COMPARISON OF CELLULOSE CONSUMPTION BETWEEN *Coptotermes formosanus* AND *Reticulitermes flavipes* (ISOPTERA: RHINOTERMITIDAE) UNDER LABORATORY CONDITIONS

A Senior Scholars Thesis

by

DENISE NICOLE LANCASTER

Submitted to the Office of Undergraduate Research
Texas A&M University
In partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2007

Major: Entomology

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Associate Dean for Undergraduate Research: Robert C. Webb

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ABSTRACT

Comparison of cellulose consumption between *Coptotermes formosanus* and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) under laboratory conditions (April 2007)

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We are interested in determining the effects of subterranean termite soldier ratios on the overall cellulose consumption of *Coptotermes formosanus* and *Reticulitermes flavipes*. In nature, *Coptotermes formosanus* is found in very large colonies and a higher soldier ratio than *Reticulitermes flavipes*. In this project, each test colony was established at a specific size and soldier ratio. The soldier ratios tested for *Coptotermes formosanus* were 0:100, 5:100, 10:100, and 15:100, and the ratios tested for *Reticulitermes flavipes* were 0:100, 3:100, 5:100, and 10:100. These ratios were selected based on the research of other scientists. Since each species is specially adapted to different environments, these studies were carried out within their optimum temperature and humidity conditions. The *Reticulitermes flavipes* test colonies were kept inside of a plastic container at 27 ± 2 ºC, and relative humidity of 40 – 60%. The *Coptotermes formosanus* test colonies were kept at 30 ± 2 ºC and 80% relative humidity. The cellulose consumption results of this project are reported as g/h, and within each species and soldier ratio there are no significant

differences. Because of this, the cellulose consumption values per species could be combined and compared on the species level. In analyzing the data this way, the *C. formosanus* test colonies consumed a significantly larger amount of cellulose per hour compared to *R. flavipes.*

DEDICATION

This thesis is dedicated to my parents, James and Keren George, and Mark and Melanie Lancaster, for whom I owe the deepest gratitude. Through their financial assistance and personal support I have been able to attend Texas A&M University, a place where my dreams have come true since the fall of 2002. Each one of them has taken a newfound interest in the field of entomology, with me eager to tell them about what fascinating topic I have just learned involving insects. Each has contributed to my life and this specific project in their own special ways, and for that I will honor and cherish them.

ACKNOWLEDGMENTS

There are numerous individuals to whom I owe acknowledgement, but none more so than my Research Advisor, Dr. Roger E. Gold, and my Academic Advisor, Mrs. Rebecca Hapes. Each of these people has helped to lead me onto the path that will be my future.

Mrs. Hapes, you encouraged me to apply for the Undergraduate Research Scholars Program, and at the time I thought it was just to add another bullet point onto my résumé but you were right - it has absolutely been one of the best experiences of my young life and I feel it has truly prepared me for what the research aspect of graduate school will require of me mentally, physically, and academically. Now I do not regret any moment, and feel honored to have been a part of the Undergraduate Research Scholars Program. In the two years that we have known each other, you have become my academic beacon of light, illuminating the path on which I walk with possibilities and experience to strengthen my academics and research background and to prepare me for a truly remarkable future.

Dr. Gold, you invited me into your facility with open arms and consoled me at times when it seemed like nothing could go right, research wise. With your instruction and guidance, I have experienced many new areas of entomology that were at one time

foreign to me, and I have learned to stand on my own with regards to my research. You have always expected greatness of me, and I hope that you will not be disappointed.

I would also like to recognize everyone associated with the Center for Urban and Structural Entomology, because you all have greatly contributed to my research experience these past two semesters. I would like to thank Bryce Bushman for taking me on termite collection trips and showing me the ropes of collecting and maintaining viable termite colonies successfully under laboratory conditions. I also owe Dr. James Austin, Laura Nelson, and Bill Summerlin my appreciation for their support throughout the course of my project.

In closing, this project may never have occurred if not for the impact each of these people have made on me, and their influence on me to succeed with this project and through the Undergraduate Research Scholars Program.

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CHAPTER I

INTRODUCTION

Termites (Order Isoptera) are considered a major economic pest because of their ability to infest and feed on man made wooden structures; most people are incredibly concerned with the state of their homes. It is sometimes forgotten that termites have been found fossilized up to 100 million years ago, and that they have a very unique and specialized social lifestyle. Each member within a termite colony has a specific role and those roles can be producing offspring, defending the colony, or working to forage and maintain the food supply. Termite colony sizes can range from thousands to millions of individuals, dependent upon their species (Grace et al. 1989).

In nature, termites act as a very beneficial insect and return nutrients to the environment through the process of cellulose digestion, which very few animals are able to accomplish. Several other benefits of termites in nature include the facts that termite feeding enhances decomposition of organic matter in their surrounding area, and their tunneling activities help to aerate the soil to promote additional plant growth. Termites are also seen as a highly available and nutritious source of protein within many cultures around the world. In some parts of Africa, termites are often oven roasted, dried or

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This thesis follows the style and format of the Journal of Economic Entomology.

fried in fat, and in other countries they are dried and ground into a powder to create a high protein paste additive to foods (Berenbaum 1995).

Termites are generally classified by the following three groups: dampwood termites, drywood termites, and subterranean termites. The dampwood termites prefer to only infest woods with high moisture content. This includes felled logs, stumps and dead trees in contact with the ground that accumulate large amounts of moisture. The drywood termites live entirely within wood, and have a lower moisture requirement allowing them to live above the ground. The only moisture required to sustain life is extracted from their food source, or regenerated through their natural metabolism. In order to conserve moisture, their cuticle is thicker and more adapted to the conservation of water compared to subterranean termites. Subterranean termites have a thin cuticle, and therefore require a moist and humid environment. This specific category of termites lives in very close association with the ground because of the high moisture content of soil. Subterranean termites are also undisputedly the most destructive and widespread termite group. The research presented in this thesis will specifically emphasize two species of subterranean termites: *Reticulitermes flavipes* and *Coptotermes formosanus*.

The Native Subterranean termites such as *Reticulitermes flavipes* is commonly referred to as the "eastern" or subterranean termite. "Because of its broad distribution, the eastern subterranean termite is probably the most economically important and damaging termite species in the United States" (Su and Scheffrahn 1990). These termites are small in

comparison to the dampwood and drywood termites. A key characteristic in determining this genus of termites is the characteristically rectangular shaped head within the soldier case. *R. flavipes* regularly swarm from February through May in Texas. This species of termite is one of the two selected primarily because of its native status to the United States, and their abundant availability in the Bryan / College Station area.

The subterranean termite known as *Coptotermes formosanus* is known as the Formosan Subterranean Termite. This termite is an introduced (non-native) species to the United States and is believed to originate from southern China, and then transported to Japan before the 1600's. It arrived in Hawaii over a century ago, and is believed to have reached the U.S. mainland in the mid-1940's on military ships returning from the Pacific following World War II.

The Formosan subterranean termite began its invasion into the United States through the major port cities like Houston and Galveston, Texas and New Orleans and Lake Charles, Louisiana. They have moved inland through infestation of railroad ties. These rail road ties, deemed unusable by the railroad commission, are sold for landscaping purposes, bringing these termites right to the front door of homeowners (Woodson et al. 2001). This termite species is a major concern to many because "[i]n Hawaii, this termite can cause major structural damage within six months, and almost complete destruction within two years" (Tamashiro 1984). The reasoning behind the increased damage associated with the Formosan termites is thought to be involved with their large colony

size compared to *R. flavipes*, and not necessarily that they eat wood faster per individual (Su and Scheffrahn 2000). The most devastating effect that the *C. formosanus* has on its immediate environment, as noted within New Orleans and surrounding area, is that it can and will attack more than 20 species of living trees and shrubs (Henderson 2001).

C. formosanus termites are creating a severe impact during its short duration within the mainland of the United States, and this is primarily why it was selected for this project. Much is unknown about the Formosan termites preferences and abilities to adapt to different environments, however not many studies have been conducted to determine if the percent soldier ratio affects the over all feeding of a colony.

CHAPTER II

METHODS

The experimental methods for this project included three major components. The first component involved learning and implementing the proper techniques for collecting viable termites to be used in laboratory research projects. The second component was to maintain the individual termite colonies, and sustaining a high quality of life among the colonies for long periods of time. The third component was the actual research experimental methods along with all of the trial and error situations involved with comparing the cellulose consumption of *C. formosanus* and *R. flavipes* under laboratory conditions.

The process of collecting termites for research was not a difficult one, but was very time consuming and labor intensive. I was taught the entire procedure by a Research Assistant with the Center for Urban and Structural Entomology. The first step in this process was learning the baiting methods.

First, a hole 25 centimeters wide and 25 centimeters deep was dug in the ground. Next, a 1 gallon plastic bucket (with the bottom removed) was placed within the hole. This bucket served to keep the baiting mechanism from caving in and being completely surrounded by dirt, and also assisted the researcher in finding the baiting stations.

Secondly, a wooden block was placed inside the bottom of the hole and used as a base on which to place the baiting system, in order to create a solid surface contact, and to keep the baiting system vertical and intact. Two different baiting systems were commonly used for the capture and collection of foraging termites. One was a wooden sandwich bait station (Figure 1), and the other was a corrugated cardboard / PVC pipe bait station (Figure 2).

Fig. 1. Wooden sandwich bait station, used specifically to capture foraging *Coptotermes formosanus* termites in Baytown and Beaumont, Texas.

Fig. 2. PVC pipe and corrugated cardboard bait station used to collect *Reticulitermes spp.* termites on a weekly basis.

The wooden sandwich bait station was composed of three wooden blocks that were secured with a large bolt, and each block was separated by a space of three washers. This baiting system was primarily used to collect *Coptotermes formosanus* because of the location of those traps (outside of the Bryan / College Station area) required less frequent visits. The corrugated cardboard / PVC pipe bait station was commonly used throughout Bryan / College Station and was used to collect termites locally. Strips of cardboard boxes were cut to a width of 5 centimeters and a length of 15 centimeters, and as many as possible were wedged into a round PVC pipe that had a wire mesh glued to one side in order to prevent the cardboard from falling out of the PVC pipe. Cardboard and any other processed paper product was, in essence, pre-digested wood in regards to termite consumption, and was quickly consumed by the termites foraging within the bait station. Because of this, these bait stations needed to be changed weekly in order to ensure that the food source provided did not run out.

After the proper baiting station was selected, it was soaked in water to provide moisture to create optimum living conditions for the termites. The baiting systems provide moisture for termite specimens to endure high temperatures in Texas. For an optimum level of water absorption, the wooden sandwich bait system needed to soak overnight, whereas the corrugated cardboard PVC pipe traps needed only to soak for about 10 minutes to obtain an adequate level of moisture. After placing the water soaked baiting system inside the bucket inserted into the hole in the ground, a lid was placed on the bucket, to assist in retaining moisture, and to keep leaf litter and debris from filling into

the hole. Leaf litter was often used to conceal the baiting system to reduce the chances of unwanted human contact with the baiting system. The location of the exact placement was recorded by the researcher.

Due to privacy and legal issues concerning the damage caused by these termites, I can only state the cities in which termite specimens were collected for this project. All termites were collected within the State of Texas. *C. formosanus* were collected in Baytown, Texas and Beaumont, Texas, and *R. flavipes* were collected locally within the Bryan / College Station Area.

As with any research project, this aspect comes with a minimal risk of non-target animal interference. Since termites are considered a valuable protein source within the animal kingdom, quite often non-target animals are collected within the baiting systems. Most are predatory insects and other arthropods such as centipedes and spiders, but other animals such as small snakes, snails and earthworms were documented and collected. These non-target animals were separated and re-introduced into to the wooded area near the laboratory.

The process of maintaining the termite colonies after collection began with removal of the individual termites from the dirt and debris located within the baiting station. The termites were removed from the wooden sandwich baiting system by gently hitting the baiting system with a hammer and shaking the termites into a large plastic shoe box.

Then the bolt was carefully removed from the bait system and checked for more termites within the cracks and crevices of the wooden blocks. Extreme was taken when handling the *C. formosanus* termite because their species has not yet been documented within Brazos County and so extreme caution was taken to isolate and retain them. Once all of the termites were removed from the wooden sandwich bait system, they were transferred onto a sorting system (Figure 3) composed of a ribbed tray with tubes leading into a plastic shoe box with a cellulose food source. The stage of the sorting platform was sloped, thus allowing the termites to trickle down the stage as the debris was retained within the groves and was filtered out. The termites traveled down the plastic tubes and into the plastic shoe box, which was filled with moist tongue depressors which served as a food source.

Fig. 3. Stage-tube-box system is used to remove the dirt and debris from the termites. Slowly the termites trickle down the slopped stage and through the tubes into the plastic box for collection.

Fig. 4. The termites collected into the plastic shoe box from figure 3 are transferred into a petri dish and fed tongue depressors.

After all of the termites were in the plastic shoe box, they were transferred into a petri dish filled with stacked, moistened tongue depressors. Each petri dish was labeled, identifying the date and location of collection. The overall colony in their new home looked very similar to Figure 4.

At this point in the maintenance process, the termites were ready to be stored in their respective containers. The plastic box that contained the termite colonies contained two layers. The first layer was sand spread to about 0.5 cm thick, and sprayed with water until slightly damp. This layer served to add moisture to the enclosed environment to keep the termites from desiccating. The second layer was a sheet of tin foil placed between the moist sand and the petri dishes containing the termites. This served to prevent the perti dishes from coming into direct contact with excess moisture. Though termites require moisture to survive, they require it in the form of water vapor versus condensed water. If a colony is exposed to too much condensed water, mold will begin to grow and cause death of the colony. Six labeled petri dishes were stacked on top of

the foil in groups of three, and then either held in the laboratory or environmental chamber. *C. formosanus* termites are specially adapted for a warmer climate and higher humidity, so these colonies were kept inside of an environmental chamber at a constant temperature (30 ± 2 ºC) and humidity (80%), whereas the *R. flavipes* are found locally and are well adapted to the local environment so they were kept at room temperature (27 ± 2 ºC) and relative humidity of 40-60%. The last part of the maintenance process involved the daily check to remove dirt buildup and check the status of the cellulose resource. If the tongue depressors were very dry or almost fully consumed, then they were replaced, and the excess dirt was scrapped out and discarded.

The third and most important component of this research project was the actual experimental design, and everything leading up to it. After determining the problem to be researched, I had four major obstacles to overcome, and spent about half of my time researching and determining the best ways to overcome these obstacles.

The first obstacle to overcome was to create a cellulose food source that was small enough to provide measurable feeding, large enough to last 5 days. This obstacle was overcome by using a scroll saw to slice a weathered pine block of wood into 1 mm thicknesses, and then cut into circles using various sizes of cork cutters to determine which size was the most effective. The thickness was selected to be 1 mm because it was the thinnest setting that was routinely consistent and allowed for easier creation of the small disks punched out from the cork cutter. The disks also needed to be thin enough to

encourage the termites to feed inward from the edges instead of only on the surface because one mode of determining consumption was visually using the surface area as a reference. The best cork cutter size was a size 6 which created a disk 6 mm in diameter. This was the disk size used for all trials and resulted in an average turnaround time of between 1-5 days, depending on termite species.

The second obstacle was to determine the most efficient way to count out test groups of 300 individual termites. First, I counted 300 individuals out and then photographed the colony to create an easier way of double checking my counting accuracy. As expected, this took an enormous amount of time (approximately 12 hours to count out 12 groups of 300 individuals each), and extreme caution needed to be taken to not harm the insects in the process of transfer. Second, I weighed out ten groups of ten worker termites and determined the mean weight per individual termite. This weight was then extrapolated to the colony size needed. There was a significant deviation in the actual value of termite individuals versus the expected value of 300. Next, I counted out five groups of 100 termites and then proceeded to determine the mean weight, which resulted in a more accurate and consistent value. The mean weight determined for the *C. formosanus* was 0.32 g / 100 workers, as compared to 0.29 g / 100 workers for the *R. flavipes* workers. These were the weights used to prepare colonies in the future. This method was much faster, and included less handling of the insects, which seemed to increase the life expectancy and vigor of the colonies.

The third major obstacle was trying to separate the members of the colony by size to ensure that only actively feeding individuals were making it into the test trial colonies. I attempted two nested sieving methods. One set of sieves used were copper nested soil sieves, and the other set were homemade sieves using different gauges of thin cloth mesh. The first set of sieves successfully removed the soldier termites from the workers, but there was no significant separation within the instars (sizes) of the workers. Cleanup using this set was difficult because the termites were crawling in every nook and cranny within the gauged wire. The second set of sieves proved to be completely unsuccessful, with no separation at all of the termites. In the end, I decided that neither method was successful enough for what I needed, and decided to use colonies collected 6 - 10 weeks prior to testing because at that age, a termite individual had begun feeding on its own, and was no longer being fed by other workers of the colony.

The last obstacle to overcome prior to beginning testing was to determine the optimum environment in which to keep both termite species so as to provide an optimum quality of life, and cellulose consumption. First, I set up colonies in the laboratory. It was at this time that I noticed that the *C. formosanus* were considerably less active, and that the colonies feeding was significantly lower than the *R. flavipes*. *C. formosanus* colonies were starving and dying off rapidly. Next, I set up the colonies within the environmental chamber set at 30 ± 2 ºC and 80% relative humidity. In this atmosphere, *C. formosanus* thrived and were actively feeding. But at the same time, the *R. flavipes* were doing poorly, due to fungal growth. At this point in time, it was evidently clear that each

species of termites were specially adapted to different environments, and that there simply was no other option than to keep the *C. formosanus* within the environmental chamber, and *R. flavipes* in the laboratory at room temperature and humidity.

Now that these obstacles were overcome, it was time to design and conduct the experimental procedures. Each test colony would contain 300 individuals by weight, with the addition of the appropriate number of soldiers added to the colony. Each trial was allowed to run until a visual amount of feeding could be detected, and the trial run time was recorded in hours to determine cellulose consumption per hour mean value per colony.

Two methods of cellulose consumption determination were used throughout this project. The first method was done by weight. Initially the feeding disks were labeled and heated to 50 ºC within an oven for 24 hours and then weighed prior to addition to test trials. After the test trials had been conducted, the disks were carefully scraped clean of any excess dirt and debris from the termite feeding, and once again heated to 50 ºC for 24 hours prior to weighing after completion of the trial. The second method of determining the cellulose consumption was by a visual method to measure the percent area lost from the wooden disks. Area loss measurements were accomplished by analyzing digital images taken of each feeding disk before and after testing. Disks were photographed from a distance of 3 cm using a digital camera (Sony Cybershot® DSC-S70, Tokyo, Japan) set on the macro close up mode with no flash. The camera was mounted onto a

copy stand (No. CS-3, Testrite Instrument Co. Inc., Newark NJ) for stability (Figure 5). All images were downloaded and opened within the computer program Adobe Photoshop (Adobe Systems Inc. 2001) where they were converted to black and white to increase the measurement accuracy. This required first translating the color image to grayscale, and then setting the contrast to the maximum level. The paint tool was used to correct small blemishes within the interior perimeter of the feeding disk after converting the image black and white. These newly altered images were analyzed using SigmaScan® Pro 5 (SPSS Inc. 1999) to determine the area of each disk. The calculations were then performed to determine the percent area loss for all disks included within these trials.

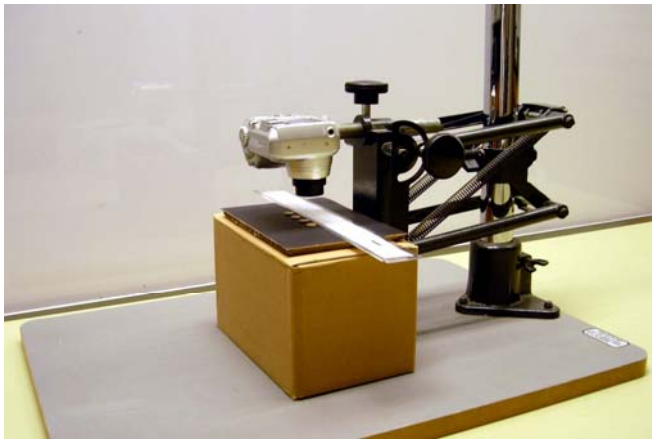

Fig. 5. Camera system for photography of wood disks. Distance between camera lens and disks equaled 3 cm.

A computer programming system called ANOVA (SAS, 2003) was used to statistically separate the mean values of this project, and determine the significance of the data.

CHAPTER III

RESULTS

Based on this research project, four different sets of results will be discussed. The first set of results pertains to the weight of the individual worker termites. Experimentally I determined that the *C. formosanus* weigh more per individual than the *R. flavipes* (Table 1).

Table 1. Mean weight of an individual termite worker (g)

Species	Weight (g)
Coptotermes formosanus	0.0032
Reticulitermes flavipes	0.0029

Secondly, I determined the environmental factors for each species to result in optimum life expectancy and feeding results. *C. formosanus* was at an optimum feeding level and life expectancy when held inside of the environmental chamber at 30 ± 2 ºC and 80% relative humidity. *R. flavipes* was at an optimum feeding level and life expectancy when kept within the laboratory at 27 ± 2 ºC and relative humidity of 40-60%.

Thirdly, I determined that of the two cellulose consumption measurements I used, that measuring the differences in weight of the feeding disks provided more accurate and representative results than the surface area measurements. Many areas on the wooden disks where termite feeding had occurred were not measured visually because the termites had not eaten completely through the disk resulting in a very large standard

deviation value (Table 2 and 3), and eventually deciding not to rely on this data for accurate cellulose consumption values.

Table 2. Mean values and standard deviation of percent area lost for *C. formosanus* based on percent soldier ratio.

Percent soldier ratio tested			
0%	**5%**	**10%**	**15%**
19.68 ± 15.34	16.98 ± 13.27	17.11 ± 14.42	18.00 ± 14.30

Table 3. Mean values and standard deviation of percent area lost for *R. flavipes* based on percent soldier ratio

Percent soldier ratio tested			
0%	**3%**	**5%**	**10%**
23.20 ± 21.43	10.67 ± 5.72	36.84 ± 16.50	14.83 ± 7.44

Lastly, statistically, there was no significant difference between the cellulose consumption of *C. formosanus* and *R. flavipes* based on mass (Table 4 and 5, Figure 6).

Table 4. Mean consumption rates (g/h) for 12 complete repetitions of the four soldier ratios tested on *C. formosnaus.*

Percent soldier ratio tested			
0%	5%	10%	15%
0.00021	0.00017	0.00014	0.00019

Table 5. Mean consumption rates (g/h) for 7 complete repetitions of the four soldier ratios tested on *R. flavipes.*

Percent soldier ratio tested			
0%	3%	5%	10%
0.00019	0.00011	0.00020	0.00019

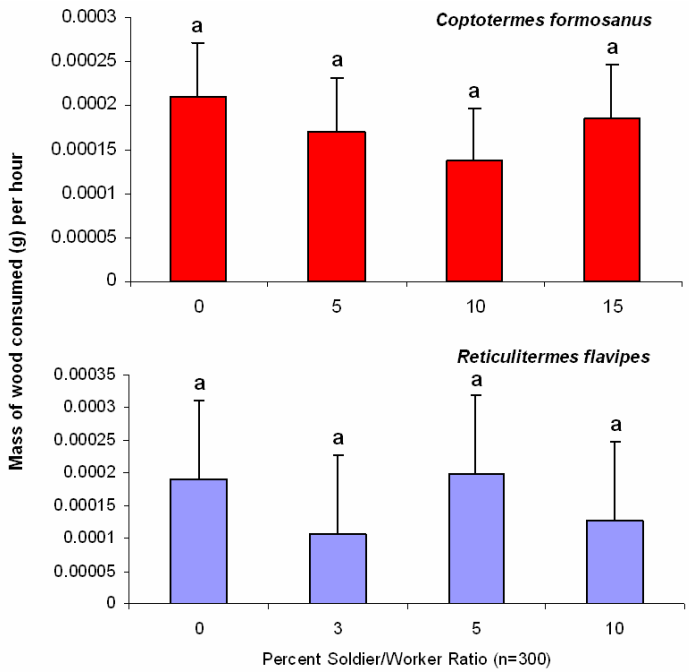

Fig. 6. Mean cellulose consumption per hour based on soldier ratio in *Reticulitermes flavipes* (bottom) and *Coptotermes formosanus* (above). Means with the same letter were not significantly different at ($p \leq 0.05$).

As there were no significant differences in cellulose consumption based on soldier ratios, it was possible to analyze the combined data from all trials for each of these two species (Figure 7). When this was done, there was a statistically significant difference ($p \leq 0.05$) in the cellulose consumption with the *C. formosanus* consuming cellulose at a higher rate than *R. flavipes.*

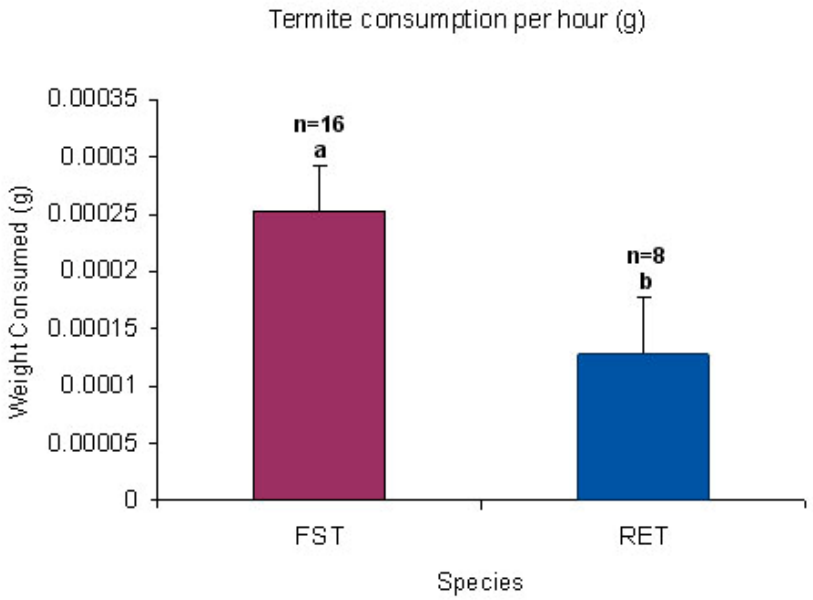

Fig. 7. Mean cellulose consumption per hour based on species of termite in *Reticulitermes flavipes* (RET) and *Coptotermes formosanus* (FST). Data assigned different letters were significantly different at ($p \leq 0.05$).

CHAPTER IV

DISCUSSION AND CONCLUSIONS

The objective for the cellulose consumption comparison between *C. formosanus* and *R. flavipes* focused primarily on percent soldier ratios was to determine if the varying percentages of soldiers would impact the feeding activity of the worker termites of *C. formosanus* and *R. flavipes.* Results demonstrated that the varying percentages of soldiers, regardless of amount, did not significantly decrease the amount of weight and percent area lost in the wood feeding disks. Consequently, the null hypothesis for this project was not rejected, because there was no significant difference in the cellulose consumption of either species based on soldier ratio.

An interesting result was that by averaging the total cellulose consumption per hour values within each species resulted in the *C. formosanus* consuming cellulose at a higher rate than *R. flavipes* (Fig. 7). The reasons for this are not fully understood, one factor (reinforced through this project), is that *C. formosanus* is a larger individual termite, thus requiring more of a food source. It is also widely known that *C. formosanus* colonies are up to four times as large as *R. flavipes*, which could have caused a genetic selector for worker termites that forage and feed more aggressively than native termite populations.

Future research with this project should include expanding the test colony size, and increasing the amount of time for the entire project. I also recommend acclimatization

the termite test groups to their new soldier ratio values for many months with an unlimited amount of resource prior to cellulose consumption studies.

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