IMPACT OF SEASONAL TEMPERATURES AND RELATIVE HUMIDITY ON CELLULOSE CONSUMPTION BY *RETICULITERMES FLAVIPES* AND *RETICULITERMES VIRGINICUS* (ISOPTERA: RHINOTERMITIDAE)

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Abstract The influence of seasonal ground temperature and relative humidity on cellulose consumption by two native subterranean termite species was evaluated in South Carolina, USA. Seven colonies of *Reticulitermes flavipes* Kollar and six colonies of *R. virginicus* (Banks) were studied. In-ground plastic bucket stations containing cardboard rolls were equipped with electronic data loggers to record daily temperature and relative humidity. Consumption data and number of termites in cardboard rolls were recorded every one or two weeks, depending on termite activity, and correlated with temperature and relative humidity data. Ground temperature 1 m away from bucket stations (10 cm deep) showed the highest correlation with consumption. Relative humidity recorded from all *R. flavipes* bucket stations was above 80%, while relative humidity in two *R. virginicus* bucket stations reached a low of 30% during winter months. Peak consumption and the number of termites in cardboard rolls were highest during summer and lowest during winter. Consumption patterns for both species were not statistically different. The number of *R. virginicus* collected was often twice as many as *R. flavipes*, resulting in approximately twice as much cardboard being consumed by *R. virginicus*. Two *R. virginicus* colonies were active below 40% relative humidity during cool months, while *R. flavipes* colonies were inactive. Predictive models of consumption as related to ground temperature and relative humidities for *R. flavipes* and *R. virginicus*.
Key Words Eastern subterranean termite, southeastern subterranean termite, seasonality, consumption

INTRODUCTION

Seasonal changes of temperature and relative humidity play an important role in termite biology and behavior. Temperature has a strong influence on termite foraging and seasonal activities (Potter, 2004; Evans and Gleeson, 2001), even though termites can, to a point, regulate temperature and moisture within their nests. Delaplane (1991) stated that subterranean termite foraging behavior is seasonal. Subterranean termites will not forage in areas where soil surface temperatures are either too hot or too cold (Haverty et al., 1974; LaFage et al., 1976; Smith and Rust, 1994). Lenz and Evans (2002) stated that daily and seasonal changes in foraging activity of subterranean termites are still not well known, but their subterranean habit is widely assumed to reduce adverse effects of weather.

Feeding activity is an important aspect of developing management strategies for termites, especially when using baiting systems. By their behavior, subterranean termites are a cryptic threat to structures that can result in significant damage. Feeding behavior of termites in the genus *Reticulitermes* has a greater impact on the environment and human economy than that of most other animals in the USA (Waller, 1991). Determining consumption amounts and patterns by *Reticulitermes* species can be complex because feeding may be influenced by factors associated with the colony, the environment, the food source or by interactions among these factors (Waller, 1991). Feeding also may be affected by caste ratio (Delaplane, 1991), the presence of competitors and predators, or through abiotic effects such as temperature (Smythe and Williams, 1972).

Additional knowledge about the effects of seasonal conditions on termite behavior and biology is important when developing improved control methods. The objective of this field experiment was to determine if cellulose consumption by two native species of subterranean termites in the United States, *Reticulitermes flavipes* and *R. virginicus*, was impacted by seasonal changes of ground temperature and relative humidity. The hypothesis was that seasonal changes of ground temperature and relative humidity influence cellulose consumption by *R. flavipes* and *R. virginicus*.

MATERIALS AND METHODS

Termites Colonies. Seven colonies of *R. flavipes* and six colonies of *R. virginicus* were used for this experiment. Colonies in the Clemson University Experimental Forest in Pickens and Anderson Counties, South Carolina and in a neighborhood in Clemson, South Carolina, USA (approximately 34º:40' N by 82º:49' W) were used. Species identification was based on soldier characters as described by Gleason and Koehler (1980). Specimens were also sent to the University of Arkansas, Fayetteville, AK, USA, for species confirmation based on DNA sequences (Austin, pers. com.).

To establish colony monitoring locations, wooden grade stakes, approximately 2.54 x 5.08 x 33.02 cm were placed 15 to 20 cm in the ground in areas where termites appear. After one month, the stakes were inspected for active termites. A roll of moistened corrugated cardboard, approximately 15 x 15 cm was placed around each active wooden stake. The cardboard and stake were then covered by a 18.9-L plastic bucket with a lid. The bottom of the bucket was removed to permit continued access by termites to the bucket station. A second method to collect termites was also used. Logs were inspected for termite foraging galleries near the ground surface beneath the logs. When galleries were found, a cardboard roll was placed and secured with a wooden stake, and covered with a plastic bucket as previously described. Termite colonies were defined by the distance of the bucket trap locations. In this experiment, groups of termites were considered different colonies if the distance between the active stations was separated by at least 100 m. The greatest distance between colonies was 8 km.

Experimental Units. Experimental units for this experiment were bucket stations, with a cardboard roll established with either *R. flavipes* or *R. virginicus*. One electronic data logger (HOBO Temp H8 Series; Onset Computer Corporation, Bourne, MA, USA) was attached to the inside wall of each bucket to record temperature and relative humidity. Thermal couples also were placed 10 cm under ground beneath the cardboard roll and 1 m outside the bucket to record ground temperature (Fig. 1).

Procedure. Cardboard rolls were oven-dried for three days at 90º C, weighed, and placed in bucket stations. Cardboard rolls in bucket stations were removed and replaced with a new roll every one to two weeks, depending on colony activity. Removed cardboard rolls were taken to the laboratory, opened carefully, cut piece by piece and put in a plastic tray (46 cm x 35.5 cm). Termites were removed from the cardboard by aspiration and held in a plastic container (32 cm x 23.5 cm x 11 cm). For rolls estimated to contain 5000 or fewer individuals, all termites were counted. If a roll appeared to contain more than 5,000 termites, the total number was estimated. To estimate, five samples of 100 termites each were taken from the total, weighed, and the mean weight of the termites was recorded. An individual mean termite weight was calculated. Samples were returned to the container and all termites were weighed. The total number of termites was estimated by dividing the total weight of all termites by the mean individual termite weight. Termites were not returned to their field colonies. Soil and other materials were cleaned from cardboard rolls by washing them with a gentle tap water spray. Dry cardboard rolls, after exposure to sun and then oven-drying for three days, at 90º C, were reweighed. Consumption was calculated by subtracting the final weight of the cardboard roll from the initial weight. Ground temperatures, ambient temperature and relative humidity in the bucket stations were recorded hourly with electronic data loggers. Data were downloaded to a field recording device (HOBO Temp H8 Series; Onset Computer Corporation, Bourne, MA), then transferred to a computer spreadsheet (Excel, Microsoft Corporation, Redmond, WA, USA).

Data Collection and Analysis. Termite consumption was measured as the amount of cardboard consumed in g/station/day. In addition to the number of termites in the cardboard rolls, total worker body weight was recorded, and mean weight per termite was calculated. Two-week consumption was correlated with the corresponding two-week mean ground temperature and relative humidity in the bucket stations. The relationship between consumption, ground temperature and relative humidity was plotted in three-dimensional graphs and modeled using linear regression with natural log transformed data. Differences in the consumption pattern between the two species were compared through interpretation of t-tests of appropriate parameter in the overall multiple regression model. Analyses were implemented using SAS (SAS Institute, 1999).

Feeding Disruption Study. To determine if termite feeding was disrupted in the field when cardboard rolls were replaced, a laboratory experiment was conducted to simulate that condition. For this experiment, five colonies of *R. flavipes* were used. Termites were collected from the Clemson University Experimental Forest in Pickens and Anderson County, South Carolina. Experimental units consisted of a 1.2 L plastic container (Rubbermaid Home Products, Wooster, OH), filled with a 250 ml mixture of sand and vermiculite (1:1 by

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volume). This mixture was moistened with 20 ml of distilled water. A cardboard roll, 8 cm in diameter and height, was placed on top of the sand-vermiculite mixture as a source of food in each container (Fig. 2).

Figure 1. Experimental unit used in the field, with the electronic data logger attached to inside bucket wall.

Figure 2. Experimental unit used for the disruptive feeding study, with a cardboard roll on top of a vermiculite-sand mixture.

This experiment was conducted as a randomized complete block design (RCBD), with termite colonies as blocks. Treatments were: replace the cardboard roll in the experimental unit every three days over a 15-day period, and keep the cardboard roll in the experimental unit continuously for 15 days. Five hundred termites were released into each experimental unit. Each colony was assigned randomly into three replicates for each treatment. Experimental units were kept in a rearing room at $23.53 \pm 0.33°$ C and $61.60 \pm 5.85\%$ relative humidity as recorded by an electronic data logger (HOBO Temp H8 Series; Onset Computer Corporation, Bourne, MA, USA). Consumption (after 15 days) was analyzed using Analysis of Variance for a RCBD ($\alpha = 0.05$) (SAS Institute, 1999).

RESULTS AND DISCUSSION

Continuous Versus Disruptive Feeding

Replacing the cardboard roll every three days in the laboratory feeding disruption experiment did not affect termite cellulose consumption (Table 1). In the laboratory, termites did not consume a different amount of cardboard when it was replaced every three days as termites not disrupted over the same 15 day period ($p = 0.6860$). Survivorship also was not statistically different between the two treatments ($p = 0.4964$).

This experiment may simulate termite feeding activity in the field. In the field, an unknown number of termites move in and out of a cardboard roll from unknown sources with fluctuating temperature and relative humidity. In the laboratory, 500 termites were isolated in each experimental unit during the study with relatively constant temperature and relative humidity. However, the need for food may make termites readily consume any available food source, either in the field or in the laboratory. In the field, once termites find a dependable food source, they are likely return to it. Lenz and Evans (2002) stated that some termites would even relocate brood and reproductives, i.e., their nest, into suitable large food resources. Even though the cardboard rolls were replaced every two weeks, in the field experiment, termites continued to return to new rolls.

Worker mortality in the field cannot be estimated, but it is assumed that mortality occurs due to a variety of factors. In the laboratory, mortality was less than 20%. The relatively low mortality may indicate that the rolls were both an adequate food source and an adequate environment for termite survivorship.

Table 1. Mean consumption of cardboard and percent survival (± SE) of termite workers either disrupted every three days (treatment 1) or not disrupted over a 15-day period (treatment 2) in a laboratory feeding study.

Treatment	Consumption (mg cardboard roll) (± SE)	Survivor (%) (± SE)
1	807.93 ± 26.66 a	81.97 ± 5.90 a
2	793.80 ± 26.04 a	86.13 ± 2.08 a

Table 2. Pearson correlation coefficients between each of three mean temperatures and mean consumption in the field feeding study conducted from July 2003 – July 2004.

Termite species	Ambient temperature	Ground temperature inside the station	Ground temperature 1 m away from the station
R. flavipes	0.6984	0.7554	0.7710
R. virginicus	0.7116	0.7307	0.7338

Consumption Pattern

All three temperatures recorded were highly correlated with each other (Pearson correlation coefficient > 0.90 with $p < 0.0001$). However, among these temperatures, ground temperature 1m away from the bucket stations showed the highest correlation with consumption for both species (Table 2). Therefore this temperature was used as one variable to explain seasonal consumption.

Termite consumption peaked in summer months with mean ground temperature at 22 - 24º C and relative humidity above 80%. The lowest consumption occurred during winter months with a mean ground temperature at 4-5º C (Figs. 3, 4). In winter, relative humidity inside the bucket stations was more variable. Inside *R. flavipes* stations, relative humidity was always above 80%, but in two *R. virginicus* stations relative humidity recorded as low as 30%. Under these conditions, the two *R. virginicus* colonies remained active, and continue to consume cardboard.

Most data reported on termite activity in commercial bait stations used for termite control are from yards and open spaces around housing areas or other buildings. From personal observation in the Clemson area using plastic termite bait stations, termites are often more active in spring and fall than in summer or winter. In spring and fall, ground temperature is favorable for termites foraging. In summer and winter, termites avoid commercial stations in open areas, which are exposed to temperature extreme and termites probably move away from the stations to areas where temperatures are more suitable. However, in our field experiment, all stations were in wooded areas where ground surfaces were shaded by tree canopies, and never reached extreme hot summer temperatures (Fig. 3 and 4). Therefore, the peak activity for *R. flavipes* and *R. virginicus* in this study occurred during summer.

Rainfall during the evaluation period also may have contributed to the termites being more active. Rain makes soil moist, and termites need moisture to survive and develop. Potter (2004) stated that subterranean termites are very vulnerable to desiccation and require a constant supply of moisture. Rainfall in summer of 2003 was 61.75 mm higher than in the summer 2004 (the first two weeks of July) (Clemson Univ. Weather Office Data 2004). This may have contributed to the higher consumption overall in 2003.
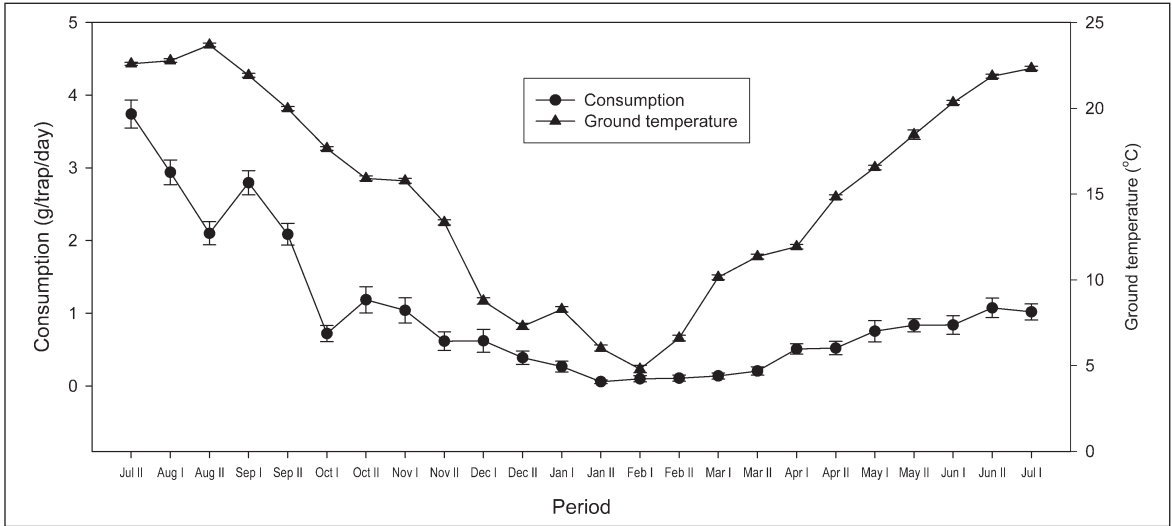
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Figure 3. Mean cellulose consumption (± SE) by *Reticulitermes flavipes* and mean ground temperature (± SE), July 2003 – July 2004.

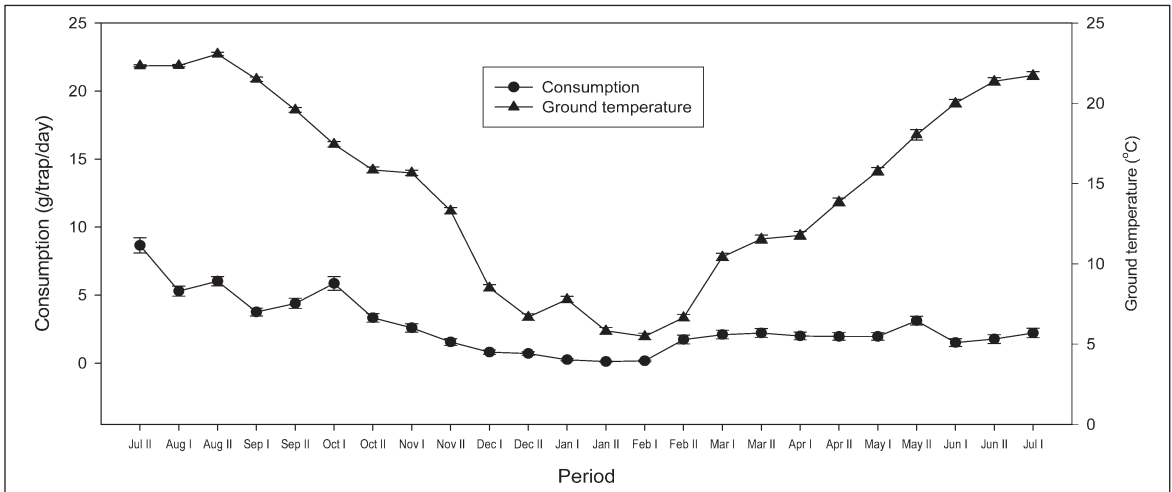**Figure 4.** Mean cellulose consumption (± SE) by *Reticulitermes virginicus* and mean ground temperature (± SE), July 2003 – July 2004.
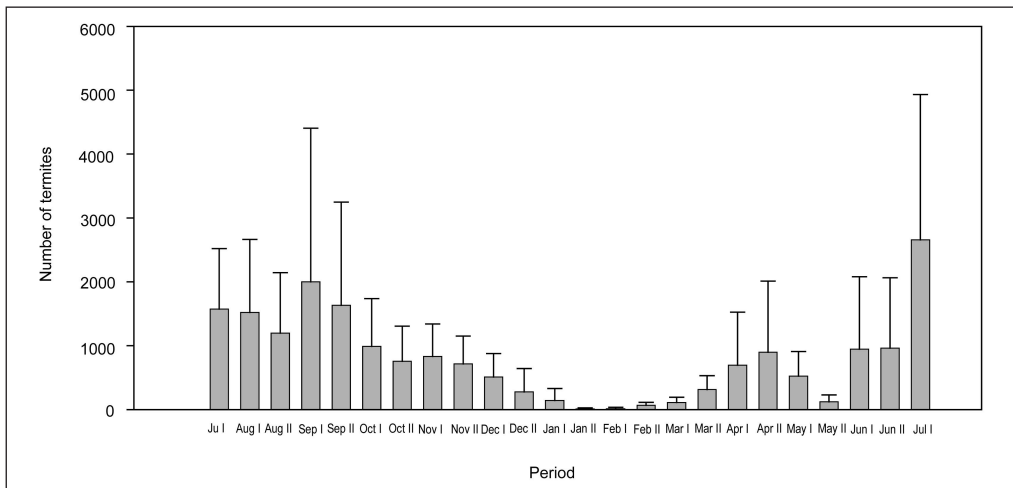

Figure 5. Mean number (± SD) of *Reticulitermes flavipes* collected from seven bucket stations, July 2003 – July 2004.
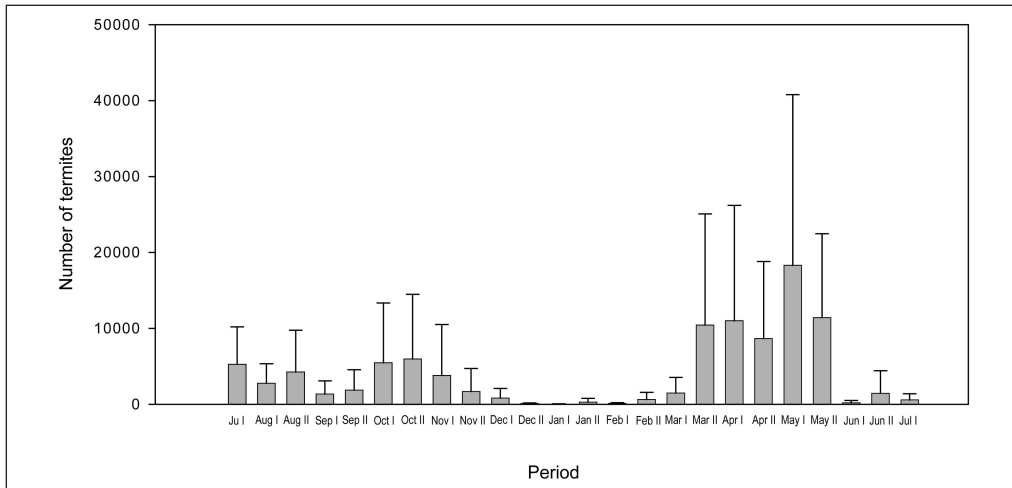
Figure 6. Mean number (± SD) of *Reticulitermes virginicus* collected July 2003 to July 2004.

Mean body weight (mean ± SE) of *R. flavipes* (3.07 ± 0.02 mg), was significantly higher than *R. virginicus* (2.08 ± 0.02 mg) (p < 0.0001) when averaged across all time periods. The body weight variance component for time period was not significant (p = 0.2395). This indicates that seasonal temperature has no impact on termite body weight. Individually, *R. flavipes*, with larger body size, consumed more food than *R. virginicus*. However, the number of termites in cardboard rolls for *R. virginicus* was much larger than *R. flavipes* (Figs. 5, 6) and in general, *R. virginicus* colonies consumed twice the amount of cardboard compared to *R. flavipes*. The number of termites in cardboard could indicate the colony size. Colonies with more individuals are likely to consume more cellulose than colonies with fewer individuals. In South Carolina, *R. flavipes* is usually considered a more serious termite pest than *R. virginicus* (Potter, 2004). The results of these data indicate that *R. virginicus* colonies can be large and damaging in the amount of cellulose they consume. In our study, two *R. virginicus* colonies were active at low humidity during cooler months. If *R. virginicus* colonies are typically large and can survive in low moisture conditions, they may pose more threat to buildings than *R. flavipes*.

A smoothed representation of the relationship between seasonal termite consumption patterns to both temperature and relative humidity can be seen in Figs. 7, 8, and 9. Until an upper temperature threshold is reached and at higher relative humidities, *R. flavipes* and *R. virginicus* will correspondingly increase consumption. *Reticulitermes flavipes* exhibited this trend consistently, with one fluctuation around relative humidity at approximately 90% (Fig. 7). *R. virginicus* showed a more complex three-dimensional pattern (Fig. 8). For two of the *R. virginicus* colonies, an average of 1,000 termites in cardboard rolls were collected during winter months, where relative humidity inside the buckets was around 30%. Since there were no other bucket stations with low relative humidity during the same period, the average consumption at that range of relative humidity was skewed to those two colonies. This makes it difficult to draw conclusions about whether *R. virginicus,* in general, shows the same consumption pattern, as *R. flavipes*. When consumption data for *R. virginicus* were plotted for relative humidity above 80% (Fig. 9), the trend was more comparable to *R. flavipes* (Fig. 7), but differences still were evident.

To compare the consumption pattern of *R. flavipes* to *R. virginicus* and build a predictive model of consumption as related to ground temperature and relative humidity, a linear regression analysis was done. Due to large consumption variability across the data space and some very large consumption values at the highest temperature and relative humidity combinations observed, it was necessary to use a natural log transformation of the consumption data. An indicator variable, species identification (specid), was incorporated with *R. flavipes* coded as 0 and *R. virginicus* coded as 1. A full second-order model was fit. Since second-order terms in temperature and the temperature by relative humidity interaction were all non-significant (p > 0.1), they were removed from the model. The resulting model was Ln (consumption) = -18.572 + 0.155 (ground temperature) + 0.271 (relative humidity) - 0.001 (relative humidity)^2 + 146.849 (specid) -3.213 (relative humidity) (specid) + 0.0175 (relative humidity)^2 (specid). For *R. flavipes*, where specid is 0, the resulting fitted surface is shown in Figure 10. Inserting specid = 1, gives the consumption response surface for *R. virginicus* shown in Figure 11.
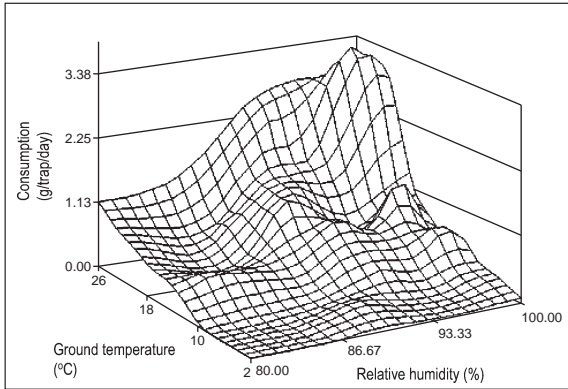
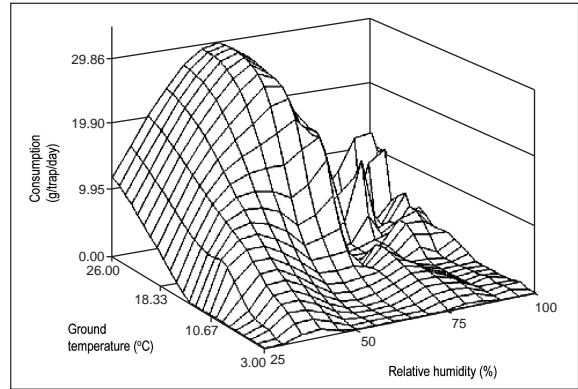**Figure 7.** Cellulose consumption by *Reticulitermes flavipes* at temperatures and relative humidities recorded.

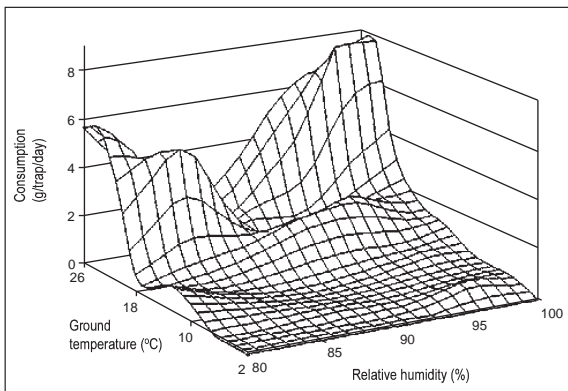**Figure 8.** Cellulose consumption by *Reticulitermes virginicus* at temperatures and relative humidities recorded.

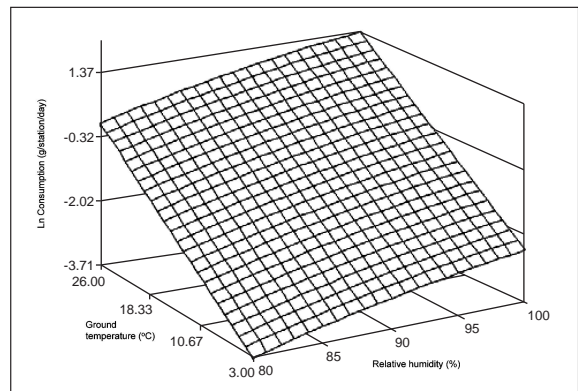**Figure 9.** Cellulose consumption by *Reticulitermes virginicus* at all temperatures and relative humidity above 80%.

Figure 10. Prediction model for cellulose consumption by *Reticulitermes flavipes* in relation to temperature and relative humidity.

While the surfaces in figures 10 and 11 result from using this best model identified by the data, it does not seem likely that the true consumption surface for *R. virginicus* should show decreasing consumption for increasing relative humidity levels between 80 and 90%. To investigate restricting this surface to be non-decreasing for increasing levels of our temperature and relative humidity ranges, a first-order model was fit resulting in the following equation: Ln (consumption) = -9.147 + 0.155 (ground temperature) + 0.064 (relative humidity) + 0.234 (specid). This model clearly demonstrated that best first-order surfaces for the two species were not greatly different (p = 0.0958 for specid term), but the response plane for *R. virginicus* was higher (greater consumption) than the response plane for *R. flavipes*. The average of these two planes is shown as Figure 12. This was the preferred predictive model since it explained about 47% of the variability in the log-transformed consumption data, only slightly less than the 50% of this variability explained by the second-order model. Illustrating the use of this model with an example where consumption is to be predicted for a temperature of 25° C and relative humidity of 86%, the following would be calculated: for *R. flavipes*, Ln (consumption) = 0.232 or consumption = 1.26 g/station/day, for *R. virginicus*, Ln (consumption) = 0.466 or consumption = 1.59 g/station/day.

Termites trapped

The number of termites in cardboard rolls followed the pattern of consumption, reaching the highest level in summer months and lowest level in winter months (Figs. 5, 6). Bucket stations for *R. flavipes* had termite activity during most months, especially during warmer periods. *Reticulitermes virginicus* activity was more variable. One station became permanently inactive in November 2003. Two *R. virginicus* bucket stations became inactive in June 2004, one month before the 12-month study ended. Those two *R. virginicus* colonies were

active in the spring of 2004. During April and May 2004, from 20,000 to 40,000 workers were collected each week. The removal of large numbers of termites ultimately may have depleted the colonies. The colonies also may have located a more preferred food source. A large swarm was observed on May 20, 2004, from a large tree (1.5 m in base trunk diameter and about 30 m tall) near one of the *R. virginicus* stations. The alates were probably from the same colony. Overall, these factors may have contributed to a decrease in *R. virginicus* trapped in June 2004.
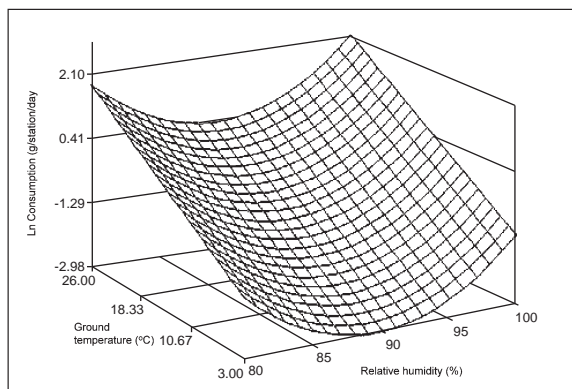

Figure 11. Prediction model for cellulose consumption by *Reticulitermes virginicus* in relation to temperature and relative humidity.
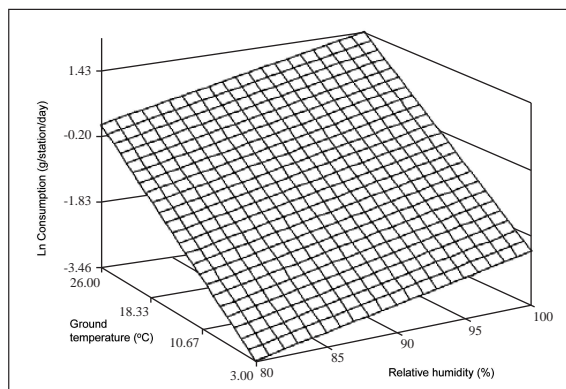
Figure 12. Combined prediction model of cellulose consumption pattern for *Reticulitermes flavipes* and *Reticulitermes virginicus* in relation to temperature and relative humidity

In summary, our original hypothesis was correct. Seasonal changes in ground temperature and relative humidity influenced cellulose consumption by *R. flavipes* and *R. virginicus*. Consumption patterns for *R. flavipes* and *R. virginicus* between July 2003 to July 2004 in the Clemson area of South Carolina were statistically not different. On average, *R. virginicus* colonies were larger and consumed more per time period than *R. flavipes* colonies. For both species, peak consumption and the number of termites collected were highest during the warmest months, when ground temperatures in shaded locations average between 22-24° C. Most colonies for both species were active at a relative humidity of 80% or higher, but two of *R. virginicus* colonies were found to be active at a relative humidity around 30% during cooler months. These findings may indicate that *R. virginicus* colonies in South Carolina are, on average, larger and active at a wider range of relative humidity than *R. flavipes*.

ACKNOWLEDGMENTS

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