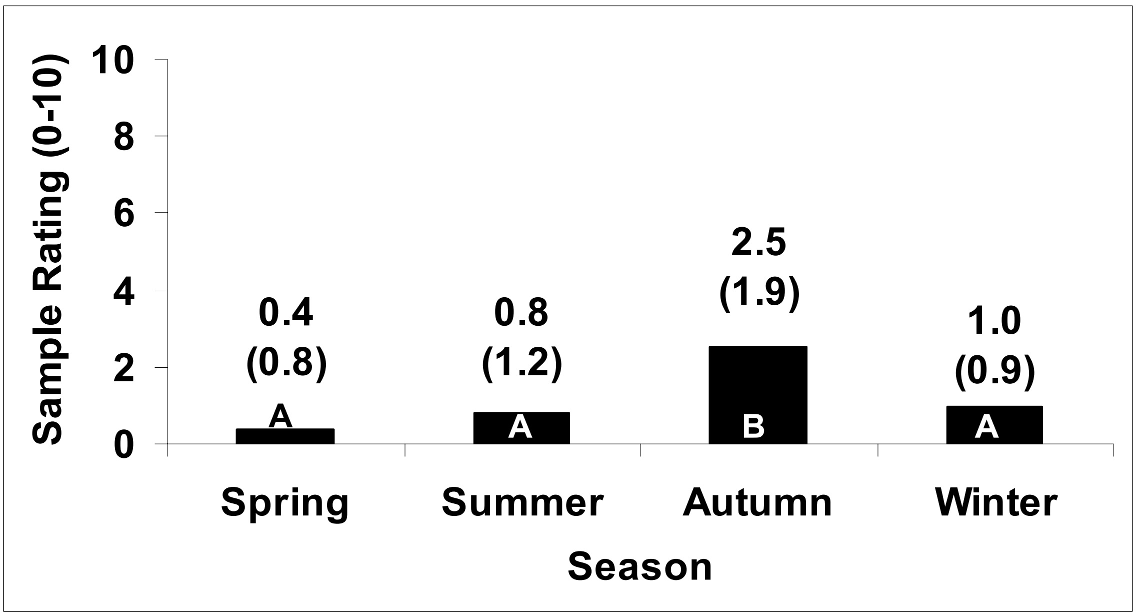
Figure 2 shows sample rating, sample weight loss, and termite mortality graphs for the four seasons in which termites were collected spring, summer, autumn and winter. The number in percent represents the mean of all values collected during that season and the number in parentheses represents the standard deviation of all values collected during that season.

Sample Rating (0-10) for Season. Figure 2A shows that the termites collected with the spring resulted in the lowest sample rating, followed by summer, winter and autumn. Spring and summer seem to be the optimal time for termite vigor, probably because they start to become more active at that time.

Sample Weight Loss (%) for Season. Figure 2B shows that the termites collected during the spring had the highest sample weight loss followed by winter, summer and autumn.

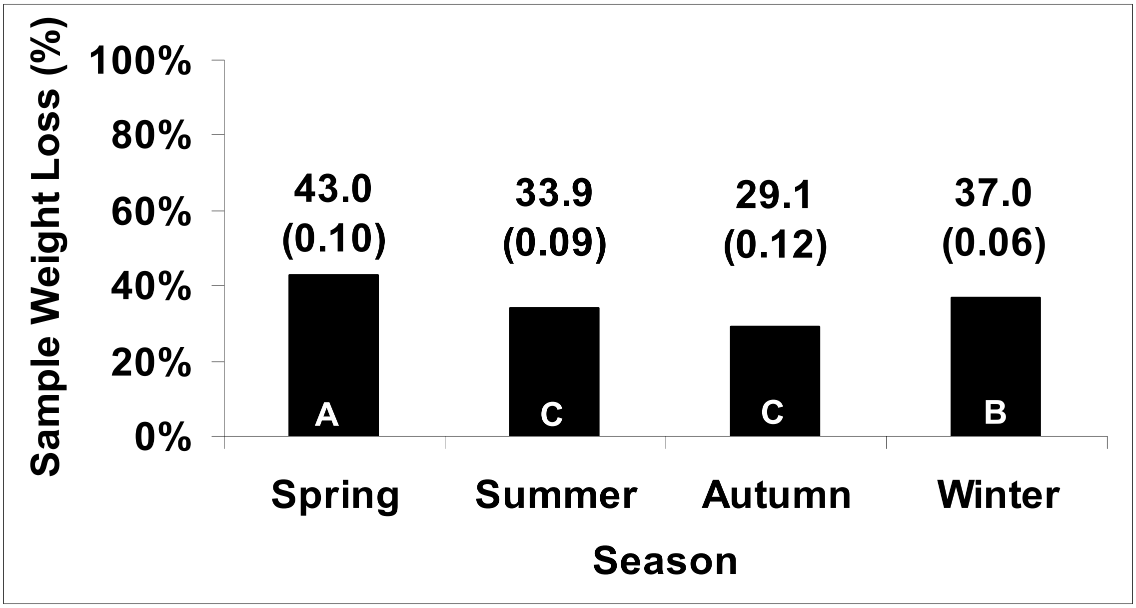
Termite Mortality (%) for Season. Figure 2C shows that the termites collected during autumn having the highest termite mortality followed by winter, spring and summer. The trend shows stronger performance from the termites collected during warmer months, but this may be attributed to other factors.

Figure 2A. Sample rating graph for the data collected over four seasons spring, summer, autumn, and winter.



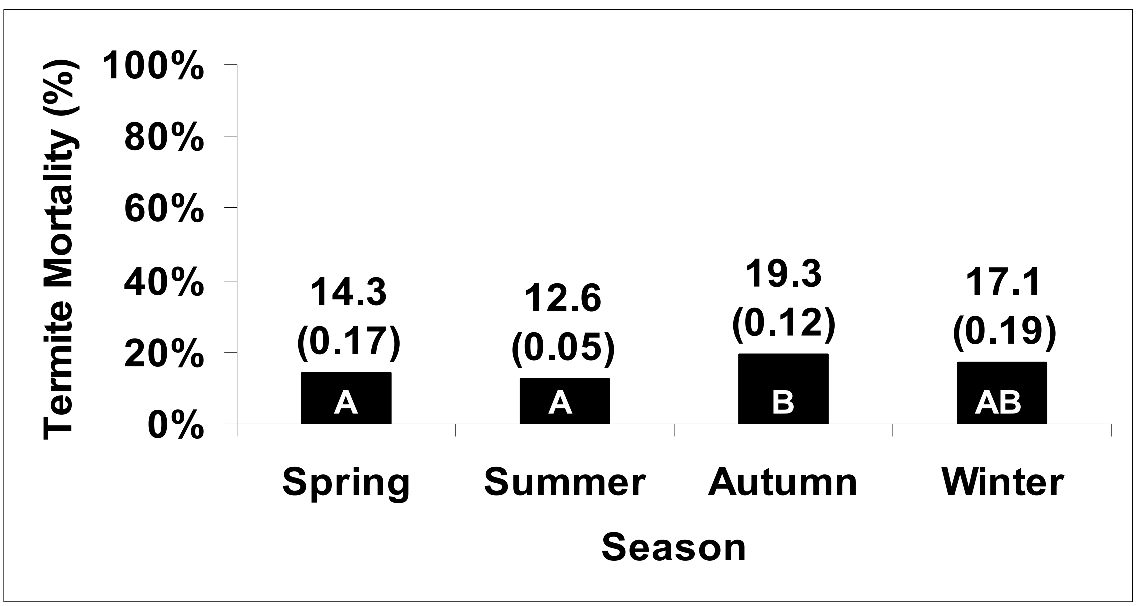
Note: Groups with same letters indicate no significance. Values in parentheses are standard deviations.

Figure 2B. Sample weight loss graph for the data collected over four seasons spring, summer, autumn, and winter.



Note: Groups with same letters indicate no significance. Values in parentheses are standard deviations.

Figure 2C. Termite mortality graph for the data collected over four season's spring, summer, autumn, and winter.



Note: Groups with same letters indicate no significance. Values in parentheses are standard deviations.

CONCLUSIONS

Based on this research, we were able to draw the following conclusions. 2005 had the best sample rating and weight loss, but results for all years indicated sufficient termite vigor. During the spring and summer months the termites seem to be more active and vigorous, and seem to slow down during the winter months. Termite mortality has decreased from 2003 to 2006 and we attribute this to our new collection method using veneer sheet rather than wood blocks to collect termites.

It is acknowledged that other factors in addition to those addressed in this study may be influential in determining termite performance in the E-1 test. Therefore, this on-going research will continue to investigate these others factors such as termite storage time in the laboratory before use, average termite size, geographic location, the genetic make up of the termites, soil type, and weather data in the different locations.

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