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Research Reports

Feeding, Weight Change, Survival, and Aggregation of *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) in Seven Varieties of Differentially-Aged Mulch[1]

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Abstract

Seven commercially-available mulches were aged outdoors for 0, 6 and 12 months then compared for suitability to the subterranean termite *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) in non-choice and multi-choice tests under laboratory conditions. Mean differences in mulch consumption, individual termite weight change, and termite worker survival were examined in non-choice tests while mulch consumption and termite aggregation were measured in multi-choice tests. Non-choice tests did not reveal differences in consumption between mulches of the same age. However, mulch consumption and survival increased with mulch age and termite weight loss decreased with mulch age. In multi-choice tests feeding and aggregation preferences shifted with mulch age.

Index words: mulch consumption, eastern subterranean termite, preferences.

Significance to the Nursery Industry

The use of mulch is a beneficial practice used to improve both the health of landscape plants and the appearance of landscaping. Organic mulches such as pine straw and ground wood provide attractive alternatives for recycling and using non-commercial wood. However, the possibility that mulch around a home may be attractive to termites has generated questions from both mulch producers and homeowners alike. In our study, all commercially-available wood mulches were equally palatable to the subterranean termite *Reticulitermes flavipes* (Kollar). Our study suggests, however, that aged mulches are more palatable, minimize termite weight loss, and improve termite survival in *R. flavipes*.

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Introduction

Concerns about mulch as a food source or attractant have motivated studies regarding its effect on the foraging activity and feeding of subterranean termites (*Reticulitermes* spp.). Several studies have examined the relative susceptibility of sound wood to attack by subterranean termites, but few studies address feeding and behavior relationships between mulch and subterranean termites (4). Under laboratory conditions, equivalent consumption rates but lower survivorship of *Reticulitermes virginicus* (Banks) workers fed on *Eucalyptus*, pine bark, cypress, or hardwood were observed (10). Similarly, under laboratory conditions Duryea et al. (4) observed differences in consumption and avoidance of commercial mulches from Florida, as well as differences in termite survival in *R. virginicus* and *R. flavipes.*

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Decayed wood may provide more nutritional benefits for subterranean termites when compared to fresh wood. Decomposition may increase the availability of nitrogen and other nutrients, break down toxic compounds in the wood, and enhance the ability of termites to metabolize cellulose by chemically modifying the wood (19). Similarly, *R. flavipes* was more likely to be associated with, and displayed higher survivorship and consumption in, decayed wood versus sound wood (9, 5). Although the effects of aging on the chemical properties of mulch are not as well known, it may be expected that mulches having high wood content experience similar decomposition processes that would be favorable for subterranean termite populations.

It has been demonstrated that mulch positively impacts other groups of insects in urban environments. Significantly more invertebrates were collected from mulched areas than areas with no mulch (7). For example, spiders were seven times more abundant and centipedes three times more abundant in mulched areas. They also found that termites were commonly found in hardwood mulch and occasionally in softwood, but were significantly more abundant in areas when mulch was four to five inches deep, regardless of mulch type (7).

The objective of this study was to examine termite feeding, weight change, survival, and aggregation preferences of *R. flavipes* subterranean termites when exposed to differentially-aged mulches from Missouri in both non-choice and multi-choice laboratory experiments.

Materials and Methods

Termite collection. *Reticulitermes flavipes* were collected in Columbia, MO, from the soil surrounding dead wood using aboveground traps. Logs were rolled back and the soil surface beneath them examined for termite activity. When termites were observed, traps constructed of rolled corrugated cardboard within PVC pipe (140 mm high, 75 mm diameter) were dipped in a 1% sucrose/water solution and placed on the ground over the spot where termites were observed. The upper end of each PVC roll was covered with aluminum foil to prevent moisture loss. The entire aboveground PVC trap was covered with a bucket turned upside down. Traps were checked 24 to 72 hr later. If termites were present, traps were removed and brought into the lab where termites were removed from the cardboard, counted, weighed, and stored inside Petri dishes (150 mm × 25 mm, Falcon®) using moistened wood tongue depressors as a food source. Petri dishes were stored inside small plastic Rubbermaid® containers (320 mm × 180 mm × 90 mm) with damp cloths in the bottom to maintain humidity levels. Termites were stored in the lab at approximately 21C (69.8F) for a maximum of one week before use. Mean individual termite weights were taken before the feeding experiments. Individual termite weight was calculated by weighing three groups of 30 workers and calculating the mean weight in grams (g).

Mulch species and aging. Seven mulches, including cedar (*Juniperus* sp.), white oak and white oak coated with iron oxide (*Quercus* sp.), oak bark (*Quercus* sp.), pine bark (*Pinus* sp.), baldcypress (*Taxodium* sp.) and pine needles or pine straw (*Pinus* sp.) were placed outdoors in contact with soil and aged for 0, 6 and 12 months. When tests were initiated, individual mulch samples were brought into the laboratory and oven-dried for 24 hours at 103C (217.4F) before being placed into non-choice or multi-choice arenas.

Experimental arenas. Termite feeding preferences were evaluated in both non-choice and multi-choice feeding containers. In both cases containers were prepared by placing 2 g of mulch into petri dishes (100 mm × 15 mm). The mulches were moistened by the addition of 0.5 cc of distilled water and placed inside humidity chambers. For non-choice tests, individual Petri dishes containing mulch were kept inside humidity chambers during feeding. To provide termites with a variety of mulch types, a multi-choice feeding container was constructed using a central Petri dish (150 mm × 25 mm) connected via 5 cm of Tygon tubing (internal diameter: 3 mm, external diameter: 6 mm) with seven evenly-distributed peripheral Petri dishes (100 mm × 15 mm) (Fig. 1). Each multi-choice chamber contained all varieties of mulch at either 0, 6, or 12 months of age. The tubing between central and peripheral dishes was inserted flush with the bottom of the dish in order to facilitate termite movement between Petri dishes. The humidity chambers containing mulch and termites were kept at 23C (73.4F) and 95% relative humidity.

Palatibility. In order to evaluate *R. flavipes* feeding, weight change, and survival on various mulches under non-choice conditions, approximately 150 *R. flavipes* workers plus two soldiers were added to each Petri dish within 24 hr after preparing the feeding chambers and allowed to feed for 15 days. There were 4 replicates of each mulch age and variety. In multi-choice feeding chambers, 1050 *R. flavipes* plus 3–4 soldiers were added to the central portion of the feeding chamber within 24 h and were allowed access to all mulches for 15 days.

We used the Su and LaFage (16) method for calculating wood consumption by *R. flavipes*. Their equation is:

$$WC = 2 \times (W_i - W_f) / (T_i + T_f),$$

where: W_i = initial dried wood weight (mg), W_f = final dried wood weight (mg), T_1 = initial termite weight (g), T_f = final termite weight (g).

Fig. 1. View of the multichoice arena after several days of feeding by *R. flavipes*.

Survival. To determine differences in *R. flavipes* survival within different mulches the number of termite workers after the 15 day feeding period was divided by the number at the beginning of the feeding period. The number of workers was estimated for each treatment before and after feeding by dividing total termite weight in each Petri dish by mean individual termite worker weight from the same dish.

Weight change. To determine the effects of individual mulches on termite weight change, a group of 30 *R. flavipes* workers from each Petri dish was weighed following the two-week feeding period in non-choice experiments. Mean final individual termite weight was calculated and subtracted from mean initial individual termite weight to determine weight change.

Aggregation. The number of termites in each mulch type and age was examined to determine *R. flavipes* aggregation preferences. The number of termites in each Petri dish at the conclusion of the multi-choice test was estimated by weighing the group of termites in each Petri dish, then dividing it by the mean individual termite weight for that arena.

Statistical analysis. One-way analysis of variance (ANOVA) for a complete randomized design with four replicates of each variety and age of mulch using SigmaStat® 2.0 (15) was used to compare mulch consumption, number of surviving termites, termite weight loss, and aggregation preferences within mulch varieties of the same age. ANOVA was also used to compare the same variables among different ages of the same mulch. Tukey's multiple comparison procedures were used to detect all significant differences ($p = 0.05$) occurring in each analysis with the exception of differences in non-choice mulch consumption experiments at six and twelve months. In these cases, Dunn's method was used.

Results and Discussion

Palatability. All mulches in this experiment were readily consumed by *R. flavipes*, although consumption varied between mulch type and age class. In the non-choice experiment, mean mulch consumption varied from 134.7 mg/g termite to 758.7 mg/g termite. There were significant differences in consumption between some mulches of the same age. At zero months, consumption of pine straw was significantly higher ($p = 0.0003$) than white oak, iron coated white oak and cedar. Consumption of oak bark was also significantly higher than white oak and iron coated white oak. At six months, pine straw consumption was higher ($p = 0.0137$) than cypress. At twelve months, there were no significant differences in consumption between mulches. Consumption of the same mulch significantly ($p < 0.05$) increased when aged from zero to twelve months for all mulches, with the exception of pine needles, which was not evaluated at twelve months due to a lack of sufficient amounts of pine straw resulting from rapid decomposition during the year (Fig. 2).

Within a few days after introducing termites to the multi-choice experimental arenas, they were moving throughout the arenas within a tunnel constructed of fecal materials and which followed the edge of the central plate. The circular tunnel connected all the entrances to Petri dishes containing mulch. Mulches of all species and ages were consumed by *R. flavipes* in the multi-choice test (Fig. 3). Mean mulch consumption varied with mulch type and age, ranging from 6 mg to 220 mg/g termite. Consumption of white oak was significantly ($p = 0.0042$) higher than consumption of cedar at zero months of age. Consumption of white oak and pine bark was significantly higher than consumption of cedar at six months ($p = 0.0016$). At twelve months cypress and oak bark were consumed in significantly ($p < 0.0001$) higher quantities than cedar, pine bark and oak bark. In addition, consumption of iron coated white oak was significantly lower than cypress but not oak bark.

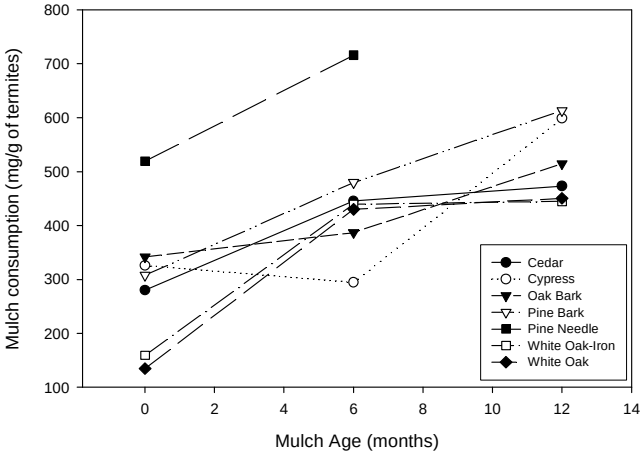

Fig. 2. Mean mulch consumed by *Reticulitermes flavipes* in non-choice experiments when provided with seven varieties of mulch aged for 0, 6, or 12 months.

Termite survival. Survival of *R. flavipes* workers varied between 73 and 100%. No significant differences in worker survival were found between mulches of the same age. With the exception of iron coated white oak, survival of termite workers did not significantly differ between ages of the same mulch. Fifty percent of iron coated white oak replicates at zero months showed high levels of mortality which were not observed in the same mulch at six and twelve months.

Termite weight loss. In non-choice experiments, termite workers lost weight in all mulches tested. Weight loss ranged from 0.15 mg to 1.15 mg/150 termites. There were no significant differences in the amount of weight lost by termites feeding on mulches of the same age. In general, *R. flavipes* workers lost less weight when feeding on aged mulches; however differences in termite weight loss among different ages were only significant for white oak and cypress ($p = 0.0463$). Termite weight loss was not examined in multi-choice experiments.

Aggregation preferences. Termites were not equally distributed among the different mulches in multi-choice arenas. After three days of feeding aggregation behavior was evident. Termites consistently aggregated in some mulches and not in others. Aggregation preferences were more evident as mulches increased in age. At zero months, aggregation on pine bark was only significantly higher ($p = 0.0005$) than cedar. At six months aggregation on pine bark was significantly ($p = 0.0014$) higher than aggregation on cedar and oak bark. At twelve months, aggregation on pine bark was

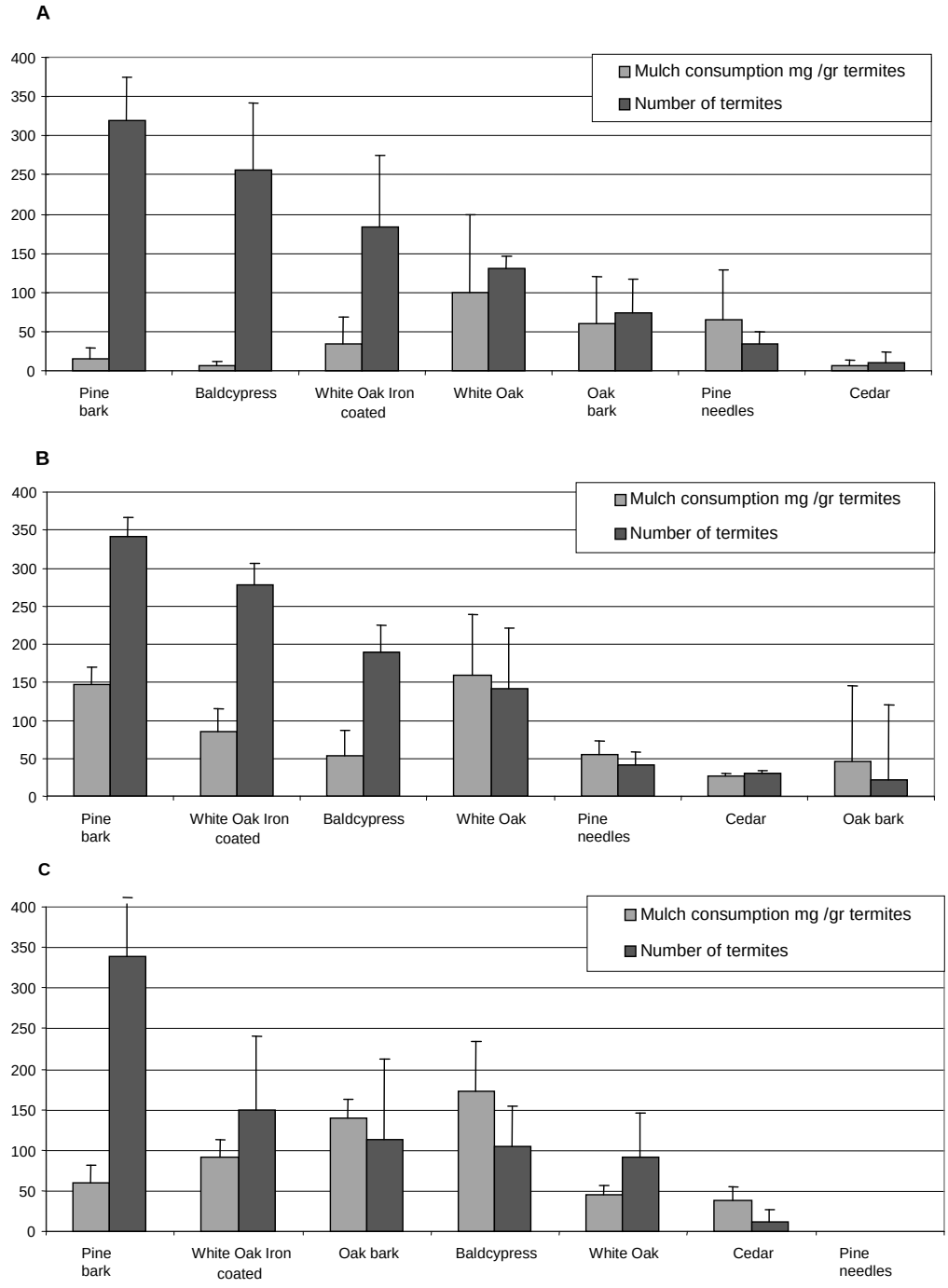

Fig. 3. Mean mulch consumption and aggregation of *Reticulitermes flavipes* in multi-choice experiments when provided with seven varieties of mulch aged for 0 (A), 6 (B), and 12 (C) months.

significantly higher ($p < 0.0001$) than cedar, oak bark, white oak, cypress, and iron oxide coated white oak.

In general, mulch consumption reported here was only slightly lower than that reported for other *Reticulitermes* sp. feeding on mulch (10) or wood (17, 12). Non-choice tests showed *R. flavipes'* ability to survive on several varieties and ages of mulch, while multi-choice tests showed the capacity of *R. flavipes* to rank food sources based on quality and availability.

Mulches of different ages were consumed by *R. flavipes* when provided in both multi-choice and non-choice experiments. In the non-choice experiments, consumption of pine needles at zero and six months was highest. Unfortunately, due to decomposition there were not enough material to test samples of pine straw at twelve months. Although cellulose may account for about 20% w/w content in needles of some pine species (11), it is unknown from this study, the physical or chemical factors that resulted in relatively higher consumption of pine needles at zero and six months. However, it is important to note that weight loss was higher for termite workers when feeding on pine needles than on other food sources in this study.

In this study *R. flavipes* demonstrated the ability to choose among food sources of varying quality (18). This capability was expressed as a switch in the preferred mulch at different ages. Cedar mulch was consistently less preferred than other mulches at all ages. This finding is consistent with the known antitermitic volatiles that occur in the wood of some *Juniperus* species (8, 6) and that may still be present in mulches made of this wood. Conversely, cypress mulch consumption, whose heartwood is considered unfavorable for *R. flavipes* (1), notably increased at twelve months of mulch age. This was also true for oak bark. The significant increase in consumption of cypress and oak bark may be attributed to the fact that these mulches showed more advanced decomposition after twelve months of aging than other varieties tested. The preference that *R. flavipes* has for consuming decayed wood is mentioned in several studies (2, 3, 9, 19) and it is likely that decomposition in the outdoor environment improved the palatability of these two mulches.

In non-choice experiments there was a trend toward increased ability of *R. flavipes* to survive and maintain weight on aged mulches. In general, there was lower weight loss and higher survival on mulches at twelve months when compared to zero months, although this trend was not related to any specific mulch. Statistically significant differences between some of the treatments were mitigated by the unexplained large variation in our experiments. However, this variation is similar to that reported in previous studies of *R. flavipes* feeding (13, 14).

Aggregation preferences of *R. flavipes* were markedly different from consumption preferences in this study. Workers of *R. flavipes* showed a consistent tendency to aggregate in pine bark while consuming mulches found elsewhere in the multi-choice arena (Fig. 3). Mobility between food sources and differences between feeding and sitting preferences are interesting observations from this study and may provide evidence that the presence or absence of termites on a substrate may not be an accurate indicator of feeding.

Most commercially-available mulches from Missouri are eaten by *R. flavipes* workers during periods of at least two weeks. Under favorable environmental conditions, aging of mulches may improve palatability, reduce termite worker weight loss, and improve termite worker survival in *R. flavipes*. Variations in mulch variety and age may affect food choice and worker aggregation behavior.

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