

Effect of Cellulose Concentration on The Feeding Preferences of the Termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

One potential variable that could affect termite wood preference is the concentration of cellulose in a food source. Here we tested the feeding preference of the termite *Reticulitermes flavipes* (Kollar) using artificial food sources with different concentrations of cellulose. In one experiment, we used foods that contained only cellulose and in a second experiment, we used foods containing different ratios of cellulose to lignin. In both experiments termites were shown to prefer foods with higher concentrations of cellulose over foods with lower concentrations. These results suggest that the concentration of cellulose could be another factor that affects termite feeding preference.

INTRODUCTION

Termites have been very successful organisms due to their ability to process wood as a food source despite its poor nutritional value with the aid of symbionts (La Fage & Nutting 1978). Different species of wood vary chemically and termites have been observed to prefer certain species of wood over others (Smythe & Carter 1970a; Waller 1988; Waller 1990; Morales-Ramos & Guadalupe 2001);. In addition, termites appear to survive better when fed certain species of wood over others (Smythe & Carter 1970b; Haverty & Nutting 1974; Haverty & Nutting 1975; Morales-Ramos & Guadalupe 2003) and the species in which termites have high survival rates are the ones that are preferred (Morales-Ramos & Guadalupe 2001). Understanding what factors influence termite food selection and survival will allow for a better understanding of termite nutritional ecology and pest management.

It is clear from previous work that termites use many cues to determine the quality of a food source. Studies have shown that sugars (Abushama & Kamal

1977; Waller & Curtis 2003; Swoboda *et al.* 2004; Saren & Rust 2005), ion levels (Botch *et al.*, unpublished), phosphorus levels (Botch *et al.*, unpublished), and nitrogen concentration (Prestwich 1980; Shellman-Reeve 1994) can affect food selection in termites. It is thought that these molecules are important for termite nutrition as termites can't live on a pure cellulose diet (Cook & Scott, 1933). Wood also contains many other molecules such as hemicelluloses, lignin, and a variety of allelochemicals that affect the properties of wood. These molecules have the potential to either enhance or deter termites from eating wood (Smythe 1969; Smythe & Carter 1970a; Smythe & Carter 1970b; Scheffrahn 1991).

The structure of the wood may also affect termite food preferences. It has been suggested that termites prefer to feed on lower density woods (Smythe 1969; Smythe & Carter 1970a; Behr *et al.* 1972). However, Waller *et al.* (1990) found that if the biomass consumed is considered then the termites consume the same or more biomass of the denser wood than less dense wood. This result suggests that there is something about the denser woods that termites prefer. One possible cue that has not been directly tested is the concentration of cellulose in the food source. The preference for higher density woods found by Waller *et al.* (1990) suggests that the density of cellulose could be a factor, however; all of the other potential cues would be concentrated as well.

In this study, we examined the effect of cellulose concentration in artificial food sources on the food selection in the termite *Reticulitermes flavipes*. With the use of artificial food sources we were able to eliminate other possible cues that may deter or stimulate termite feeding. We first tested cellulose concentrations and then tested termite feeding preferences with different ratios of cellulose and lignin. The latter experiment examined the effects of the ratio of indigestible to digestible foods on termite feeding preferences.

METHODS AND MATERIALS

Experiment 1:

In experiment 1, we examined the feeding preference of termites using an artificial food source that only varied in the amount of cellulose added.

Food

Three different types of artificial foods were created that varied in the amount of cellulose: The total contents of the food types are as follows: Non-cellulose Food

(Non CF): 1.5g Agar in 100ml distilled H₂O. Low Cellulose (Low CF): 1.5g Agar in 100ml distilled H₂O with 1g α -cellulose powder. High Cellulose (High CF): 1.5g Agar in 100ml distilled H₂O with 10g α -cellulose powder.

To prepare each food type, the agar-water mixture was brought to a boil and then the appropriate amount of cellulose was added. No cellulose was added to the Non CF. The mixture was stirred and immediately poured into a Petri dish. Because cellulose does not dissolve, the mixture was cooled on a shaker to keep the cellulose evenly suspended in the solution until the food was solidified.

Trials

Ten colonies from Cape Girardeau County were sampled using termite traps describe in Judd & Fasnacht (2007). From each of the 10 colonies, two groups of 200 termites were each placed in individual 17.78cm x 17.78cm x 5cm (739ml) plastic sealable containers $\frac{1}{4}$ filled with topsoil. The containers insured the humidity remained the same throughout the experiment. Termites were allowed to acclimate for 48 hrs. After the acclimation period, food was added to the bins. Each piece of food was 3g and placed on a small square cut from a note card. In one set, each bin received a 3g piece of High CF and 3g of Non CF. In the second set, each bin received a 3g piece of High CF and 3g of Low CF. For both sets, each food type was placed in opposite corners of the bin. At the same time, 2 termite-free bins were created $\frac{1}{4}$ filled with topsoil and 3g of each food type was placed in each bin. This controlled for natural water loss in the food in the same environment.

The termites were allowed to feed for 12 days. On every 2nd day, each piece of food was weighed. Termites and soil were removed from the food when it was weighed. Food in the control bins were also weighed each time. In the High CF-Low CF experiment, two colonies died and were eliminated from the analysis.

Experiment 2

During experiment 1, the amount of water loss was higher in the Non CF and Low CF diets than the High CF diets. In the following trials lignin was added to counterbalance the cellulose in order to reduce water loss and investigate the affect of the presence of a non-digestible molecule in the food source.

Food

Four different food types were created varying the amounts of cellulose and lignin. In each food type, the following amounts of cellulose and/or lignin were added to 1.5g agar in 100ml distilled H₂O and suspended as described in Experiment I.

100% Cellulose:	10g α -cellulose powder.
100% Lignin:	10g Lignin Powder
50% Cellulose:	5g α -cellulose powder and 5g lignin
10% Cellulose:	1g α -cellulose powder and 9g lignin

Trials

Five trials were set up in the same manner as in experiment I. The food choices were as follows:

Trial 1: 100% Cellulose and 100% Lignin Trial 2: 50% Cellulose and 100% Lignin Trial 3: 100% Cellulose and 50% Cellulose Trial 4: 10% Cellulose and 100% Lignin Trial 5: 50% Cellulose and 10% Cellulose

Termites were allowed to feed 20 days in trial 1 and 18 days in trials 2-4. All trials had an additional five control bins to control for water loss.

Data analysis for Experiments 1 and 2

To determine how much of each diet termites consumed, the percent weight loss was calculated for each piece of food. However, the natural water loss was not even for each type of food. For example, in experiment 1, The High CF loss less water (37.8%) than the Low CF (56.7%) and the Non CF (56.1%) in the control bins. To compensate for the uneven water loss in food types, the average percent water loss for each food type was determined from the control bins and this number was subtracted from the total loss in weight for the experimental bins. Any negative number resulting from these calculations was converted to a zero to signify the food was not eaten by the termites. This created a conservative estimate of the loss in weight due to termite feeding. Although the difference in weight loss in each food type was less in experiment 2, the same calculations were done. For each experiment, the weight loss of the food types was compared using the Wilcoxin Sign rank test (Daniel 1990). The results of the analyses for experiments 1 and 2 were corrected using the Bonferoni Table Wide Correction (Rice 1989).

RESULTS

Experiment 1

High CF vs. Non CF: There was significantly higher weight loss in the High CF than the control food (N=9, T=2, P=0.004; Fig. 1) after the adjustment for natural water loss was made. After the weight adjustment from the controls the median estimated percent weight loss due to termite feeding was 13.6% in the high food and the median percent weight loss was 0% in the Non CF. This means that the termites only fed on the High CF.

High CF vs. Low CF: After the adjustment for water loss was made, there was a significantly higher weight loss in the High CF than the Low CF (N=8, T=1, p= 0.0078; Fig. 2). The High CF had a median 33.3% weight loss due to termite feeding while the Low CF had a median of 15.8% weight loss. This suggests that the termites preferred the food source that had a higher amount of cellulose. Unlike the previous experiment, the termites did feed on both food sources but fed more on the food with a higher concentration of cellulose.

Experiment 2

In trial 1 all colonies preferred the 100% cellulose over the 100% lignin (N=12, T=0, P=0.0002). Termites in trial 2 showed a significant preference for 50% cellulose to 100% lignin (N= 12, T=11, P=0.0269). The results of trials 1 and 2 suggest that termites prefer cellulose over pure lignin.

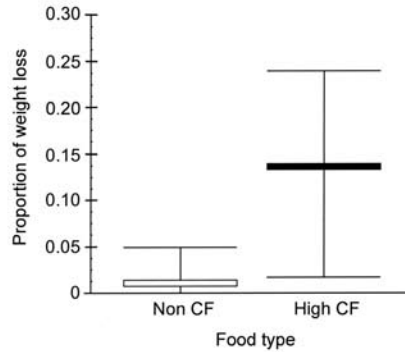


Fig. 1. Medians and quartiles of the proportion of weight loss due to termite feeding (adjusted for natural water weight loss) for Non Cellulose food and High Cellulose Food. The High Cellulose Food showed a significantly higher weight loss (N=9, P=0.004; Wilcoxin Signed Rank Test).

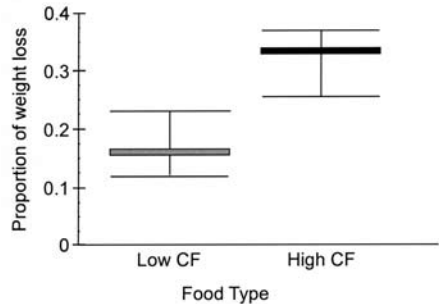


Fig. 2. Medians and quartiles of the proportion of weight loss due to termite feeding (adjusted for natural water weight loss) for Low Cellulose Food and High Cellulose Food. The High Cellulose Food showed a significantly higher weight loss (N=8, p= 0.0078; Wilcoxin Signed Rank Test).

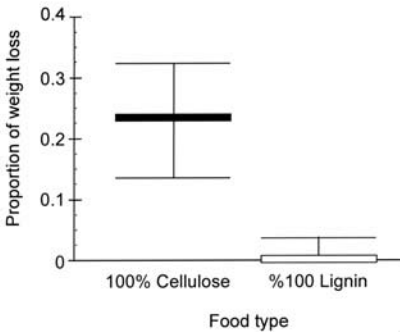


Fig. 3. Medians and quartiles of the proportion of weight loss due to termite feeding (adjusted for natural water weight loss) for 100% Cellulose and 100% Lignin Food. The 100% Cellulose Food showed a significantly higher weight loss ($N=12$, $P=0.0002$; Wilcoxin Signed Rank Test).

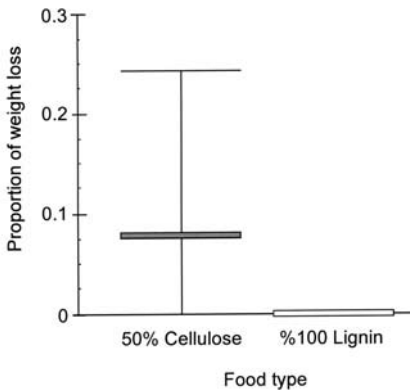


Fig. 4. Medians and quartiles of the proportion of weight loss due to termite feeding (adjusted for natural water weight loss) for 50% Cellulose and 100% Lignin Food. The 50% Cellulose Food showed a significantly higher weight loss ($N=12$, $P=0.0269$; Wilcoxin Signed Rank Test).

In trial 3, the termites fed significantly more on the 100% Cellulose food than the 50% cellulose food ($N=15$, $T=0$, $P=0.0001$). This result matches the results of experiment 1. The termites preferred food with higher cellulose content.

In trial 4, the termites did not show an interest in the 10% cellulose or the 100% lignin. There was no significant difference in weight loss between the two food types. The experiment was done later in the fall and the termites did not feed much on either food. There was no significant difference in food weight loss for the 50% cellulose and the 10% cellulose in trial 5. As with trial 3 this experiment was also done later in the fall and the termites were not feeding as much as in the earlier trials.

DISCUSSION

Overall, the termites in these experiments did prefer foods containing higher levels of cellulose. In experiment 1 there was a clear preference for High CF over the Non CF and Low CF. In experiment 2 the 100% and 50% cellulose foods were preferred over the control food (100% lignin). In addition, the 100% cellulose food was preferred over the 50% cellulose food. These results suggest that the termites were able to taste the cellulose and perceive the relative amount of cellulose in the food. The last two trials in experiment 2 failed to show differences. There are two explanations for these results. First, the termites cannot taste the difference between the two foods when the lignin is present. Second, the termites were not feeding because

these trials were conducted late in the season. The latter explanation seems more likely in this case because the termites were noticeably less active. In addition, the termites did feed on the 50% cellulose food when presented with the 100% lignin food.

The interesting question that emerges from this study is exactly how the termites are able to detect the concentration of cellulose in a food source. Cellulose is an extremely large molecule and it is unlikely that termites have cellulose receptors. The most likely explanation is that the termites are breaking down the cellulose in their mouths and then determining the sugar concentration. Cellulases have been found in the saliva of *Reticulitermes* (Inoue *et al.* 1997; Tokuda *et al.* 2005). Indeed, termites have been shown to prefer foods enriched with sugars including glucose (Abushama & Kamal 1977; Waller & Curtis 2003; Swoboda *et al.* 2004; Saren & Rust 2005). Perhaps termite preference for sugars is tied to an indication of the presence of cellulose. If a termite is breaking down the cellulose a little in its mouth, higher concentrations of cellulose in a bite of food should produce more concentrated sugar solutions in the mouth.

On an ultimate level, the preference for greater cellulose concentrations could be adaptive for termites and their symbionts. Food with higher concentrations of cellulose is probably more digestible for termites. Wood has many components



Fig. 5. Medians and quartiles of the proportion of weight loss due to termite feeding (adjusted for natural water weight loss) for 100% Cellulose and 50% Cellulose Food. The 100% Cellulose Food showed a significantly higher weight loss ((N=15, P=0.0001); Wilcoxin Signed Rank Test).

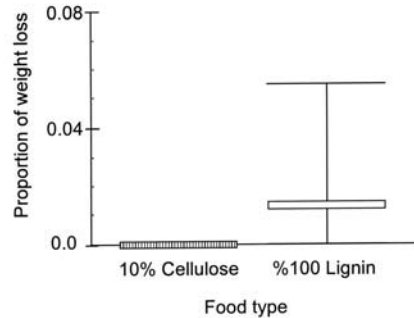


Fig. 6. Medians and quartiles of the proportion of weight loss due to termite feeding (adjusted for natural water weight loss) for 10% Cellulose and 100% Lignin Food. There was no significant difference in weight loss between the two food sources.

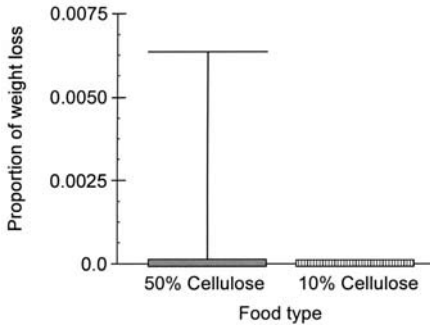


Fig. 7. Medians and quartiles of the proportion of weight loss due to termite feeding (adjusted for natural water weight loss) for 50% Cellulose and 10% Cellulose Food. There was no significant difference in weight loss between the two food sources.

such as lignin that is not easily digested by termites (Waller & La Fage 1987). In experiment 2 of this study, the termites didn't feed much on the 50% and 10% foods. Perhaps the high levels of lignin inhibited the feeding rate of the termites. Although carbon is plentiful in most wood termites encounter, the higher the non-digestible to digestible components ratio is, the harder it will be for termites to gain access to the other important nutrients such as nitrogen and micronutrients that may be bound up in the non-digestible molecules.

Diets that are difficult to digest could adversely affect the gut symbiont populations as well.

Poor termite diets can cause losses of certain protozoan species in a termite gut (Inoue *et al.* 1997). Thus, ultimately the termites maybe making their food choices based on nutrient value (levels of nitrogen phosphates and micronutrients), allelochemicals, and the proportion of digestible components in the food source. Morales-Ramos and Guadalupe (2003) found that *C. formosanus* survived better on wood from trees such as yellow birch and sugar maple better than others such as Douglas fir and red oak. The former two species of trees have higher cellulose to lignin ratios (2.2:1) than the latter two (1.7:1) (Browning 1963). *C. formosanus* also prefers yellow birch and sugar maple to Douglas fir or red oak (Morales-Ramos & Guadalupe 2001). Thus, it is possible that wood with high lignin content is harder for termites to feed on and are ultimately less palatable. Waller *et al.* (1990) found that *C. formosanus* ate significantly more mahogany that was compressed to 40% greater density than uncompressed mahogany. Mahogany has a low cellulose to lignin ratio (1.5:1) compared to many trees (Browning 1963) and perhaps compressing the wood increased the amount of cellulose available per bite and made it more viable as a food source. Although when a similar test was done using pine the same amount of biomass was eaten from compressed and uncompressed pine sources (Waller *et al.* 1990). Thus, cel-

lulose concentration alone is probably a contributing factor to wood selection but other factors play a role as well.

Termites do select certain species of wood over others. Much work has presumed that the important factors affecting this food selection are elements that are not common in wood and allelochemicals. Although both factors have been shown to play an important role in the food selection in termites, future work will also have to consider the role of cellulose concentration. The results of this study reinforce the conclusions of Waller *et al.* (1990) in that the density of wood, specifically the amount of cellulose per unit area, has to be taken into account in any food selection study. Thus, it is more likely that rare nutrients, allelochemicals, and the relative amount of cellulose all play a role in food selection in termites.

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