Aggregation and Feeding Behavior of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) on Wood Decayed by Three Species of Wood Rot Fungi

by

Mary L Cornelius*, Kelley S. Williams, Mary P. Lovisa & Anthony J. De Lucca II

ABSTRACT

Aggregation and feeding behavior of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was evaluated on wood decayed by three species of fungus, the brown rot fungus, *Gloeophyllum trabeum* and two white rot fungi, *Phanerochaete chrysosporium* and *Pycnoporus cinnibarinus*. Although termites aggregated on decayed sawdust from all three species in at least some of the tests, sawdust decayed by *P. chrysosporium* elicited aggregation behavior by termites over the greatest range of incubation periods. In some tests, termites avoided sawdust decayed by *G. trabeum*. Termite feeding on blocks decayed for 90 d was significantly greater than on control blocks for all three species of fungi, despite the significantly lower decay rate of *P. cinnibarinus*. Increasing our understanding of the interaction of termites with wood rot fungi could lead to the identification of chemicals that attract termites to bait stations.

Key Words: *Coptotermes formosanus*, fungus, aggregation, consumption, decay

INTRODUCTION

Lignocellulose is the major component of plant cell walls. The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, uses cellulases secreted from salivary glands and symbiotic flagellates to digest cellulose (Nakashima*et al.* 2002, Zhang*et al.* 2011). Although there is evidence that termites produce cellulases, hemicellulases, and lignases, lignin is not metabolized by termites and acts as a physical barrier to cellulases (Breznak & Brune 1994, Brune

United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124

^{*}Corresponding author: Mary.Cornelius@ars.usda.gov;

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2006, Geib *et al.* 2008, Coy *et al.* 2010, Ke *et al.* 2011, Scharf *et al.* 2011). Wood rot fungi may facilitate termite digestion of cellulose by modifying or metabolizing lignin.

Brown rot fungi circumvent the lignin barrier and metabolize cellulose and hemicellulose without removing the lignin. Therefore, lignin remains the major component of plant cell walls degraded by brown rot fungi. However, brown rot fungi modify the lignin by demethylation and oxidation (Green III & Highley 1997). In contrast, white rot fungi simultaneously degrade the three major components of the plant cell wall: lignin, cellulose, and hemicellulose (Geib *et al.* 2008). Two species of white rot fungi have different enzyme systems for lignin degradation. Ligninolytic activity by, *Phanerochaete chrysosporium* Burdsall, is closely correlated with secretion of two specific peroxidases, lignin peroxidase (LiP) and manganese peroxidase (MnP), whereas neither LiP nor MnP was detected in the ligninolytic activity of the white rot fungus, *Pycnoporus cinnabarinus* (Jacq.:Fr.) Karst. Ligninolytic activity by *P. cinnabarinus* was characterized by a single dominant laccase (Eggert *et al.* 1996, Alves *et al.* 2004).

Many studies have documented the preference of subterranean termites for wood decayed by the brown rot fungus, *Gloeophyllum trabeum* (Persoon: Fries) Murrill over sound wood (Amburgey 1979, Lenz *et al.* 1991). Studies have also demonstrated that chemicals in *G. trabeum* elicit trail following and directional tunneling behavior (Esenther *et al.* 1961, Matsumura *et al.* 1968, Rust *et al.* 1996, Su 2005). Other researchers have found that the interaction between subterranean termites and *G. trabeum* is antagonistic because of the competition for cellulose (Jayasimha & Henderson 2007a, 2007b).

In previous research, the Formosan subterranean termite has shown a significant preference for spruce, *Picea* sp., sawdust inoculated with both the brown rot fungus, *G. trabeum* and the white rot fungus, *P. chrysosporium*, over control sawdust. However, termites strongly preferred sawdust inoculated with *P. chrysosporium* over sawdust inoculated with *G. trabeum*. Also, there was significantly greater aggregation of termites on spruce sawdust that had only been decayed for three weeks by *G. trabeum* compared to sawdust decayed for 12 week (Cornelius *et al.* 2002).

The objective of this study was to examine the interaction of Formosan subterranean termites with three species of wood rot fungi, the brown rot fungus, *G. trabeum* and two species of white rot fungi, *P. chyrsosporium* and *P. cinnabarinus* at different stages of decay. Because the two species of white rot fungi use different enzyme systems for lignin degradation, their effect on termite behavior may be different. This study examines the aggregation and feeding behavior of Formosan subterranean termites on wood decayed by these three species of wood rot fungi.

MATERIALS AND METHODS

Termite collection and fungus cultures

Termites were collected from field colonies in City Park, New Orleans, LA, using cylindrical irrigation valve boxes (22.5 by 14.8 cm; NDS, Lindsay, CA) buried in the ground so that the lids are level with the surface of the soil and filled with blocks of wood (spruce [*Picea* sp.]). Collections from different stations in the same section of City Park were considered to be separate colonies based on a mark-release-recapture technique using the dye markers Nile Blue A and neutral red (Sigma- Aldrich, Milwaukee, WI) to determine which stations were part of a single, interconnected tunneling system. Any termites from stations containing dyed termites were considered to be part of the same colony as the termites in the station from which the dyed termites were released. Termites were kept in the lab in 5.6-L covered plastic boxes containing moist sand and blocks of spruce until they were used in experiments. Termites were used for experiments within two months of collection.

The brown rot fungus, *G. trabeum* (Madison 617), the white rot fungus, *P. chrysosporium* (46235) and the white rot fungus, *P. cinnabarinus* (10242) were obtained from the American Type Culture Collection (ATCC, Manassas, VA).

Fungal inoculation of sawdust

Northern red oak, *Quercus rubra* L., sawdust was inoculated with the mycelium from one of the three species of fungus and decayed for different incubation periods (7, 14, 30, 60, 90, 120, or 150 d). There was no sporulation by any of the fungi in any of the tests. Termites were only exposed to mycelium. Nutrient plates were inoculated with one of the fungus species and placed in an incubator set at a temperature of 25° C with a photoperiod of 12:12 (L:D) h for 3 d. For *P. chrysosporium*, potato dextrose agar (PDA)

plates were used and for G. trabeum and P. cinnabarinus, potato dextrose yeast (PDY) plates were used. Five liters of nutrient broth were inoculated with (1-cm square) plugs from the plates at a ratio of four plugs per liter of broth. The flasks were incubated at 25° C in an orbital shaker at 120 rpm. Phanerochaete chrysosporium was cultured in Sabouraud dextrose broth for 7 d, whereas G. trabeum and P. cinnabarinus were cultured in PDY broth for 14 d. Afterwards, the fungal biomass was strained through a wire mesh strainer, and finally vacuum filtered through a Buchner funnel using a sterile Whatman #4 filter paper. Sawdust was placed in an autoclavable polypropylene vent bags (32 by 40 cm) (Unicorn Imp. and Mfg. Corp., Commerce, TX) with a single 0.2 micron filter (7 by 25 cm), 100 grams per bag. The bags were heatsealed and autoclaved vent side up, for 1 h on each of two consecutive days. An amount of mycelium equal to 43.4 g of mycelia per 100 g of sawdust was weighed and suspended in 18 ml of sterile water and blended in a Stomacher 400 Mark II Lab Blender (Spiral BioTech, Bethesda, MD) on high for 60 s. After the autoclaved bags were cooled to room temperature, one corner of the bag was cut open, and the filtered mycelium was added to the bag using a pipette. After the sawdust was inoculated with the filtered mycelium, the opening in the vent bag was heat-sealed. The bags were kept in an incubator set at 25° C with 12 h photoperiod for different lengths of time (7, 14, 30, 60, 90, 120, or 150 d).

Aggregation behavior on decayed sawdust

Tests were conducted to determine if termites aggregated on sawdust inoculated with living fungal mycelium after different incubation periods. Different batches of sawdust were inoculated at each incubation period to evaluate variability between different batches decayed for the same incubation period. Three plastic screwtop containers (5cm diam and 4.7 cm height) were connected with 5 cm length pieces of PVC tubing. Each distal container was filled to a height of 1 cm with either treated or control sawdust or with sawdust decayed by two different fungus species. A soil moisture meter (Spectrum Technologies, Plainfield, IL) was used to make sure that moisture levels were equivalent in treated and control sawdust. The center container was filled with 10 g of sand, moistened with 2 ml of distilled water. For each replicate, termites (190 workers and 10 soldiers) were released into the center container. For each test, termites were collected from four different colonies, with three replicates of each colony. For 7 d, containers were kept in an unlit environmental chamber set at 28°C, 97% RH. At the end of the test, the number of termites in the tubing and in each container was counted.

Two tests were conducted with the first batch of sawdust decayed by *G. trabeum* for 30 d and 60 d because the behavior of termites toward the sawdust decayed for 30 d was different than toward the sawdust decayed for 60 d. Tests were repeated with this batch to evaluate this change in termite behavior towards the decayed sawdust.

Fungus inoculation of wood blocks

Blocks of red oak (4 cm by 3.5 cm by 1 cm) were oven-dried at 90° C for 24 h, weighed, and numbered. Blocks were then soaked in water for 3 d, wrapped in moist paper towels and two layers of aluminum foil, and then autoclaved for 60 minutes on two consecutive days. After cooling, each individual block was placed in a Petri dish (100 mm by 20 mm) containing PDA inoculated with one of the three fungus species. Block numbers and fungus species were written on the lid of each Petri dish. Fungus-inoculated blocks were kept in an environmental chamber set at a temperature of 25° C with 12 h photoperiod for 30, 60, or 90 d. The fungal mycelium of each species gradually covered the block during the incubation period. At the end of each time period, blocks were cleaned, oven-dried at 90° C for 24 h, and re-weighed. Decay rates were determined by calculating the weight loss of each block during the incubation period.

Feeding behavior on decayed blocks

A no-choice test was conducted using blocks decayed by one of the three fungus species for 30 d, 60 d, or 90 d and undecayed control blocks. The assay was conducted using polystyrene, cylindrical screwtop containers (9 cm high by 7cm diam.) (Consolidated Plastics, Twinsburg, Ohio) filled with 50 g of sand (Play Sand, Quikrete, Atlanta, GA) and moistened with 10 ml of distilled water. After weight loss due to decay was determined, a block was placed on top of the sand in each container. For each replicate, termites (190 workers and 10 soldiers) were released into the container. For six weeks, containers were kept in an unlit environmental chamber set at 28°C, 97% RH. After six weeks, blocks were cleaned, oven-dried at 90° C for 24 h, and re-weighed. Wood consumption was determined by calculating the weight loss of each block. Termites were collected from three different colonies, four replicates of each colony for each fungus species at each decay period, except that there were only two replicates of each colony for control blocks and for *P. chrysosporium* and *G. trabeum* at 30 and 60 d.

Statistical analysis

In aggregation assays, the number of termites in treated and control containers was compared using a paired choice t-test. Termites, located in the tubing and the center container, were not included in the analysis. In the nochoice feeding test, proportional weight loss due to decay and feeding, and proportional survival were compared for each treatment (fungus species + decay period) and for each fungus species at each decay period (30, 60, or 90 d) separately using a one-way Kruskal-Wallis ANOVA. Means were separated by Dunn's test with ranked sums due to unequal sample sizes. If the tests for both normality and equal variances passed, a one-way ANOVA was used and means were separated with Dunn's test (Systat Software 2008).

RESULTS AND DISCUSSION

Aggregation behavior on decayed sawdust

Average survival of termites in the aggregation tests was 92.4 ± 2.3 . Sawdust decayed by *P. chrysosporium* had a highly significant effect on aggregation behavior for all batches and incubation periods from 7 d -120 d. When sawdust was decayed for 150 d, there was no significant difference in the number of termites on decayed and control sawdust (Table 1).

Termite responses to sawdust decayed by *G. trabeum* were highly variable. In the first batch, termites aggregated on the control sawdust when sawdust was decayed for 30 d (P = 0.05). In contrast, termites aggregated on sawdust decayed for 60 d. When these tests were repeated with sawdust from the same bags, termites aggregated on control sawdust after an incubation period of 30 d (P = 0.003) and aggregated on the decayed sawdust after an incubation period of 60 d (P = 0.001). In the second batch, termites aggregated on sawdust decayed for 30 d, and showed no response to sawdust decayed for 40, 50, or 60 d. In the third batch, they aggregated on control sawdust compared to sawdust decayed for 14 d. Termites also appeared to aggregate on control sawdust after 40 d (P = 0.05) (Table 2). Aggregation behavior on sawdust

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Incubation Time (days)	Fungus	Control	P Value ¹
Batch 1			
7	139.9 ± 11.6	4.7 ± 1.1	< 0.0001
14	110.8 ± 6.2	30.6 ± 5.7	< 0.0001
Batch 2			
14	116.7 ± 7.6	41.0 ± 8.4	< 0.0001
30	135.8 ± 11.9	13.7 ± 3.9	< 0.0001
40	110.9 ± 9.5	30.6 ± 7.9	< 0.0001
50	99.5 ± 9.4	32.8 ± 5.2	< 0.0001
60	125.6 ± 7.6	19.6 ± 4.6	< 0.0001
Batch 3			
14	127.5 ± 8.7	12.2 ± 3.2	< 0.0001
30	112.2 ± 12.1	19.6 ± 4.5	< 0.0001
60	131.4 ± 9.4	14.2 ± 4.3	< 0.0001
90	98.3 ± 9.9	28.7 ± 5.6	< 0.001
120	123.2 ± 4.2	19.7 ± 3.0	< 0.001
150	87.4 ± 10.5	55.6 ± 11.4	0.12

Table 1. Mean $(\pm SE)$ number of termites in containers filled with red oak sawdust inoculated with *Phanerochaete chrysosporium* and decayed for different lengths of time compared with containers filled with control sawdust after 7 d.

¹The number of termites in treated and control containers were compared using a paired choice t-test.

Table 2. Mean (\pm SE) number of termites in containers filled with red oak sawdust inoculated with *Gloeophylum trabeum* and decayed for different lengths of time compared with containers filled with control sawdust after 7 d.

Incubation Time (days)	Mean (±SE) I Fungus	Number of Termites in Control	Containers P Value ¹	
Batch 1				
14	26.7 ± 12.3	67.4 ± 16.9	0.14	
30 (test 1)	21.5 ± 6.7	63.3 ± 18.3	0.05	
30 (test 2)	25.6 ± 7.5	81.3 ± 10.4	0.003	
60 (test 1)	67.8 ± 14.2	23.8 ± 10.8	0.04	
60 (test 2)	99.3 ± 13.6	31.8 ± 6.3	0.001	
Batch 2				
30	68.0 ± 10.7	25.9 ± 5.1	0.01	
40	58.2 ± 11.3	47.5 ± 10.8	0.63	
50	46.9 ± 9.8	50.2 ± 9.9	0.84	
50	37.1 ± 6.6	26.8 ± 6.8	0.30	
Batch 3				
14	46.3 ± 9.7	79.8 ± 8.5	0.08	
30	38.2 ± 5.0	53.4 ± 8.6	0.17	
40	46.1 ± 8.2	82.2 ± 11.5	0.05	
50	58.4 ± 7.2	58.1 ± 7.0	0.97	
60	42.4 ± 8.3	61.2 ± 5.2	0.09	

decayed by *G. trabeum* was not consistent between batches and did not correspond with incubation period. In some cases, termites aggregated on control sawdust. Because termites were exposed to living fungal mycelium in these tests, fungal growth rates were not measured. However, there may have been substantial differences in growth rates of *G. trabeum* between batches. Because *G. trabeum* does not metabolize lignin, it can have a negative effect on termites by depleting the cellulose without removing the lignin. Therefore, the range of conditions under which termites would aggregate on sawdust decayed by *G. trabeum* appears to be much narrower than for *P. chrysosporium*.

Termites aggregated on sawdust decayed by *P. cinnabarinus* for 14 d in both batches, but did not show a significant response to sawdust decayed for longer incubation periods in either batch (Table 3). In choice tests of sawdust decayed by either *P. chrysosporium* or *P. cinnabarinus* for the same length of time, termites only displayed significant aggregation behavior on *P. chrysosporium* when sawdust was decayed for 120 d. Termites were distributed equally between the two containers of sawdust decayed by the two fungi when sawdust was decayed for 14 or 60 d (Table 4). These were the first tests conducted to evaluate the response of termites to *P. cinnabarinus*. Although many factors, such as fungal growth rates and wood species, affect the interaction of subterranean termites with wood rot fungi, *P. cinnabarinus* did elicit aggregation behavior in termites when sawdust was only decayed for 14 d, but not when sawdust was decayed for a longer period.

Sawdust decayed by *P. chrysosporium* elicited aggregation behavior by Formosan subterranean termites the most consistently and over the greatest range of incubation periods of the three fungus species tested. Aggregation behavior of termites on a food source could increase the efficacy of baiting programs for termite control by increasing consumption of toxic baits.

Feeding behavior on decayed blocks

In comparisons of rates of decay and feeding for the three fungi at each decay period, percent weight loss due to decay was significantly different at 30 d (H = 12.0, df = 2, P = 0.002), 60 d (H = 12.4, df = 2, P = 0.002), and 90 d (H = 19.7, df = 2, P < 0.001), but percent weight loss due to termite feeding was not significantly different at 30 d ($F_{2,23} = 1.5$, P = 0.24), 60 d ($F_{2,33} = 1.4$, P = 0.27), or 90 d ($F_{2,35} = 3.1$, P = 0.06). Percent weight loss due to

In such setting Times (dame)	Mean (±SE) Nu	P Value ¹	
Incubation Time (days)	Fungus	Control	P value
Batch 1			
14	125.5 ± 11.4	62.3 ± 9.8	0.01
60	95.3 ± 16.4	48.2 ± 10.8	0.07
120	93.5 ± 15.3	80.0 ± 13.1	0.64
Batch 2			
14	131.3 ± 12.1	30.0 ± 7.1	0.0001
30	82.0 ± 12.5	73.1 ± 9.4	0.65
120	68.2 ± 9.9	55.8 ± 8.1	0.32

Table 3. Mean $(\pm SE)$ number of termites in containers filled with red oak sawdust inoculated with *Pycnoporus cinnabarinus* and decayed for different lengths of time compared with containers filled with control sawdust after 7 d.

¹The number of termites in treated and control containers were compared using a paired choice t-test.

Table 4. Mean $(\pm SE)$ number of termites in containers filled with red oak sawdust inoculated with *Pycnoporus cinnabarinus* compared with containers filled with *Phanerochaete chrysosporium* when inoculated sawdust was decayed for different lengths of time after 7 d.

	Mean (±SE)	Number of Termites in C	ontainers
Incubation Time (days)	Pycnoporus	Phanerochaete	P Value
14	68.3 ± 12.2	76.3 ± 18.1	0.79
60	80.5 ± 6.5	95.8 ± 5.6	0.22
120	31.0 ± 8.7	145.8 ± 8.5	< 0.001

¹The number of termites in treated and control containers were compared using a paired choice t-test.

decay by *P. cinnabarinus* was significantly less than percent weight loss due to decay by the other two species at all three decay periods (Table 5).

In comparisons of rates of survival, decay and feeding over all treatments, there were no significant differences in the survival of termites in containers with different treatments (H = 15.6, df = 9, P = 0.08). There were significant differences in the percent weight loss of blocks due to fungal decay (H = 56.5, df = 8, P < 0.001) and due to feeding damage (H = 51.2, df = 9, P < 0.001) for the different treatments. Percent weight loss due to decay was significantly greater for *P. chrysosporium* and *G. trabeum* after 90 d decay compared to percent weight loss for *P. cinnabarinus* at 90 d. In addition, percent weight loss due to decay by *P. chrysosporium* was also significantly greater after 60

d than percent weight loss for *P. cinnabarinus* at 60 d. Termite feeding on decayed blocks was significantly greater than on control blocks for all three fungi after 90 d of decay (Table 5).

The effect of decay on feeding rates by termites is complex and affected by multiple factors. Although termites avoided sawdust decayed by *G. trabeum* in some aggregation tests, termite consumption of blocks decayed by *G. trabeum* was significantly greater than consumption of control blocks after 90 d of decay. Even though *G. trabeum* does not metabolize lignin, it seems to cause chemical changes to blocks that increase feeding rates by Formosan subterranean termites. The chemical modification of lignin by *G. trabeum* appears to facilitate termite feeding under at least some conditions. By removing cellulose while leaving the lignin behind, *G. trabeum* gradually makes the wood less palatable to termites as the rate of decay increases (Lenz *et al.* 2007, Cornelius *et al.* 2002).

The weight loss of blocks due to decay by *P.cinnabarinus* was much lower than the weight loss due to decay by the other two fungus species. Even though weight loss of red oak blocks due to *P. cinnabarinus* was < 3% for all three incubation periods, the fungus may have been causing chemical changes to the blocks that facilitated termite feeding. After 90 d of incubation, wood consumption on decayed blocks was 12.6% compared to 4.8% on control blocks. Other studies have also demonstrated that termite feeding rates on decayed blocks do not always correlate directly with amount of decay or length of incubation periods (Amburgey & Smythe 1977, Lenz*et al.* 1980). Although there are many factors that could influence the differences in termite behavior towards the two white rot fungi, *P. chrysosporium* and *P. cinnabarinus*, the difference in the enzymatic pathways utilized for lignin degradation may be important. Further research is needed to elucidate the factors affecting termite aggregation and feeding behavior towards wood rot fungi.

The interactions of termites with wood rot fungi range from obligate mutualism (Darlington 1994, Aanen *et al.* 2002) to antagonism (Becker 1976, Amburgey & Beal 1977, Jayasimha & Henderson 2007a, 2007b). By breaking down or chemically modifying lignin, wood rot fungi can facilitate the ability of termites to consume lignocellulose. Because of their symbiotic association with lignin-degrading fungi, fungus-growing termites are able to utilize virtually all of the lignocellulose they consume and they expel only a

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Table 5. Percent $(\pm SE)$ weight loss of red oak wood blocks decayed by three species of fungi for 30, 60, or 90 d. Percent $(\pm SE)$ weight loss of blocks due to termite feeding and percent $(\pm SE)$ termite survival in a no-choice feeding test after six weeks.

Fungus Species	Percent (±SE) Weight Loss of Blocks Due to Decay	Percent (±SE) Weight Loss of Blocks Due to Termite Feeding	Percent (±SE) Termite Survival
Undecayed control blocks		4.8 + 0.3A	78.5 + 1.5
30 d decay			
Phanerochaete chrysosporium	5.4 ± 0.4 aABC	$10.9 \pm 0.6 A$	88.7 ± 1.5
Gloeophylum trabeum	5.3 ± 0.3 aABC	9.8 ± 0.2A	86.0 ± 1.8
Pycnoporus cinnabarinus	$2.4 \pm 0.5 bA$	$10.7 \pm 0.4 \mathrm{A}$	86.9 ± 1.7
60 d decay			
Phanerochaete chrysosporium	$8.1 \pm 0.8 \mathrm{aC}$	$9.5 \pm 1.7 A$	85.3 ± 4.6
Gloeophylum trabeum	$7.9 \pm 0.5 aBC$	9.5 ± 1.1A	83.6 ± 7.7
Pycnoporus cinnabarinus	$2.9 \pm 0.7 \text{bAB}$	$11.3 \pm 0.4 A$	87.2 ± 1.3
90 d decay			
Phanerochaete chrysosporium	$11.11 \pm 3.1 aC$	$15.4 \pm 1.1B$	85.4 ± 1.7
Gloeophylum trabeum	10.3± 0.4aC	$14.5 \pm 0.7B$	87.8 ± 1.0
Pycnoporus cinnabarinus	2.6 ± 0.1bAB	$12.6 \pm 0.4B$	88.0 ± 0.9

Means followed by the same lowercase letter within an incubation period within a column were not significantly different (Dunn's test: $P \ge 0.05$). Means followed by the same uppercase letters within a column were not significantly different (Dunn's test: $P \ge 0.05$)

small volume of feces (Darlington 1994). In an analysis of feces of Formosan subterranean termites fed southern pine, *Pinus australis* F. Michx, it was determined that lignin was modified, but not degraded, and that it retained its aromatic properties (Ke *et al.* 2011). Scharf *et al.* (2011) provide evidence that termites could gain a fitness advantage by utilizing gut symbionts to overcome end-product inhibition caused by the degradation of lignin. Formosan subterranean termites may be able to utilize a greater percentage of lignocellulose from decayed wood due to the biochemical changes in the wood. Therefore, feeding on decayed wood may provide a fitness advantage by allowing the wood rot fungi to partially degrade lignin prior to consumption.

Many researchers have investigated the potential of using attractants from wood rot fungi in termite baits (Esenther & Beal 1974, Amburgey *et al.* 1981, Rust *et al.* 1996, Getty & Haverty 1998, Cornelius *et al.* 2009). However, optimal conditions for eliciting aggregation and feeding behavior by termites depends on many factors including, decay rate, wood species, and differences in fungal strains and species. Elucidation of the biochemical changes in wood due to decay by different fungus species may result in the identification of chemicals that could be used to improve baiting programs for termites.

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REFERENCES

- Aanen, D. K., P. Eggleton, C. Rouland-Lefevre, T. Guldberg-Froslev, S. Rosendahl & J. J. Boomsma. 2002 The evolution of fungus-growing termites and their mutualistic fungal symbionts. Proceedings of the National Academy of Sciences 99 (23): 14887– 14892.
- Alves, A. M. C. R., E. Record, A.K Lomascolo, A. Scholtmeijer, M Asther, J. G. H Wessels & H. A. B. Wösten. 2004. Highly efficient production of laccase by the basidiomycete *Pycnoporus cinnabarinus*. Applied and Environmental Microbiology 70 (11): 6379-6384.
- Amburgey, T. L. 1979. Review and checklist of the literature on interactions between wood-inhabiting fungi and subterranean termites: 1960-1978. Sociobiology 4 (2): 279-296.
- Amburgey, T. L. & R. H. Beal. 1977. White rot inhibits termite attack. Sociobiology 3 (1): 35-38.
- Amburgey, T. L. & R. V. Smythe. 1977. Factors influencing termite feeding on brown-rotted wood. Sociobiology 3 (1): 3-12.
- Amburgey, T. L., G. N. Johnson & J. L. Etheridge. 1981. A method to mass produce decayed-wood termite bait blocks. Journal of Georgia Entomological Society 16 (1): 106-112.
- Becker, G. 1976. Termites and fungi. Material und Organismen, Bieheft 3: 465-478.
- Breznak, J. A. & A. Brune. 1994. Role of microorganisms in the digestion of lignocellulose by termites. Annual Review of Entomology 39: 453–487.
- Brune, A. 2006. Symbiotic associations between termites and prokaryotes. pp. 439-474. *In*: M. Dworkin, S. Falkow, E. Rosenberg, K-H. Schleifer & E. Stackebrandt [eds.]. Prokaryotes, Springer, New York, NY, USA.
- Cornelius, M. L., D. J. Daigle, W. J. Connick, Jr., A. Parker & K. Wunch. 2002. Responses of *Coptotermes formosanus* and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) to three types of wood rot fungi cultured on different substrates. Journal of Economic Entomology 95 (1): 121-128.

Cornelius, M.L. *et al.* — Feeding Behavior of Termites on Decayed Wood

- Cornelius, M. L., Lyn, M., K. S. Williams, M. P. Lovisa, A. J. De Lucca, II & A. R. Lax. 2009. Efficacy of bait supplements for improving the rate of discovery of bait stations in the field by Formosan subterranean termites (Isoptera: Rhinotermitidae). Journal of Economic Entomology 102 (3): 1175-1181.
- Coy, M. R., T.Z. Salem, J. S. Denton, E. S. Kovaleva, Z. Liu, D. S. Barber, J. H. Campbell, D. C. Davis, G. W. Buchman, D. G. Boucias & M. E. Scharf. 2010. Phenol-oxidizing laccases from the termite gut. Insect Biochemistry and Molecular Biology 40: 723-732.
- Darlington, J. P. E. C. 1994. Nutrition and evolution in fungus-growing termites. pp. 105–130. *In*: J. H. Hunt & C. A. Nalepa [eds.]. Nourishment and Evolution in Insect Societies, Westview Press, Boulder, CO., USA.
- Eggert, C., U. Temp & K-E. L. Eriksson.1996. The ligninolytic system of the white rot fungus *Pycnoporus cinnabarinus*: Purification and characterization of the laccase. Applied Environmental Microbiology 62 (4): 1151–1158.
- Esenther, G. R & R. H. Beal. 1974. Attractant-mirex bait suppresses activity of *Reticulitermes* spp. Journal of Economic Entomology 67 (1): 85-88.
- Esenther, G. R., T. C. Allen, J. E. Casida & R. D. Shenefelt. 1961. Termite attractant from fungus-infected wood. Science 134 (3471): 50.
- Geib, S. M., T. R. Filley, P. G. Hatcher, K. Hoover, J. E. Carlson, M. del Mar Jimenez-Gasco, A. Nakagawa-Izumi, R. L. Sleighter & M. Tien. 2008. Lignin degradation in wood-feeding insects. Proceedings of the National Academy of Sciences 105 (35): 12932-1237.
- Getty, G. M. & M. I. Haverty. 1998. Consumption of sound and decayed Ponderosa pine and douglas-fir by *Reticulitermes* spp. (Isoptera: Rhinotermitidae) from Northern California. Journal of Economic Entomology 91 (3): 650-654.
- Green III, F. & T. L. Highley. 1997. Mechanism of brown-rot decay: Paradigm or paradox. International Biodeterioration and Biodegradation. 39:113-124.
- Jayasimha, P. & G. Henderson. 2007a. Suppression of growth of a brown rot fungus, *Gloeophyllum trabeum*, by Formosan subterranean termites (Isoptera: Rhinotermitidae). Annals of the Entomological Society of America 100 (4): 506-511.
- Jayasimha, P. & G. Henderson. 2007b. Fungi isolated from integument and guts of *Coptotermes formosanus* and their antagonistic effect on *Gloeophyllum trabeum*. Annals of the Entomological Society of America 100 (5): 703-710.
- Ke, J., D. Dhrubojyoti, D. Laskar, D. Singh & S. Chen. 2011. *In situ* lignocellulosic unlocking mechanism for carbohydrate hydrolysis in termites: crucial lignin modification. Biotechnology for Biofuel, 4 (1):17.
- Lenz, M., T. L. Amburgey, D. Zi.Rong, J. K. Mauldin, A. F. Preston, D.Rudolph & E. R. Williams. 1991. Interlaboratory studies on termite-wood decay fungi associations: II. Response of termites to *Gloeophyllum trabeum* grown on different species of wood (Isoptera: Mastotermitidae, Termopsidae, Rhinotermitidae, Termitidae). Sociobiology 18 (2): 203-254.

- Lenz, M., D. B. A. Ruyooka & C. D. Howick. 1980. The effect of brown and white rot fungi on wood consumption and survival of *Coptotermes lacteus* (Frogatt) (Isoptera: Rhinotermitidae) in a laboratory bioassay. Zeitschrift fur Angewandte Entomologie. 89: 344-362.
- Matsumura, F., H. C. Coppel & A. Tai. 1968. Isolation and identification of termite trailfollowing pheromone. Nature 219 (5157): 963-964.
- Nakashima, K., H. Watanabe & J. I. Azuma. 2002. Cellulase genes from the parabasalian symbiont *Pseudotrichonympha grassii* in the hindgut of the wood-feeding termite Coptotermes formosanus. Cellular and Molecular Life Sciences 59: 1554-1560.
- Rust, M. K., K. Haagsma & J. Nyugyen. 1996. Enhancing foraging of western subterranean termites (Isoptera: Rhinotermitidae) in arid environments. Sociobiology 28 (3):275-286.
- Scharf, M. E., Z. J. Karl, A. Sethi & D. G. Boucias. 2011. Multiple levels of synergistic collaboration in termite lignocellulose digestion. PLoS ONE 6 (7): e21709.
- Su, N.-Y. 2005. Directional change in tunneling of subterranean termites (Isoptera: Rhinotermitidae) in response to decayed wood attractants. Journal of Economic Entomology 98 (2): 471-475.
- Systat Software, Inc. 2008.SYSTAT statistical package, version 12.0. Systat Software, Inc., San Jose, CA, USA.
- Zhang, D., A. R. Lax, J. M. Bland & A. B. Allen.2011. Characterization of a new endogenous endo-b-1,4-glucanase of Formosan subterranean termite (*Coptotermes formosanus*). Insect Biochemistry and Molecular Biology 41: 211-218.

