5-11-2013

# Implications for the detection, utilization, and degradation of bark beetle-attacked southern pines by subterranean termites

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# Implications for the detection, utilization, and degradation of bark beetle-attacked southern pines by subterranean termites

By

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A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Agriculture & Life Sciences (Entomology)
in the Department of Biochemistry,
Molecular Biology, Entomology and Plant Pathology

Mississippi State, Mississippi

May 2013

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Title of Study: Implications for the detection, utilization, and degradation of bark beetle-attacked southern pines by subterranean termites

Pages in Study: 82

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Bark beetles regulate forest succession by removing weakened or stressed trees and exposing understory species to light from canopy gaps. Subterranean termites are predominate decomposers of coarse woody debris in southern pine forests; however, little is known about their role in forest health and succession. Both groups of insects rely heavily on fungal symbioses to fill their respective ecological niches in southern pine forests. During recent inspections of southern pine timber, we observed that trees in the early stages of bark beetle attack often had subterranean termites in blue-stained portions of the trunk. The frequency of subterranean termite presence in blue-stained areas of trees increased proportionally to the stage of bark beetle attack. However, practically no research has undertaken the challenge of describing how woody resources created by bark beetles are identified and utilized by subterranean termites before any signs of stress are visible. Therefore, this study examined possible facilitative interactions between subterranean termites, bark beetles and their blue-stain fungal associates, and other invertebrates, and investigated the effect of blue-stain fungi on surface properties of wood. Both native (*Reticulitermes* spp.) and Formosan subterranean termites exhibited a

higher feeding preference for blue-stained sapwood than for unstained sapwood in laboratory assays. Native subterranean termites also consumed blue-stained sapwood at a higher rate than decayed wood. This study was the first to demonstrate that wood containing a non-decay fungus could elicit a feeding response from subterranean termites greater than that observed for decayed wood. Additionally, the surface properties of bark beetle-attacked southern pine were initially reduced by blue-stain fungal infection; however, the process of kiln-drying reversed this effect, resulting in a surface that was more conducive to wood product manufacturing.

# DEDICATION

I would like to dedicate this dissertation to my wife Mary J. (Jan) Little. My academic progression was driven by her love and support and the anticipated arrival of our first child. I also dedicate this dissertation to the one who will someday stand on my shoulders, our daughter Sadie L. Little. I hope she will realize that all of her hopes and dreams are within reach. I love you girls with all my heart.

ACKNOWLEDGEMENTS

My gratitude is extended to all who helped direct me through my academic progression. I want to thank the Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology and Mississippi State University for providing me the opportunity to continue my education. I wish to express thanks to Dr. John J. Riggins, my major professor, for his guidance and assistance during the formation and completion of this research as well as the preparation of the dissertation. I would also like to show appreciation to Drs. Andrew J. Londo, M. Blake Layton Jr., Michael D. Ulyshen and Thomas G. Shelton for their direction and guidance throughout my academic development. My thanks are extended to my family for their continued support. I am particularly grateful to my wife, Jan. Her love and encouragement gave me the confidence and ability to pursue a dream I never thought possible.

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# CHAPTER I

# INTRODUCTION

## Problem

The role of bark beetles in the regulation of forest succession is well known; as the primary herbivores of southern pine forests they remove weakened or stressed trees. Conversely, little is known about a predominant decomposer of southern pine forests, subterranean termites, in relation to temperate forest ecology. Recent observations of subterranean termites in blue-stained portions of bark beetle-attacked trees indicate that previously unknown interactions may occur between these insects. However, practically no research has undertaken the challenge of describing how woody resources created by bark beetles are identified and utilized by subterranean termites.

## Symbioses

Bark beetles and subterranean termites both rely heavily on fungal symbioses to fill their respective ecological niches in southern pine forests. Most bark beetles vector one or more species of fungi, either phoretically on the exoskeleton or in specialized structures on the integument called mycangia (Six 2003, Scott et al. 2008). These fungi perform a variety of symbiotic functions for bark beetles, ranging from larval nourishment (mycangial fungi) to reducing host defense responses during initial attack (phoretic fungi) (Kendall et al. 1989, Nebeker et al. 1993, Paine et al. 1997, Klepzig et al.

2001). Subterranean termites benefit from their associations with fungi through the fragmentation of structural components of wood by decay fungi. In turn, these fungi are transported by the insects to ephemeral and otherwise inaccessible plant resources.

**Bark Beetles**

The relationships between conifer-infesting bark beetles and fungi are complex (Graham 1967, Whitney 1982, Harrington 1993). Most conifer-attacking bark beetles vector one or more species of blue-stain fungi. Blue-stain fungi can be highly phytopathogenic, while others are far less virulent (Harrington 1993, Paine et al. 1997). Six and Wingfield (2011) noted that bark beetles often vectored blue-stain fungi with virulence inversely proportional to the aggressiveness of their attacking behavior. The hyphae of blue-stain fungi penetrate deep into the sapwood by growing through pit pairs between ray cells from one wood storage cell to another (Kirk and Cowling 1984). The non-structural breakdown of pathways in the sapwood by blue-stain fungi is often followed by a succession of structurally-degrading wood-boring insects (mechanical degradation) and decay fungi (enzymatic degradation) (Hickin 1975).

**Subterranean Termites**

The literature is replete with reports of subterranean termites being attracted to woods containing various brown-rot decay fungi (reviewed by Amburgey 1979) and repelled by wood containing certain species of white-rot decay fungi (Amburgey and Beal 1977). Esenther et al. (1961) observed that subterranean termite shelter tubes on the bark of living trees inevitably led to a decaying branch. Upon further investigation, wood that was decayed by a brown-rot fungus, *Gloeophylum trabeum* Murrill, was shown to

elicit aggregation and trail-following behaviors from subterranean termites (Esenther and Beal 1979). The subsequent identification and extraction of the behavior altering compound from decayed wood led to the development of modern bait delivery systems for subterranean termites. Following this discovery, an inordinate amount of research ensued, which focused solely on subterranean termite attraction to wood containing decay fungi.

## Synthesis

Trees inoculated with blue-stain fungi are predisposed to decay by a succession of invertebrates (Boddy and Rayner 1983, Franklin et al. 1987). Additionally, the decay cycle is likely hastened by the deposition of excess nitrogen-rich excrement in woody debris by subterranean termites (Hickin 1975). During recent inspections of southern pine timber, we observed that trees in the early stages of bark beetle attack often had subterranean termites in blue-stained areas of the trunk. Moreover, trees in the latter stage of bark beetle attack and foliage chlorosis almost always had subterranean termites present in blue-stained areas of the trunk. Researchers in different regions of the U.S. have also reported that other families of termites are often present in bark beetle-attacked trees. Our observations indicate that subterranean termites are able to detect woody resources created by bark beetles during the early stages of attack. Therefore, it is plausible that one or more water soluble chemical(s) are produced by non-decay (blue-stain) fungi, which may be similar in function to one produced by the decay fungus, *G. trabeum*, and that these compounds are detectable by subterranean termites foraging in the soil.

Although multi-trophic associations may exist between bark beetles and subterranean termites, practically no research has considered the possibility that blue-stain fungi facilitate these interactions. Therefore, in the following chapters (II & III) we investigate whether different genera of subterranean termites feed preferentially on blue-stained wood over unstained wood. Additionally, we compared subterranean termite feeding preferences for wood containing non-structurally degrading fungi (blue-stain) to those elicited by the decay fungus *G. trabeum* in a field setting (chapter IV). The possibility that wood containing a non-decay fungus could elicit a greater feeding preference from subterranean termites than that observed for decayed wood remains entirely unexplored.

**Blue-Stain Fungi and Wood Properties**

Blue-stain fungi do not impart any losses in strength to wood (Schirp et al. 2003; Valiev et al. 2009); however, the resultant stain lowers the economic value of the wood. This decrease in solid-sawn wood product value is solely due to the lack of aesthetic appeal to consumers. Additionally, the accelerated degradation of bark beetle-attacked timber by subterranean termites and other invertebrate decomposers limits the window of opportunity for the utility of blue-stained wood. However, some of the value lost in blue-stained wood may be recoverable through the manufacture of wood composite products, which can be engineered to overcome a number of limitations. In chapter V, we investigate the alterations in the surface of wood caused by the non-structural breakdown of pathways by blue-stained wood. By altering the surface of wood, blue-stain fungi may increase the surface area, improving its utility as furnish for wood composite products.

## Objectives

With the knowledge gained from field observations and subsequent examinations of prior literature, several objectives were formed: 1) determine if native subterranean termites (*Reticulitermes* spp.) exhibit a feeding preference for blue-stained wood, 2) determine if this preference exists for a non-native genera of subterranean termites, 3) examine the extent of these feeding preferences relative to other fungi that are known to elicit these behaviors, and 4) examine the effect of blue-stain fungi on the surface properties of wood. If actualized, these objectives have the potential to give insight into previously unknown multi-trophic interactions and improve the utility of bark beetle-attacked timber. Therefore, the ensuing chapters examine possible facilitative interactions between subterranean termites, bark beetles and their blue-stain fungal associates, and other invertebrates, and investigate the effect of blue-stain fungi on the surface properties of wood.

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# CHAPTER II

# FEEDING PREFERENCE OF NATIVE SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE: *RETICULITERMES*) FOR WOOD CONTAINING BARK BEETLE PHEROMONES AND BLUE-STAIN FUNGI

*Previously published in Journal of Insect Behavior (2012) 25:197-206. The final publication is available at www.springerlink.com.*

**Abstract**

Surprisingly little research has been conducted to investigate interactions between subterranean termites and bark beetles. Facilitative interactions between these organisms could easily alter stand dynamics and impact wood utilization strategies. A series of American Wood Protection Association Standard E1-09 "choice tests" were carried out to determine the feeding preference of *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitidae) for blue-stained sapwood and sapwood impregnated with various bark beetle pheromones. *Reticulitermes flavipes* exhibited a feeding preference for both air-dried and kiln-dried blue-stained sapwood, unstained sapwood treated with frontalin, and air-dried blue-stained sapwood treated with a 0.02% solution of both frontalin and *endo*-brevicomin. The implications of these results are far reaching, with particular relevance to forest health, ecology, and utilization.

## Introduction

Traditionally, subterranean termites (Isoptera: Rhinotermitidae: *Reticulitermes*) have been considered to be important in forest succession and nutrient cycles in the southeastern U.S. nearly exclusively from a decomposition standpoint. Little is known about the role of subterranean termites in relation to forest health and succession. In particular, no investigations of the role of subterranean termites during the early stages of tree death are present in the literature. The possibility that subterranean termites interact with bark beetles, for instance, remains largely unexplored despite the economic and ecological importance of both organisms.

Although usually considered pests of pine plantations in the southeastern U.S., members of the southern pine bark beetle guild and their associated microorganisms play a beneficial role in regulating forest succession (Thatcher et al. 1980). While the five scolytine species, *Dendroctonus frontalis* Zimmermann (southern pine beetle), *Dendroctonus terabrans* Oliver, *Ips calligraphus* Germar, *Ips grandicollis* Eichhoff, *Ips avulsus* Eichhoff, are historically considered to be the most economically important, other species, such as *Hylastes* spp. (Coleoptera: Curculionidae) cause significant damage to the root structure of pine trees (Klepzig et al. 1991).

One of the most destructive pest of pine trees in the southeastern U.S. is *D. frontalis* (Londo 2010). The attacking behavior of *D. frontalis* is mediated by both host volatiles and beetle-produced pheromones (Smith et al. 1993). After penetrating the bark of a tree, the female beetle releases aggregation pheromone components, frontalin and *trans*-verbenol, which combine with small amounts of *endo*-brevicomin produced by the male (Sullivan et al. 2007). Frontalin, *trans*-verbenol, and *endo*-brevicomin combine with

terpenes from the host tree to become a signal for mass attack (Sullivan et al. 2007). Once the defense mechanisms of the host tree are overwhelmed, the male pheromone component concentrations of *endo*-brevicomin and verbenone increase and begin to inhibit aggregation response.

Once *D. frontalis* reaches the inner bark and overcomes the oleoresin defense mechanism of the affected tree (Nebeker et al. 1993), they inoculate at least three fungi, *Ophiostoma minus* (Hedgc.) H. and P. Sydow, *Ceratocystiopsis ranaculosus* Perry and Bridges, and *Entomocorticium* sp. A, onto the host (Klepzig et al. 2001). Both *O. minus* and *C. ranaculosus* are carried on the exoskeleton of *D. frontalis* or phoretically through symbiotic mites (Klepzig et al. 2001). *Entomocorticium* sp. A is transported in a pouch-like structure, the mycangium, located in the prothorax of the beetle (Happ et al. 1971; Klepzig et al. 2001). *Ophiostoma minus* may assist adult *D. frontalis* in overcoming host defenses (Nelson 1934; Caird 1935; Bramble and Holst 1940; Mathre 1964; Basham 1970); however, it eventually reduces developmental success of the beetle (Barras 1970; Franklin 1970).

Blockages of water-conducting passages in the sapwood are caused through internal wounding by *O. minus*, which results in resin formation (Tisdale et al. 2003). This resin formation is part of the host defense mechanism that impedes fungal lesion size (Tisdale et al. 2003). Hyphae of *O. minus* are initially confined to the radial parenchyma tissue of the sapwood, but eventually enter the ray parenchyma and propagate (Whitney 1971; Ballard et al. 1983). The hyphae subsequently penetrate the tracheids and maneuver from cell to cell via pit pairs (Ballard et al. 1983) making the wood more permeable. Researchers have observed that stained portions of the sapwood

of colonized trees were generally drier than unstained portions and assumed that water conduction was impaired in the stained portions of the tree (Nelson 1934; Bramble and Holst 1940), a characteristic which could benefit subterranean termite colonization.

Levi and Dietrich (1976) reported that native subterranean termites (*Reticulitermes* spp.) rarely attack the wood of living trees. Once a tree has been killed via successful *D. frontalis* attack, both the specific gravity and moisture content of the wood are reduced (Humar et al. 2008). Barron (1971) reported that both fungal fruiting bodies and termites appeared on some *D. frontalis*-killed trees, both standing and felled, near the end of the third month and caused measurable strength losses after five months. Recent observations indicate that subterranean termites are commonly found infesting blue-stained portions of living trees during early stages of tree death.

The objective of this research was to investigate possible facilitative interactions between a native subterranean termite (*Reticulitermes flavipes* Kollar) and *D. frontalis*. We hypothesized that the feeding behavior of *R. flavipes* would be affected by the presence or absence of blue-stain and *D. frontalis* pheromone components. In a series of choice assays, we compared the feeding response of *R. flavipes* to 1) blue-stained sapwood from both a *D. frontalis*-attacked *Pinus taeda* L. tree and a kiln-dried *P. taeda* board with an unknown blue-stain source, 2) the main components of both male and female *D. frontalis* pheromones, frontalin and *endo*-brevicomin, and 3) combinations of *D. frontalis*-inoculated blue-stained *P. taeda* sapwood and the two major male and female pheromones components listed above.

## Materials and Methods

*Dendroctonus frontalis*-attacked blue-stained *P. taeda* sapwood was obtained from Talladega National Forest in central Alabama, while all other wood samples were obtained from a local lumber supplier. *Pinus taeda* wafers, 2.54 x 2.54 x 0.635cm (tangential x radial x longitudal), were cut from 1) an untreated sapwood lumber control, 2) a kiln-dried board containing blue-stain from an unknown source, and 3) a *D. frontalis*-killed tree of comparable density. The $SG_{12}$ (specific gravity at 12% moisture content) of wood wafers differed between treatments. However, wood wafers within a specific treatment and their respective controls were matched to within ± 0.01 $SG_{12}$. *Dendroctonus frontalis* pheromone components, *endo*-brevicomin and frontalin, were obtained from Synergy Semiochemicals (Burnaby, BC, Canada) and dissolved in acetone for treatment.

The first round of treatments was employed to determine the response of *R. flavipes* to conditions likely encountered in a *D. frontalis*-attacked tree. These treatments included: 1) the male *D. frontalis* aggregation pheromone, *endo*-brevicomin, 2) the major component of the female *D. frontalis* aggregation pheromone, frontalin, 3) blue-stained sapwood from a *D. frontalis*-killed tree, 4) blue-stained kiln-dried sapwood, and 5) all possible combinations of treatments one through three listed above. Wafers were treated with a 0.02% solution of each respective pheromone with a vacuum/atmospheric pressure method. As specified in American Wood Protection Association (AWPA) Standard E1-09, each treatment combination contained five replicates. Controls were treated with the solvent only. Retentions for each compound were calculated based on initial and final weight of each wafer and the treating solution concentration. After treatment, the wafers

were placed in a fume hood for 2 days to evaporate the solvent. Samples were subsequently weighed and employed in AWPA Standard E1-09 (Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites) laboratory choice test (AWPA 2009).

The AWPA sets industrial standards applicable worldwide for evaluation of wood products subjected to biological attack. AWPA Standard E1-09 can be used as a no-choice test (primarily used to determine chemical efficacy) or a choice test (used to determine feeding deterrence of subterranean termites). Conversely, this study used AWPA Standard E1-09 choice test as a preference test.

AWPA laboratory Standard E1-09 specifies using a single colony of subterranean termites to determine resistance/repellency of wood treated with a preservative to subterranean termite attack. Based on the results of the primary experiment, certain treatments were selected for further testing with additional colonies of subterranean termites. These treatments included 1) air-dried blue-stained wood and 2) kiln-dried blue-stained wood. In addition to testing these select treatments against multiple colonies of *R. flavipes*, ten replicates were conducted for each treatment.

A standard AWPA E1-09 choice test consists of a 100 x 100mm sterilized glass jar filled with 150g of screened and sterilized sand. Twenty five ml of deionized water are added to the sand, and allowed to stand for 2 hours. Two wood wafers are placed on opposite sides of the jar (one treatment and one control), and 400 termites from the same colony are placed in the center of the jar. Soldier termites are then added to each jar to represent the original percentage in the colony, but not to exceed ten percent (AWPA 2009). The lid is placed loosely on the glass bottles which are subsequently placed in an

environmental chamber at 25°C and 55% relative humidity for 28 days. Multiple colonies of *R. flavipes* were obtained from the Dorman Lake area located near Starkville, MS. Immediately following the 28 day test, samples were air-dried for 14 days to determine mass loss due to *R. flavipes*. Samples were subsequently photographed and retained for future reference.

**Analysis**

Mass losses for each treatment and its respective controls were compared using analysis of variance (ANOVA) with JMP 8 (1989-2008). Wafers within each treatment and control group were randomly selected. Each choice test was analyzed at an $\alpha=0.05$ significance level.

## Results

Percent treatment solution for each compound, average retentions and average percent mass losses are given in Table 2.1.

Table 2.1 Results from choice tests with *R. flavipes*

| Treatment | % Solution | Avg. Retention, kg/m³ | Avg % Mass Loss | | |
|---|---|---|---|---|---|
| | | | Colony 1 | Colony 2 | Colony 3 |
| *Endo*-brevicomin | 0.02 | 0.079 | 33.8 | | |
| Control | 0.0 | 0.00 | 30.4 | | |
| Frontalin | 0.02 | 0.078 | 41.6* | | |
| Control | 0 | 0.00 | 30.6* | | |
| *Endo*+Frontalin | 0.02 + 0.02 | 0.166 | 32.1 | | |
| Control | 0 | 0.00 | 34.8 | | |
| AD Blue Stain | 0 | | 32.0* | 39.0* | 58.3* |
| Control | 0 | | 24.4* | 7.8* | 8.9* |
| AD Blue Stain+*Endo* | 0.02 | 0.095 | 27.9 | | |
| Control | 0 | 0 | 23.3 | | |
| AD Blue Stain+Frontalin | 0.02 | 0.095 | 33.1 | | |
| Control | 0 | 0.00 | 27.0 | | |
| AD Blue Stain+*Endo*+*Frontalin* | 0.02 + 0.02 | 0.192 | 30.9* | | |
| Control | 0 | 0 | 21.7* | | |
| KD Blue Stain | 0 | | 18.2* | 25.6* | 35.4* |
| Control | 0 | | 3.8* | 18.3* | 18.6* |

[1]Average percent treatment solution for each compound, retentions, and mass losses from AWPA Standard E-1 choice termite tests with *R. flavipes*.
[2]Average percent mass loss indicates the amount of wood consumed from wafers during the experiment.
[3]The SG12 (specific gravity at 12% moisture content) varied between tests, but wafers within a specific treatment and their respective controls were matched to within +- 0.01 $SG_{12}$.
[4]Values for colony 1 reflect the average of five replicate wafers, values for colonies 2 and 3 reflect the average of ten replicates.
[5]Asterisks denote significant differences between values.
[6]Pairwise comparisons noted significantly different at $P < 0.05$.
[7]AD = air-dried; KD = kiln-dried

**Blue-Stained Wood**

Blue-stained wafers yielded significantly higher mass losses to termites than unstained kiln-dried control wafers of comparable density ($F_{1, 8}$=31.7, P=0.0005). A significant feeding preference was shown by *R. flavipes* for air-dried blue-stained sapwood over unstained control wafers of similar density ($F_{1, 8}$=7.8, P=0.0317).

Continued testing using two additional colonies of *R. flavipes* yielded similar results. The two additional colonies of *R. flavipes* yielded significantly higher mass losses for both air-dried blue-stained sapwood over unstained air-dried controls ($F_{1, 18}$=238.1, P<0.0001 and $F_{1, 18}$=594.3, P<0.0001) and kiln-dried blue-stained sapwood over unstained kiln-dried controls ($F_{1, 18}$=8.9, P=0.0080 and $F_{1, 18}$=51.3, P<0.0001).

**Wood Treated with SPB Pheromone Components**

Wafers treated with the 0.02% solution of frontalin, the main component of the female *D. frontalis* pheromone complex, yielded a significant feeding preference by *R. flavipes* over their respective untreated controls ($F_{1, 8}$=13.4, P=0.0064). No significant feeding preference was shown *R. flavipes* for wafers treated with the 0.02% solution of *endo*-brevicomin, the major component of the male *D. frontalis* pheromone, over untreated control wafers.

**SPB Blue Stain/Pheromone Combinations**

Wood blue-stained by *D. frontalis* treated with frontalin and *endo*-brevicomin produced varying results. A significant feeding preference was shown by *R. flavipes* for air-dried sapwood blue-stained by *D. frontalis* treated to 0.02% with both frontalin and *endo*-brevicomin over unstained air-dried control wafers ($F_{1, 8}$=12.9, P=0.0071). There

was a positive feeding response to blue-stained wood treated with *endo*-brevicomin (+4.6% average mass loss over control wafers) and frontalin (+6.1% average mass loss over control wafers) alone; however, the results were not significant at $\alpha$=0.05.

## Discussion

### Implications for Forest Health and Ecology

Both *D. frontalis* and *R. flavipes* are native to coniferous forests in the southeastern U.S. Bark beetles and subterranean termites play distinct, although complimentary roles in forest ecosystem function. The most destructive forest insect pest of southern pine forests, *D. frontalis*, accounts for $1.5 billion in damage on more than 1 million acres in the southeastern U.S. from 1999 through 2003 (Londo 2010). Bark beetle outbreaks initiate a series of events which regulate forest succession and nutrient cycling. Schowalter (1981) indicated that bark beetle outbreaks are an ecosystem's regulatory response to an imbalance in forest succession. During normal population periods, bark beetle attack is limited to stressed or damaged conifers (Coulson 1979). After a bark beetle outbreak, trees with suppressed/unresponsive defense mechanisms may become more suitable for colonization by subterranean termites.

Early breakdown of bark beetle-killed trees can considerably influence organic matter content and nutrient dynamics in the forest floor (Levia and Frost 2006). Premature colonization by subterranean termites accelerates humic acid formation and subsequent nutrient availability in the affected areas. Subterranean termites excrete partially degraded lignin as a byproduct (Garnier-Sillam et al. 1992), which is still relatively recalcitrant and carbon rich. Although wood is low in nitrogen, an increase in humus formation due to subterranean termite feeding would boost water holding capacity

and increase nitrogen-fixing bacteria in the soil. Since hardwoods and softwoods utilize nutrients in different ways (Waring and Franklin 1979), accelerated degradation of bark beetle-attacked trees by subterranean termites could significantly affect an increase in C:N ratios, and, therefore, are likely to influence forest succession.

Total N input on an ecosystem scale for *Reticulitermes* spp. has yet to be quantified (Pandey et al. 1992). Yamada et al. (2006) reported that both *Microcerotermes crassus* and *Globitermes sulphureus,* tropical wood feeding subterranean termites native to Thailand, contribute between 7% and 22% of total N input to the local forest ecosystem. The total N input from soil and fungus feeding termites in the same area was negligible, however (Yamada et al. 2006). Although C:N ratios of wood may differ between tropical and temperate forests, Curtis and Waller (1997) found no correlation between nitrogen fixation rates of *Reticulitermes* spp. and N content in their food source. Native subterranean termites, *Reticulitermes* spp., nurture a symbiotic relationship with hind-gut fauna that fix atmospheric N, and could contribute a significant proportion of total N input to temperate forests. Since xylem is relatively low in N, deposition of N rich subterranean termite fecal matter in downed branches, stems, etc. may be an important sole source of N for some decay organisms, and therefore important during initiation of coarse woody debris decay (Yamada et al. 2006).

Despite their vital role in forest ecosystems, little is known about the interactions between *D. frontalis* and *R. flavipes*. The impact of these unknown interactions on forest ecology, nutrient cycling, and succession could be vitally important to forest ecosystems in the Southeastern U.S. Subterranean termites are attracted to certain wood decay fungi (Cornelius et al. 2002, and references therein), and are generally known to communicate

with each other through a complex of chemicals (pheromones) and vibrations (Inta et al. 2009). Since subterranean termites are attracted to some wood decay fungi, we hypothesized that they may also respond to wood inoculated with blue-stain fungi.

The results of this study indicate a feeding preference of *R. flavipes* for air-dried and kiln-dried blue-stained SYP sapwood, kiln-dried unstained sapwood treated with 0.02% frontalin, and air-dried blue-stained sapwood treated with frontalin and *endo*-brevicomin (0.02%, respectively). Worker termite preference for blue-stain sapwood and non-blue-stained sapwood treated with the *D. frontalis* pheromone component (frontalin) strongly suggests that termites may have evolved behaviors that allow them to locate and rapidly colonize bark beetle-attacked trees. Subterranean termites may prefer blue-stained sapwood over unstained sapwood due to residual water soluble chemical(s) in blue-stained sapwood derived from *D. frontalis* or its fungal hosts, or a chemical change in the host tree. Although blue-stain fungi do not impart any significant strength loss to the wood (Humar et al. 2008), they may make the tree more susceptible to subsequent attack and fungal decay due to increased wood permeability.

The strongest (most highly significant) feeding preference exhibited by worker termites for a treatment over a control during this feeding choice experiment was for air-dried blue-stained sapwood treated with frontalin and *endo*-brevicomin (0.02%, respectively). We believe this is because air-dried wafers containing blue-stain fungi and treated with two major components of the *D. frontalis* pheromone complex most closely resemble a tree heavily infested with bark beetles, since blue-stain fungi take time to grow through the tree after inoculation and the presence of *endo*-brevicomin and frontalin indicate both male and female beetles have attacked the tree. Although subterranean

termites are random foragers and opportunistic feeders (Su and Bardunias 2005; Su et al. 1984), they will recruit other colony members using trail marking pheromones to preferred food sources once they are located (Reinhard and Kaib 2000), a point where random foraging ceases. Feeding preference for wood affected by bark beetles and their associated fungi may allow termite colonies to quickly locate and utilize trees during early stages of death. Early colonization of major food sources such as bark beetle-killed trees could increase colony proliferation, and indicates a potential co-evolutionary link between native subterranean termites and bark beetles such as *D. frontalis*.

Although the results indicate a feeding preference of *R. flavipes* for frontalin in clear SYP sapwood and blue-stained sapwood treated with frontalin and *endo*-brevicomin, we wish to emphasize that pheromones are volatile and, during the 28 day test, may have spread throughout the control wafer and the entire test jar. If this were the case, one might expect negative results from this laboratory test even though pheromones might well attract termites in the field. Additionally, the results presented herein are relevant only to worker termites. The response of the winged reproductive alate caste of *R. flavipes* to bark beetle pheromones and blue-stain wood is still unknown, but it could be an important influence affecting termite colony dispersion in relation to bark beetle infestations.

In this study, we focused only on native subterranean termites. However, the non-native Formosan subterranean termite (*Coptotermes formosanus* Shiraki) is now widely established in portions of the native range of *D. frontalis* and is known to infest the heartwood of living hardwood and coniferous trees as well as coarse woody debris (La Fage 1987; McMichael 1998). By inhabiting living trees, *C. formosanus* could make

them more susceptible to *D. frontalis* by reducing tree vigor. It is unknown whether *C. formosanus* compete with native *Reticulitermes* spp. for foraging territory. Adams et al. (2005) reported that *C. formosanus* also nurture a symbiotic relationship with nitrogen fixing bacteria; however, the extent to which they fix N is unknown. If shown to offset *Reticulitermes* spp., *C. formosanus* could alter nutrient dynamics and forest succession in infested areas throughout the southeastern U.S.

**Commercial and Industrial Implications**

The potential for rapid and early termite infestation of bark beetle-killed timber holds important implications for the wood products industry. Commercial outlets for blue-stained timber exist, but only before termite feeding and strength loss occur. Timely utilization of bark beetle-killed timber is critical for maintaining wood quality and merchantability in both solid-sawn and composite products. Termite feeding can cause significant strength loss and render severely affected timber unusable for solid-sawn wood products. Blue-stained wood has been effectively used in wood composite products such as hardboard and oriented strand board (OSB) (Kelly et al. 1982), often yielding superior properties as long as timber is harvested and processed before termite damage is too extensive.

The results we present in this study indicate that both blue-stain fungi and bark beetle pheromones can potentially attract subterranean termites to wood products. However, southern pine products are kiln-dried at temperatures from 100 to 120˚C (210 to 250˚F) for about 24 hours, which would likely volatilize any *D. frontalis* pheromones, making bark beetle pheromones an unlikely termite attractant in consumer products.

**Future Work**

This study is the first to investigate possible avenues by which subterranean termites may be attracted to bark beetle-killed trees. The results herein indicate that *R. flavipes* prefer sapwood treated with certain *D. frontalis* pheromone components, blue-stained sapwood, and combinations of blue-stained sapwood treated with certain pheromone components over untreated sapwood. Additional studies are investigating the response of the invasive Formosan subterranean termite to treatments outlined in this study. More research is needed to determine the magnitude of each pheromone component needed for subterranean termite attraction, and to investigate the mechanism(s) by which subterranean termites are attracted to wood containing blue-stain fungi.

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# CHAPTER III

# PREFERENCE OF FORMOSAN SUBTERRANEAN TERMITES FOR BLUE-STAINED SOUTHERN YELLOW PINE SAPWOOD

*Previously published in Journal of Economic Entomology (2012) 105(5):1600-1644. The final publication is available at www.bioone.org.*

**Abstract**

Little research has been conducted to investigate interactions between the invasive Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and pine bark beetles native to the southeastern U.S. Facilitative interactions between these organisms could alter stand dynamics and impact wood utilization strategies. American Wood Protection Association Standard E1-09 choice tests were carried out to determine the feeding preference of Formosan subterranean termites for blue-stained versus unstained southern yellow pine sapwood. Three separate colonies of Formosan subterranean termites consumed on average twice as much air-dried blue-stained southern yellow pine sapwood over unstained air-dried controls. Additionally, Formosan subterranean termites consumed over five-times more kiln-dried blue-stained sapwood than unstained kiln-dried control wafers. The implications of these results are particularly relevant to pine forest ecology, nutrient cycling, and the utilization of blue-stained southern pine building

products in the southeastern U.S., where Formosan subterranean termites have become established.

## Introduction

Since its introduction during the 1960s, the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, has inflicted millions of dollars of damage to wooden structures in the United States (Su and Scheffrahn 1990; Su and Tamashiro 1987). It is known to be established in at least nine states in the gulf coastal plain region in the southern U.S. (Cabrera et al. 2005) and California (Atkinson et al. 1993; Haagsma et al. 1995). Although these insects have been intensively studied by wood scientists due to their destructive capability on wooden structures, little regard has been given to their effect on local forest ecosystems.

Respectively, bark beetles and termites are the primary insect herbivores and decomposers in most forest ecosystems, and therefore strongly influence nutrient cycles and forest succession. However, relatively little is known about termite ecology in relation to bark beetle-killed trees. It is well known that subterranean termite activity late in the tree decomposition cycle is strongly facilitated by a preference for wood infected with various white- and brown-rot decay fungi (Cornelius et al. 2002, and references therein). However, our knowledge of termite activity during the early stages of tree decomposition is very limited.

Older, less vigorous pine forests of the southeastern U.S. are often affected by bark beetles. When these trees are attacked by bark beetles, blue-stain fungi rather than decay fungi are most likely to mediate use of wood by subterranean termites. Our previous research has shown a connection between native subterranean termites and blue-

stained wood (Little et al. 2012), suggesting that subterranean termites may have the ability to rapidly detect and exploit resources created by bark beetle attacks. Our study indicates this preference for blue-stained wood exists for at least one species of native subterranean termite in North America; however it remains unknown if other species of subterranean termites throughout the world exhibit a similar preference for wood inoculated with blue-stain fungi.

Like North American subterranean termites, Formosan subterranean termites co-occur with several bark beetles species in the genera *Dendroctonus* and *Ips* (*D. armandi* Tsai and Li, *I. acuminatus* Gyllenhal, and *I. sexdentatus* Boerner, among others) in their native range in southern China (Gay 1969; Wood and Bright 1992). As with North American bark beetles, each species is associated with various ophiostomatoid fungi. Therefore, Formosan subterranean termites may exhibit a feeding preference for blue-stained sapwood similar to that of subterranean termites endemic to the southeastern U.S.

Formosan subterranean termites are believed to have been introduced into the U.S. through ports in the South by ships returning with wooden cargo from Japan after World War II (Austin et al. 2006). Since its introduction into the southeastern U.S., Formosan subterranean termites have spread principally via transportation of used railroad ties and firewood (Jenkins et al. 2002), and now occur within the native ranges of the five species that make up the southern pine bark beetle guild and their associated ophiostomatoid fungal symbionts (Su and Scheffrahn 1990; Wood and Bright 1992; Sun et al. 2007).

Formosan subterranean termites are well known pests of wooden structures (Hardy 1988; Henderson 2001; Woodson et al. 2001; Messenger et al. 2002; Su 2003)

and living trees (Harris 1966a; Harris 1966b; La Fage 1987; McMichael 1998), and also serve a vital role in many forest ecosystems (Harris 1966a; Lax and Osbrink 2003). Economic losses of standing timber due to *Coptotermes* species infestations have been reported across multiple continents and tree species (Nakajima and Shimizu 1959; Grieves et al. 1965; Williams 1965; Harris 1966a; Harris 1966b). Although their ecological and economic importance is well known, Formosan subterranean termite presence has yet to be quantified in forested areas of the United States (Howard et al. 1982).

A recent alate survey by Sun et al. (2007) indicated that Formosan subterranean termite populations were higher in forested areas than in urban settings throughout infested counties in Mississippi. Unlike native species, Formosan subterranean termites have a proclivity for infesting the heartwood of living trees (La Fage 1987; McMichael 1998). Sun et al. (2007) also suggested that forested areas could provide better habitats than urban areas. In particular, a lack of termiticide applications, stable ambient temperature and humidity, and ample food sources may dramatically assist colony establishment and spread in forested settings (Sun et al. 2007).

The addition of Formosan subterranean termites to local forest ecosystems may alter nutrient cycling and stand dynamics, due to several biological and behavioral differences from native subterranean termites. The ability of Formosan subterranean termites to infest living trees and create aerial nests using carton material (Osbrink et al. 1999; Henderson and Forschler 1996; Su and Scheffrahn 1990; Haagsma et al. 1995) may alter historical distribution and resource utilization patterns typically exhibited by native subterranean termites. Once established, Formosan subterranean termite colonies utilize a

greater percentage of a woody resource before initiating extensive foraging expansions (Delaplane and La Fage 1989), while native subterranean termites expend more resources foraging for secondary feeding sites (Puche and Su 2001). Additionally, Formosan subterranean termite colony size can easily exceed ten times that of native subterranean termites (Su and Tamashiro 1987). Compared to native subterranean termites, these differences in Formosan subterranean termite biology and behavior likely alter the rate in which N is made available to other decomposers in localized forested areas.

The objective of this research was to investigate if Formosan subterranean termites exhibit a feeding preference for blue-stained sapwood. The presence of non-native Formosan subterranean termites in the southeastern U.S. offers the ability to investigate whether feeding preference for blue-stained wood exists in subterranean termites not endemic to North America. Wood containing blue-stain fungi was offered to Formosan subterranean termites in laboratory tests. We hypothesized that the feeding behavior of Formosan subterranean termites would be affected by the presence or absence of blue-stain fungi in sapwood wafers.

## Materials and Methods

Bark beetle-attacked blue-stained loblolly pine (*Pinus taeda* L.) and an unstained control tree of comparable density were obtained from Talladega National Forest in central Alabama, while all other samples were obtained from a local lumber supplier. Wafers, 2.54 x 2.54 x 0.635cm (tangential x radial x longitudal), were cut from 1) an unstained kiln-dried sapwood lumber control, 2) a kiln-dried (120$^{o}$C dry bulb temperature) board containing blue-stain from an unknown source, 3) an unstained tree, and 4) a southern pine beetle-killed tree of comparable density. The $SG_{12}$ (specific

gravity at 12% moisture content) of wood wafers differed between treatments. Therefore, wood wafers within a specific treatment and their respective controls were matched to within ± 0.01 $SG_{12}$. The comparisons were as follows: 1) air-dried blue-stained sapwood from a bark beetle-attacked southern yellow pine tree versus air-dried unstained sapwood controls, 2) kiln-dried blue-stained sapwood versus kiln-dried unstained sapwood controls, and 3) air-dried blue-stained sapwood versus kiln-dried blue-stained sapwood.

The American Wood Protection Association (AWPA) sets international standards for evaluation of wood products subjected to biological attack. AWPA Standard E1-09 can be used as a no-choice test (primarily used to determine chemical efficacy) or a choice test (used to determine feeding deterrence or preference of subterranean termites) (AWPA 2009). The AWPA Standard E1-09 choice test was used as a feeding preference choice assay between various configurations of blue-stained southern yellow pine sapwood wafers and unstained control wafers. As specified in AWPA Standard E1-09, each treatment combination contained five replicate wafers.

AWPA Standard E1-09 specifies using a single colony of subterranean termites to determine resistance/repellency of wood treated with various compounds to subterranean termite attack. However, to corroborate the findings from the initial termite colony, certain treatments were selected for further verification using two additional colonies of Formosan subterranean termites. These treatments included air-dried and kiln-dried blue-stained sapwood wafers with unstained sapwood wafers as controls.

A 100 x 100mm sterilized glass jar was filled with 150g of screened and sterilized sand. Twenty five ml of deionized water was added to the sand, and allowed to stand for 2 hours. Two wood wafers are placed on opposite sides of the jar (one treated and one

control), and 400 termites from the same colony were placed in the center of the jar. Soldier termites were then added to each jar until they comprised ten percent of the total number contained within each test jar (AWPA 2009). The lids were placed loosely on the glass bottles which were subsequently placed in an environmental chamber at 25°C and 55% relative humidity for 28 days, with five replicate jars per treatment. Colonies of Formosan subterranean termites were obtained immediately prior to testing from infested logs in a wooded area located near the Mississippi State University Agricultural and Forestry Experiment Station (MAFES) at McNeill, MS. Testing was conducted at the MAFES Formosan Termite Research Facility. Following the 28 day test, samples were air dried for 14 days to determine mass loss due to Formosan subterranean termites. Samples were subsequently photographed and retained for future reference. Mass losses for each treatment and its respective controls were compared using a series of one-way analysis of variance (ANOVA) tests with JMP 8 (SAS 2009). Significant difference within each choice test was judged at $\alpha \leq 0.05$.

## Results

Average percent mass losses for each treatment and its respective control are given in Table 3.1.

Table 3.1 Results from choice tests with *C. formosanus*

| | Avg % Mass Loss | | |
|---|---|---|---|
| Treatment | Colony 1 | Colony 2 | Colony 3 |
| AD Blue Stain | *24.6±3.7 | *23.3±2.4 | *26.8±1.7 |
| Control | *6.9±3.7 | *14.4±2.4 | *15.7±1.7 |
| KD Blue Stain | *22.1±1 | *26.7±1.8 | *27.3±1.1 |
| Control | *6.1±1 | *2.8±1.8 | *5.5±1.1 |
| AD Blue Stain | *18.4±1.1 | | |
| KD Blue Stain | *12.2±1.1 | | |

[1]Average percent mass losses from AWPA Standard E-1 choice termite tests with *C. formosanus*.
[2]Average percent mass loss indicates the amount of wood consumed from wafers during the experiment.
[3]The SG12 (specific gravity at 12% moisture content) varied between tests, but wafers within a specific treatment and their respective controls were within +- 0.01 $SG_{12}$.
[4]Values reflect the average of five replicate wafers.
[5]Asterisks denote significantly greater mass losses for treated wafers compared to untreated control wafers ($P < 0.05$).
[6]AD = air-dried; KD = kiln-dried with dry bulb temperature of about 120°C

Blue-stained wafers from kiln-dried southern yellow pine sapwood had significantly higher mass losses to Formosan subterranean termites than unstained kiln-dried control wafers of comparable $SG_{12}$ ($F = 127.5$; df = 1, 8; $P < 0.0001$). Formosan subterranean termites also had a significant feeding preference for air-dried blue-stained sapwood over unstained control wafers of similar $SG_{12}$ ($F = 142.4$; df = 1, 8; $P < 0.0001$). In addition, Formosan subterranean termites exhibited a significant feeding preference for

air-dried blue-stained sapwood over kiln-dried blue-stained sapwood ($F = 21.3$; $df = 1, 8$; $P = 0.0017$).

Further testing was conducted with two additional Formosan subterranean termite colonies to minimize the possibility of a feeding preference for blue-stained wood only occurring in a single termite colony. Termites from these two additional colonies exhibited significantly greater feeding preference for air-dried blue-stained sapwood over unstained air-dried control wafers ($F = 6.6$; $df = 1, 8$; $P = 0.0329$ and $F = 20.4$; $df = 1, 8$; $P = 0.0019$) and kiln-dried blue-stained sapwood over unstained kiln-dried control wafers ($F = 84.4$; $df = 1, 8$; $P < 0.0001$ and $F = 181.9$; $df = 1, 8$; $P < 0.0001$), supporting results obtained with the first colony.

## Discussion

This study investigated the feeding behavior of Formosan subterranean termites with regard to the presence or absence of blue-stain fungi in sapwood wafers. The feeding response of Formosan subterranean termites was positively affected by the presence of blue-stain fungi in sapwood wafers, regardless of drying method. Blue-stained air-dried sapwood was preferred over blue-stained kiln-dried sapwood, which may be due in part to a semi-volatile compound produced by the fungi that was partially removed during the kiln-drying process.

Blue-stain fungi are intrinsically linked to pine tree mortality caused by bark beetles, and are initially the primary fungi present during and after tree death. Once dead, bark beetle-killed trees undergo measurable strength loss in less than six months due to wood borers and decay fungi (Barron 1971). Although blue-stain fungi are known to utilize various non-structural components of wood, such as sugars, lipids, proteins, and

other extractives (Abraham et al. 1993, 1998; Brush et al. 1994; Breuil and Huang 1994; Breuil et al. 1995; Gao and Breuil 1995; Abraham and Breuil 1996; Gao and Breuil 1998), they are not known to produce any extracellular cellulases, and are therefore not capable of degrading structural components of wood (Schirp et al. 2003; Valiev et al. 2009).

It is well established that chemicals produced by decay fungi during the decomposition of structural components of wood elicit feeding preferences and, in some instances, trail following behavior from subterranean termites (Cornelius et al. 2002, and references therein; Esenther and Beal 1979). Cornelius et al. (2002) suggested that any fungal species capable of partially degrading cellulose, hemi-cellulose, or lignin could affect termite feeding and possibly elicit trail following behavior. To our knowledge, Little et al. (2012) presents the first evidence that chemicals produced during interactions between non-decay fungi and non-structural components of wood can elicit a feeding attraction from subterranean termites. We are currently investigating how chemicals produced during metabolism of wood extractives by blue-stain fungi affect subterranean termite trail following behavior.

Termites are highly selective feeders (Evans et al. 2005). Subterranean termites are known to feed discriminately based on many factors, of which, wood species, hardness, and size are considered to be essential (Smyth and Carter 1970). It is common for termite species that coexist in the same habitat to target different sizes of wood sources to reduce competition (Evans et al. 2005). Bark beetles and their blue-stain fungal associates can provide large volumes of a highly palatable resource for subterranean termites in this respect. Blue-stain fungi metabolize certain biologically

active, non-structural extractive wood components that may interfere with termite digestion, rendering the affected wood more palatable to subterranean termites, earlier in the succession cycle.

Decay fungi and other invertebrate decomposers limit the length of time that wood is suitable for subterranean termite utilization. Eventually, wood moisture and cellulose content become suboptimal and only recalcitrant compounds remain. The ability to recognize and utilize a large diameter food source soon after attack by bark beetles could drastically increase the period of wood availability to subterranean termites and reduce competition.

## Conclusions

This study investigated potential feeding preferences of Formosan subterranean termites for bark beetle-killed pine trees in the southeastern U.S. The results indicate that Formosan subterranean termites significantly prefer blue-stained sapwood over unstained sapwood, regardless of drying method. However, among blue-stained wafers, Formosan subterranean termites had a significant preference for air-dried sapwood over kiln-dried sapwood. We are continuing our studies on the interactions between subterranean termites, bark beetles, and blue-stained wood, as well as investigating the possible mechanism(s) driving subterranean termite preference for blue-stained sapwood.

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# CHAPTER IV

# FIELD EVALUATIONS OF SUBTERRANEAN TERMITE PREFERENCE FOR SAP-STAIN INOCULATED WOOD

*Previously published in Journal of Insect Behavior DOI: 10.1007/s10905-013-9380-x. The final publication is available at www.springerlink.com.*

**Abstract**

Few studies have focused on interactions between subterranean termites and the ophiostomatoid fungal associates of pine bark beetles or root feeding weevils. Field stake tests were employed at four locations throughout Mississippi to determine the feeding preference of subterranean termites for blue-stained, unstained, and partially decayed southern pine sapwood stakes. This study also utilized wood decayed by *Gloeophyllum trabeum*, a fungus previously shown to elicit a positive subterranean termite feeding response, as a positive control. Stakes inoculated with *G. trabeum* received significantly more attacks than all other treatments after sixteen weeks. Of the stakes attacked by subterranean termites, stakes inoculated with *Ophiostoma minus* were degraded faster than any other treatment. Subterranean termite preference for stakes treated with either of two *Leptographium* spp. and the untreated negative controls did not differ; however, each was fed upon less than all other treatments. The probability of stakes inoculated with *O. ips* and *G. trabeum* being fed upon by subterranean termites did not differ. These results

represent the first evidence of wood containing non-structurally degrading fungi (*O. ips* and *O. minus*) eliciting a feeding preference from subterranean termites greater than that of decayed wood. The implications of these results are particularly relevant to pine forest ecology, nutrient cycling, subterranean termite control, and the utilization of blue-stained southern pine building products in the southeastern U.S.

## Introduction

Subterranean termites and decay fungi are the predominate woody decomposers in most forest ecosystems. Although these organisms appear to provide a similar service to ecosystems, intricate interspecific, mutualistic, commensal, and parasitic relationships occur, which increase the efficiency of decomposition and nutrient cycles. Insight into previously unknown interspecific symbiotic relationships may alter our perception of the processes involved in tree death and early stages of wood degradation.

Subterranean termites are well documented as associates of decay fungi (e.g. Hendee 1934; Sands 1969; Becker 1976; Amburgey 1979; Zoberi and Grace 1990). Wood inhabited by some brown-rot decay fungi has been shown to elicit trail following behaviors and feeding preferences from subterranean termites (Esenther et al. 1961; Esenther and Beal 1979; Grace and Wilcox 1988; Rust et al.1996). Conversely, wood containing various white-rot decay fungi are sometimes avoided (Amburgey and Beal 1977), but *Reticulitermes* spp. are known to feed directly on basidiocarps of various wood decay fungi (Waller et al. 1987).

No positive or negative feeding response with subterranean termites was found in downed woody debris infested with non-decaying stain or mold fungi in four forest habitats in Mississippi (Kirker et al. 2012). However, other studies (Little et al. 2012a,b)

showed that wood inhabited by blue-stain fungi (*Ophiostoma* spp.), which are not known to degrade any structural components of wood (Schirp et al. 2003; Valiev et al. 2009), yielded a significant positive feeding response from both Eastern (*R. flavipes* Kollar) and Formosan subterranean termites (*Coptotermes formosanus* Shiraki) in laboratory assays. The mechanism(s) behind subterranean termite feeding preference for wood inhabited by various fungi is still unclear; however, it may involve a biologically active water soluble fungal metabolite(s) or a fungal-modified extractive.

Our previous laboratory findings on subterranean termite feeding preference for southern yellow pine sapwood containing *Ophiostoma* species (Little et al. 2012a,b) inspired new research regarding subterranean termite interactions with *Leptographium* species, a genus closely related to *Ophiostoma*, which is also non-wood degrading. Many *Ophiostoma* species are vectored by above-ground bark beetles that attack southern pines (Table 4.1). Likewise, many species of *Leptographium* are vectored by below-ground feeding beetles (Table 4.1) and at least one species of above-ground bark beetle, the black turpentine beetle (*Dendroctonus terebrans* Oliver). Some species of *Leptographium* are prevalent in roots and root collars of pine trees, often for a long time before above-ground non-structurally degrading fungi associated with various pine bark beetles are present. The affiliation of *Leptographium* species with below- and certain above-ground attacking beetles, some of which also carry *Ophiostoma* species, makes it plausible that other ophiostomatoid species may elicit a positive feeding response from subterranean termites similar to that observed in previous studies (Little et al. 2012a,b).

Table 4.1 Select vectors and hosts of fungi

| Fungus | Select Vectors | Preferred Host(s) | Preferred Portion of Host | References |
|---|---|---|---|---|
| *O. minus* | *D. frontalis; D. terebrans* | *Pinus taeda* and *Pinus echinata* | Lower-bole; Mid-bole | Rumbold 1931; Wagerner and Mielke 1961; Payne 1980; Paine et al. 1981; Drooze 1985; Paine et al. 1997 |
| *O. ips* | *I. calligraphus*; *I. grandicollis*; *I. avulsus*; *D. terebrans* | *P. taeda* | Lower-bole; Mid-bole; Upper-bole | Paine et al. 1981; Rane and Tattar 1987; Paine et al. 1997; Harrington and Cobb 1988 |
| *L. terebrantis* | *D. terebrans; Ips* spp.; *Hylastes* spp. | *Pinus* spp. | Root-collar; Lower-bole; Mid-bole; Upper-bole | Wagerner and Mielke 1961; Goheen 1976; Wood 1982; Rane and Tattar 1987; Harrington and Cobb 1988 |
| *L. procerum* | *D. terebrans*; *Ips* spp.; *Hylastes* spp. | *Pinus* spp. | Root-collar; Lower-bole; Mid-bole; Upper-bole | Wagerner and Mielke 1961; Goheen 1976; Wood 1982; Rane and Tattar 1987; Harrington and Cobb 1988 |

[1]Select vectors, their preferred hosts, and portions of host(s) for blue-stain fungi used in this study.

The objectives of this study were to determine if results similar to Little et al. (2012a,b) could be observed with another closely related fungal genus, and to corroborate findings from earlier laboratory studies (Little et al. 2012a,b) in a field setting. This study employed AWPA Standard E7 field tests with unstained, stained, and partially decayed southern pine sapwood stakes with a wood decay fungus which has previously been shown to elicit a positive subterranean termite feeding response (Esenther et al. 1961; Esenther and Beal 1979; Grace and Wilcox 1988; Rust et al.1996).

## Materials and Methods

Wooden stakes were prepared from defect-free green southern yellow pine sapwood lumber obtained from a local sawmill. Two hundred forty field stake samples, 1.9 x 1.9 x 45.7 cm (r x t x l), were sawn from green lumber, and placed into autoclave bags in groups of 20 stakes. The stakes were autoclaved for two consecutive 45 minute cycles to ensure phytosanitization of the wood. The sealed autoclave bags were then transferred to a biological safety hood.

Previously identified cultures of *Leptographium terebrantis* Barras and Perry and *L. procerum* (W.B. Kendr.) M.J. Wingf. were secured from a laboratory at Auburn University, AL. A culture of *Ophiostoma ips* (Rumb.) Nannf., a sap-stain fungal associate of local *Ips* species, was obtained from a tree infested primarily by *I. calligraphus* (Germar). Mycelia (0.05 g) from pure cultures were extracted for DNA using the Nucleospin Plant II kit protocol for fungi (Macherey-Nagel, Düren, Germany). DNA fragments were amplified by PCR using ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS4R (TCCTCCGCTTATTGATATGC) (Gardes and Bruns 1993). PCR protocols included a 4 min hot start at 94 °C, followed by 39 cycles of 94 °C for 35 s, 55 °C for 55

s, and 72 °C for 1 min, ending with a 72 °C extension for 10 min. Fragment amplifications were verified on a 2% agarose gel. Fragment DNA was cleaned using the Nucleospin Extract II kit following the protocol for direct purification of PCR products (Macherey-Nagel, Düren, Germany). DNA fragment concentrations were determined by a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Fragment DNA samples were prepared for sequencing following the Beckman Coulter dye terminator cycle sequencing protocol using the sample appropriate forward or reverse primer (Beckman Coulter, Fullerton, CA). Both forward and reverse fragments were sequenced for each sample using a Beckman Coulter CEQ 8000 capillary sequencer. All sequence data were checked for quality and the forward and reverse sequences for each sample were aligned using LaserGene MegAlign software. The consensus sequence for each sample was submitted to a National Center for Biotechnology Information (NCBI) GenBank Blast search for match identifications and validated visually using morphological characters. Local cultures of *O. minus* (Hedgc.) Syd. & P. Syd. a sap-stain fungal associate of the southern pine beetle, and *Gloeophyllum trabeum* (Pers.) Murrill, a brown-rot decay fungus known to elicit feeding preference and trail following behavior from subterranean termites (Esenther et al. 1961; Esenther and Beal 1979; Grace and Wilcox 1988; Rust et al.1996), were obtained from laboratories at Mississippi State University. All fungi used in this study, with the exception of *O. ips*, were grown from cultures previously deposited in the American Type Culture Collection (ATCC). The fungal isolate identified during this study, *O. ips*, will be deposited with ATCC.

All five species of fungi were cultured individually on malt extract agar plates. Ten plugs from one petri dish, which contained hyphae from a single fungus on malt

extract agar, were used as starting growth stock in three separate 500 ml flasks containing 200 ml of liquid malt extract media. Mycelia were allowed to grow at 28˚C on an incubated orbiting table for seven days to achieve peak growth. The content from each of the three flasks was filtered under a biological safety hood using a vacuum funnel and 125 mm filter to separate the mycelia from the growth media. The mycelia were scraped from the filter with a sterile spatula, separated from the agar plugs, and weighed under the biological safety hood. One gram of mycelia was placed into a sterilized laboratory blender with 100 ml of deionized (DI) water. The blender was operated for twelve seconds to fully macerate and evenly distribute the fungal mycelia within the sterile water, creating a fungal slurry. A paint brush was used to apply the fungal slurry to field stakes contained within sealable tubs that were lined with sterilized cheese cloth material, moistened with sterile DI water, and vented with cotton plugs. One hundred ml of the inoculation slurry was used to treat each of the two tubs that contained twenty field stakes to achieve a total of 40 field stakes per treatment. Untreated control stakes were subjected to the same methodology; however they were treated with 100 ml of sterile DI water instead of the fungal slurry.

Ten field stake replications for each of the four locations were inoculated with six different fungal treatments; 1) *O. minus*, 2) *O. ips*, 3) *L. terebrantis*, 4) *L. procerum*, 5) *G. trabeum*, and 6) untreated controls. With the exception of the decay fungus *G. trabeum*, which had to be monitored carefully due to rapid strength loss caused by brown-rot decay fungi, the tubs were placed in an incubator at 28˚C until the fungal hyphae had visually stained the entire cross section of the stakes. The fungal matt of *G. trabeum* had to be monitored to allow for some holo-cellulose degradation but where some strength of the

stakes remained. The stakes were subsequently tagged and installed in four separate forest locations throughout MS: one site in Harrison Experimental Forest near Saucier, MS, two different sites near McNeill, MS, and one site in the Dorman Lake area, near Starkville, MS.

Field stakes were arranged in a grid pattern, with ten rows of six stakes for each location. There was 6.1 m between each row and 2.4 m between stakes within a row. Each stake was installed in the soil to ½ of its total depth. Each row of six stakes within a location contained one replicate from each treatment, which yielded a total of ten treatment replications per location. The within-row position of each treatment replicate was randomly assigned. Stakes were installed on April 22, 2011 and rated every four weeks for subterranean termite degradation, ending December 2, 2011. The study was terminated after eight inspections to prevent wood decay fungi from becoming established in the stakes and, perhaps, thus affecting the results. Stakes were visually rated using American Wood Protection Association (AWPA) Standard E7-09 termite rating scheme of 10 to 0, beginning with 10 (sound), 9.5 (trace, surface nibbles), 9 (slight attack with no more than 3% of cross-sectional area affected), 8 (moderate attack, 3-10% of cross-sectional area affected), 7 (moderate/severe attack and penetration, 10-30% of cross-sectional area affected), 6 (severe attack, 30-50% of cross-sectional area affected), 4 (very severe attack, 50-75% of cross-sectional area affected), and ending with 0 (failure) (AWPA 2009).

**Statistical Analyses**

Initial analyses were performed using Pearson's $\chi^2$ Test in the SAS program PROC FREQ (SAS Institute 2009) to determine percent of stakes attacked by

subterranean termites (ratings of 9.5 or lower) on all stakes throughout the 32 week test ($P \leq 0.05$). Additional analyses were then conducted on wood degradation due to subterranean termite feeding using the SAS program PROC GLMMIX (SAS 2009) for increasing subterranean termite degradation of wood stakes over time for each treatment. The degradation analyses were made on a modified AWPA Standard E7-09 index, with scores ranging from 10 to 3 instead of 10 to 0. Although the AWPA E7-09 index was initially utilized, it resulted in a serious skew to the lower values. Changing the 0 (failure) score to 3 removed much of the skew, which better approximated the desired Gaussian distribution for the response variable. Stakes that were not degraded by subterranean termites throughout the entire sampling period were omitted from the wood degradation analyses; however, some stakes of all treatments received attacks at each location. Mean stake ratings for each treatment were calculated so that each location was utilized as one replicate. The data were analyzed with location and row within a location as random effects. Treatment significance was determined at $\alpha \leq 0.05$.

## Results

Beginning at 20 weeks after installation, stakes treated with the positive control fungus *G. trabeum* had significantly more subterranean termite attacks than all other treatments ($\chi^2 = 14.96$; df = 5; P = 0.01) (Figure 4.1). This trend continued throughout the remainder of the 32 week test. The mean infestation rate of the stakes of the remaining five treatments was 31.8%. Subterranean termite attacks on the five remaining treatments did not significantly differ from each other at any point of inspection ($\chi^2 \leq 8.61$; df = 4; P $\geq 0.07$).

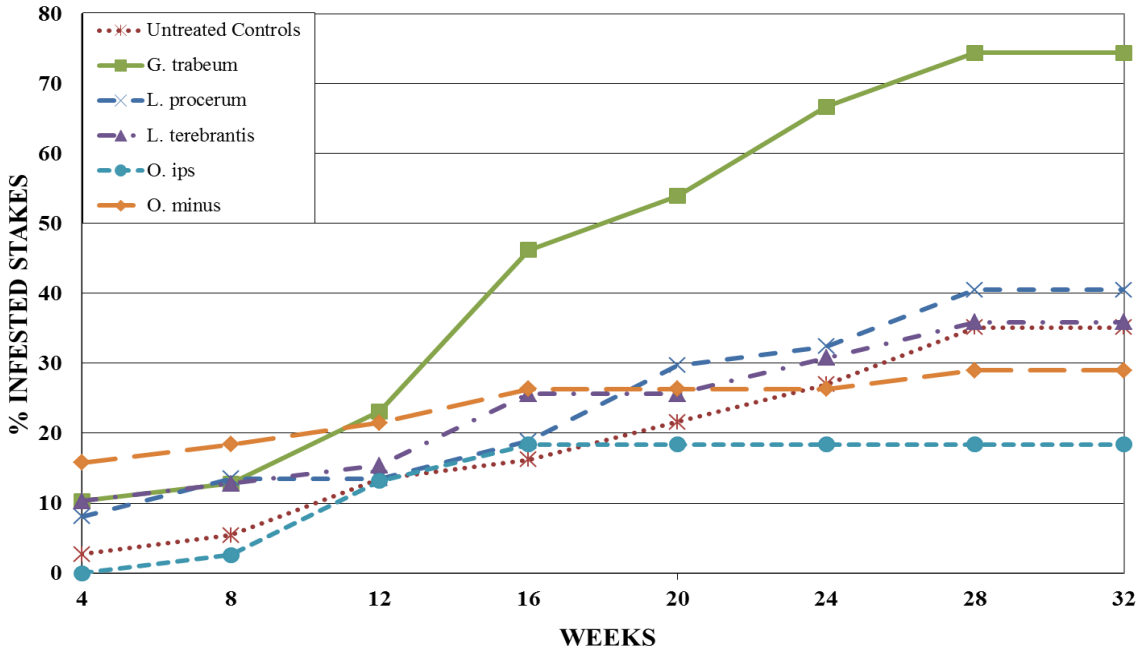


Figure 4.1 Percent of stakes with subterranean termite damage (9.5 rating or lower) over the entire test for each fungal inoculation treatment.

Diagnostic testing was performed for quadratic and linear responses of wood feeding over time and their interactions with the treatments. This testing showed a significant interaction between treatment and time. Individual regressions were fit for each treatment and the quadratic term was not significant for any treatment. The data were then tested to determine which of the linear regressions were significantly different from others. The original six regressions were reduced to three significantly different regressions: *O. minus* ($F = 64.17$; $df = 1, 22$; $P < 0.0001$), *O. ips* and *G. trabeum* ($F = 101.96$; $df = 1, 62$; $P < 0.0001$), and *L. procerum, L. terebrantis*, and the untreated controls ($F = 38.16$; $df = 1, 94$; $P < 0.0001$) (Figure 4.2).

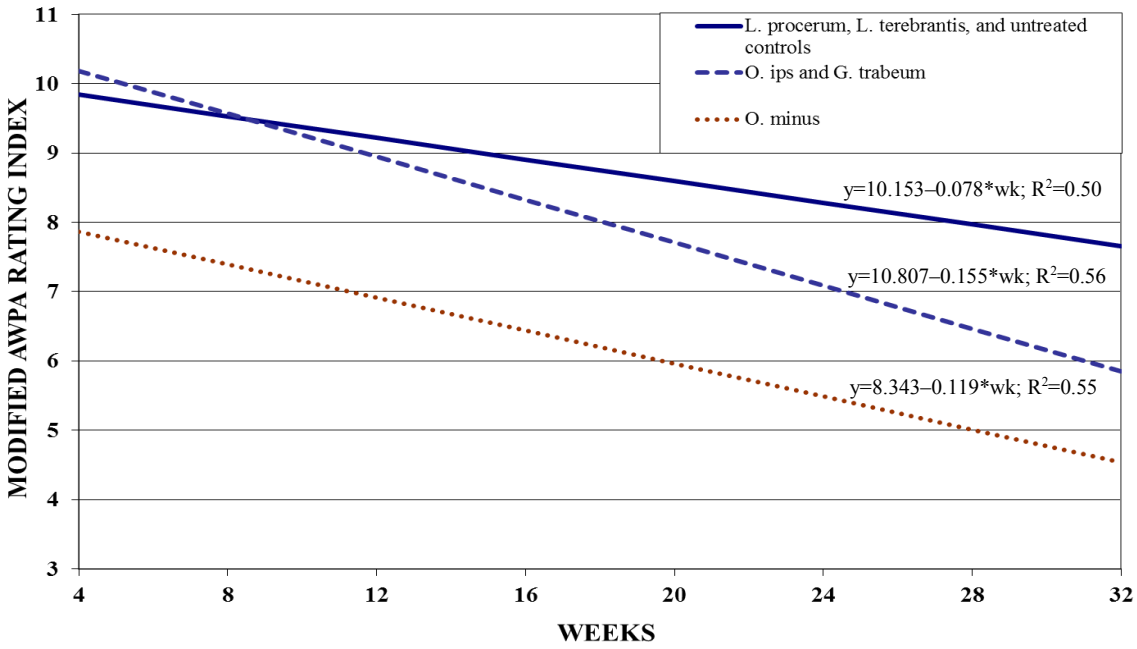


Figure 4.2 Reduced regressions of modified AWPA rating means fit to linear models for fungal inoculation treatments.

Stakes inoculated with *O. minus* had consistently greater degradation [lower ratings] due to subterranean termites than any other treatment. Subterranean termite feeding damage on stakes treated with either of the two *Leptographium* spp. and the untreated controls did not differ; however, each experienced less feeding damage than all other treatments throughout the study. Although the feeding damage of stakes inoculated with *O. ips* or *G. trabeum* did not significantly differ from each other, they had greater degradation than stakes treated with either of the two *Leptographium* spp. or the untreated control stakes.

## Discussion

Stakes inoculated with the positive control treatment *G. trabeum*, a fungus known to elicit a significant feeding attraction from subterranean termites, received more attacks

than all other treatments. However, stakes inoculated with *O. minus* were degraded consistently faster than all other treatments. The rate of degradation due to subterranean termites on stakes inoculated with the treatment *O. ips* was not significantly different than that observed on the positive control treatment *G. trabeum*. Stakes inoculated with *O. ips* and *G. trabeum* were both degraded faster by subterranean termites than stakes inoculated with either of the two *Leptographium* spp. and the untreated controls. The results from this study are indicative of the production of a compound(s) at the wood/fungal interface by non-decay fungi (*Ophiostoma* spp.).

A compound that mimics the trail following behavior of Eastern and Formosan subterranean termites, (Z,Z,E)-3, 6, 8-dodecatrien-1-ol, has been isolated and identified from wood decayed by *G. trabeum* (Smythe et al.1967; Matsumura et al.1968; Matsumura et al.1969; Matsumura et al.1976), which was used as the positive control treatment in this study. Subterranean termite responses to wood inhabited by some decay fungi vary between fungal strains, wood species inhabited, and decay rate (Amburgey and Smyth 1977; Lenz et al. 1980; Lenz et al. 1991). However, only positive responses/orientations have been reported in the literature for wood inhabited by *G. trabeum*. Fungal extracts from wood decayed by other species of brown-rot fungi are reported to elicit trail-following activity from Formosan subterranean termites (Matsuo and Nishimoto 1974); however, these responses were not due to the compound (Z,Z,E)-3, 6, 8-dodecatrien-1-ol (Ohmura et al.1995). It is unknown whether ophiostomatoid fungi produce (Z,Z,E)-3, 6, 8-dodecatrien-1-ol or an additional compound(s) that mimics a subterranean termite trail following pheromone. However, the high rate of subterranean termite feeding on stakes inoculated with *O. minus* in this study and laboratory feeding

preferences observed by Little et al. (2012a,b) indicate that a compound(s) may be produced by stain fungi.

Many sap-stain fungi produce masses of spores at the tops of long stalks, which are adapted for dispersal by different species of bark beetles or their phoretic arthropod symbionts. Once a beetle and its symbiotic fungus have penetrated the inner bark of a host tree, oleoresin is produced and compartmentalization within the tree occurs. Sap-stain fungi metabolize simple sugars, lipids, proteins, and other non-structural compounds in wood (Abraham et al. 1993; Breuil and Huang 1994; Brush et al. 1994; Breuil et al. 1995; Gao and Breuil 1995; Abraham and Breuil 1996; Abraham et al. 1998; Gao and Breuil 1998). During infection, sap-stain fungi degrade defensive barriers within the tree (Whitney 1971; Ballard et al. 1983; Tisdale et al. 2003), lowering its defensive capabilities against other organisms.

Subterranean termite bait enhancements derived from decayed wood have been largely unsuccessful. A bait matrix using decay fungus-infected sawdust, bagasse dust, potato dextrose agar, and mirex was developed for control of Formosan subterranean termites in China (Gao 1985); however, it was developed before the mechanism(s) of attraction was fully understood. Cornelius et al. (2002) demonstrated that extent of wood decay was inversely related to Formosan subterranean termite feeding preference, while Eastern subterranean termites showed no difference in response over time to decayed wood. Although Formosan subterranean termite preference for decayed wood wanes over time (Cornelius et al. 2002), it is unknown whether the degradation rate of blue-stained wood effects subterranean termite preference. A better understanding of the

mechanism(s) of subterranean termite preference for wood-inhabiting fungi is needed to optimize the efficacy of any bait matrix produced from fungal metabolites.

The results presented herein affirm findings reported by Little et al. (2012a,b) from similar studies conducted on wood wafers in a laboratory setting. Furthermore, results for this study represent the first instance of subterranean termite feeding preference for an ophiostomatoid fungus in a field setting. Further research is needed for the following: 1) determine if a water-soluble compound(s) is produced by ophiostomatoid fungi, 2) investigate the ecological interactions between ophiostomatoid fungi, their vectors, and subterranean termites in roots or stems of living or recently dead pines, and 3) investigate the prevalence of this fungally-mediated behavior in subterranean termites native to other regions of the world.

## Conclusions

Subterranean termite preference for wood inoculated with ophiostomatoid fungal associates of bark beetles and root feeding weevils common in the southeastern U.S. was investigated in four forested settings in Mississippi. These results for subterranean termite feeding on wood infected by ophiostomatoid fungi are similar to those reported by Little et al. (2012a,b). However, results for this study using *Ophiostoma* spp. were obtained outside of a laboratory setting. After locating a stake, subterranean termites fed more aggressively on wood inoculated with the blue-stain fungus *O. minus* over all other treatments, including wood inoculated with the decay fungus *G. trabeum*. This study corroborated findings from previous laboratory assays by Little et al. (2012a,b). Additionally, this study represents the first evidence of wood containing a non-structurally degrading ophiostomatoid fungus eliciting a feeding response from

subterranean termites greater than observed for decaying wood. The implications of these results are particularly relevant to pine forest ecology, nutrient cycling, subterranean termite control, and the utilization of blue-stained southern pine building products in the southeastern U.S

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# CHAPTER V

# SURFACE FREE ENERGY OF BLUE-STAINED SOUTHERN PINE SAPWOOD FROM BARK BEETLE-ATTACKED TREES

*Previously published in Wood and Fiber Science (2013) 45(2): 1-9. The final publication is available at www.swst.org.*

**Abstract**

Blue-stained wood cut from bark beetle-attacked southern pines has a lower economic value than unstained wood. Wood composite products containing blue-stained wood may offer an opportunity to recover some lost timber value. This study investigated the surface free energy of blue-stained wood. Southern pine sapwood samples with and without blue-stain from both green and kiln-dried sources were obtained. Dynamic contact angle analyses were performed using three probe liquids- ethylene glycol, formamide, and deionized water. Surface free energy was determined by applying the geometric mean model using two-liquid pairs with deionized water. The polar forces were higher across all wood types and in water-ethylene glycol versus water-formamide. The surface free energy of air-dried blue-stained sapwood was lower than all other wood types. However, kiln-dried blue-stain sapwood had a higher surface free energy than all other wood types. These results were indicative of a tree's wound response to bark beetle-attack, the volatilization of naturally occurring hydrocarbons in southern pine

sapwood, and the resulting increase in wood permeability due to blue-stain fungal colonization across the sapwood. However, the improvements in wetting observed for kiln-dried blue-stained sapwood may lead to cost and quality issues in wood composite manufacturing associated with over-drying and over-penetration of an adhesive.

## Introduction

Bark beetles, such as the southern pine beetle (SPB), *Dendroctonus frontalis* Zimmermann, have historically been the major insect threat to the economically important southern yellow pines (*Pinus* spp.) in the southeastern United States (Cook and Hain 1987). Forest landowners and managers most recently dealt with a SPB outbreak during the late 1990's, and the regional economic impact was estimated to be over $1B (Nowak et al. 2008). Southern yellow pine in the southeastern U.S. comprises nearly half of the total softwood lumber supply produced nationally (Haygreen and Bowyer 1996). Therefore, impacts of SPB outbreaks have the ability to disrupt residential and commercial construction markets on a national scale.

The SPB initially overcomes the oleoresin defenses in pines through mass-attacks and inoculation of a variety of fungi onto the host (Nebeker et al. 1993). One constituent of this fungal complex, a blue-stain fungus (*Ophiostoma minus* (Hedgc.) Syd. & P. Syd.), is the most prolific in the early stages of host colonization. Moisture losses in bark beetle-attacked southern pine trees can approach 52% in within one month of visible foliage chlorosis, and specific gravity (SG) reductions of 16% can occur within 6 months (Barron 1979). Solid-sawn lumber has been produced from timely salvaging of SPB-attacked trees, albeit with some losses in lumber recovery (Sinclair and Ifju 1979). Reductions in grade associated with SPB-attacked timber are not due to the presence of blue-stain fungi,

which do not deteriorate wood (Schirp et al. 2003; Valiev et al. 2009). Rather, these grade reductions are usually related to untimely salvage and utilization strategies that result in strength-reduction via the subsequent activities of wood-destroying borers and their associated decay fungi (Barron 1979).

Wood decay fungal fruiting bodies have been identified in SPB-attacked timber as early as the end of the third month following tree death (Barron 1979). The southern pine grading rules restrict the presence of decay in structural lumber since loadings potentially would not meet design specifications. Blue-stain alone is permitted within the southern pine grading rules for structural light framing, joists, and planks (Sinclair and Ifju 1979). However, decay in the early stages can be difficult to detect during visual inspection in the presence of heavy staining (Sinclair et al. 1979). Toughness, the mechanical property most sensitive to the presence of blue-stain fungi, can significantly decrease in as little as two months (Sinclair et al. 1979). The value of bark beetle-attacked trees to lumber manufacturers therefore diminishes greatly over a short period of time (Levi and Dietrich 1976).

A portion of the reduced value of blue-stained southern pine timber may be recoverable through manufacturing wood composite products, which can be engineered to overcome a number of wood quality limitations. The recent massive outbreaks of Mountain Pine Beetle (MPB), *Dendroctonus ponderosae* Hopkins, in the western United States and Canada have led to numerous studies regarding the suitability of blue-stained lodgepole pine, *Pinus contorta* Douglas ex Louden, for use in wood composite products. Utilization of blue-stain wood in composite products is highly dependent on an understanding of the resultant alterations in wood permeability and diffusion rates.

Increases in wood permeability and diffusion rates are commonly reported with blue-stained portions of bark beetle-attacked trees, which can significantly affect usability (Cai and Olivera 2008). Blue-stained wood from MPB-attacked lodgepole pine is suitable for the manufacture of cement-bonded particle board and wood-plastic composites (Chang and Lam 2009; Chang and Lam 2010; Chang et al. 2010). Additionally, increases in stiffness and strength properties have been observed in the manufacture of veneer and plywood (Wang and Dai 2008; Wang et al. 2008).

Wood composite products manufactured from SPB-attacked timber have received limited attention, especially in recent decades. Kelly et al. (1982) investigated the effect of SPB-attacked southern pine wood mixed with wood from healthy trees for production of particleboard. Significant increases were reported in modulus of rupture, internal bond, and screw withdrawal when SPB-attacked wood was utilized at a 25% or 100% mixture compared to control/unstained wood. In addition, significant decreases in 2 and 24 hour thickness swell and water absorption were reported at both furnish levels. These changes were found using wood from trees that had been dead for 3 and 27 months. No proposed explanations, such as increased permeability, better resin penetration, or higher compaction ratio, were suggested for the observed benefits. Additionally, no research has quantified the surface property changes of wood subjected to SPB fungal associates, such as *O. minus*.

The wettability of materials can be determined by dynamic contact angle analysis (DCA) and the subsequent calculation of surface free energy (SFE). The DCA measures the downward force of a wood sample hanging perpendicular to the liquid's surface. This accounts for the entire surface area of a sample by taking into consideration the variable

topography of the wood's surface on a microscopic scale (Son and Gardner 2004). Using liquids with known surface properties to measure the DCA allows the SFE of the wood surface to be determined by use of one of several models (Gardner et al. 2000). The geometric mean, a combination of Young's equation (1805), Girifalco's and Good's geometric mean law (1957), and Fowkes' equation (1962), describes the sum of the physical and chemical molecular interactions occurring between the liquid and wood surface. The physical, or nonpolar, interactions are explained by dispersion forces while the chemical, or polar, interactions are expressed as polar forces (Gardner 1996; Wålinder and Gardner 2000).

An adhesive must adequately wet the wood surface by penetrating into the micropore structures of the wood cell wall to achieve an optimum wood-adhesive bond (Bryant 1968). This intimate contact is critical for adhesive bonding strength and wood composite durability. The wetting of wood occurs through the wicking of liquids by capillary forces (Wålinder and Johansson 2001). The greater the movement of a liquid into voids and along rays and channels, the more wetting will occur.

Sixty percent of U.S. forest products manufacturing occurs in the South. This production depends on wood from southern pines (Prestemon and Abt 2002). A better understanding of the work of adhesion at the resin-wood interface may offer new economic outlets for southern pine timber affected by bark beetle attack, particularly those not suitable for solid-sawn lumber. The objective of this work was to examine the potential effects of blue-stain fungal attack on the surface properties of the affected southern pine sapwood. The SFEs of air- and kiln-dried wood with and without blue-stain were calculated using DCA analysis.

## Materials and Methods

Ten wafers of southern pine sapwood measuring 25.0 mm x 25.0 mm x 7.0 mm (t x r x l) along with ten miniature beams measuring 5.0 mm x 15.0 mm x 150.0 mm (t x r x l) were randomly selected from those cut from each of the following wood sources: 1) three unstained green boards (25.0 mm x 200.0 mm x 2.4 m), 2) three unstained kiln-dried boards (25.0 mm x 200.0 mm x 2.4 m), 3) three blue-stained kiln-dried boards (25.0 mm x 200.0 mm x 2.4 m), and 4) three 1.2 m long bolts (with comparable rings per inch) from the butt logs of three SPB-attacked trees in Talladega National Forest in central Alabama. The lumber (excluding the three bolts sawn from SPB trees) was obtained from a mill in Choctaw County, Mississippi. Green boards were removed from the sawmill production line in 10 minute intervals to ensure that they were milled from separate trees. The kiln-dried boards were obtained from different stacks at the mill, which were dried using a conventional southern pine drying schedule (105°C dry bulb, 55°C wet bulb for approximately 20 hours). Sample bolts were collected from three different SPB-attacked trees within two months of visible foliage chlorosis, and there were no obvious signs or symptoms of woodborers or decay fungi. Visual inspections were conducted to ensure that all samples were cut from sapwood and that no pith was present. Following machining, the wafers and miniature beams were placed in a conditioning chamber at 24 ± 2°C and 55 ± 5% relative humidity until a constant mass was attained, thus air-drying the green samples.

Mean wood porosity was determined for each wood type using wafers and following the oven-dry method of Usta (2003) based on specific gravity (SG) = 1.54 for wood cell walls. Wafers sawn from each wood source were individually measured in

radial, tangential, and longitudinal planes three separate times to obtain green volumes. Wafers were then placed in a convection oven at a temperature of 103 ± 2°C for 24 hours to obtain oven-dry mass. The SG was then calculated for each wood type. The amount of wood cell wall material, (K), was measured as a function of each wafers' SG (K = SG / 1.54). The porosity was then determined as (P = 1 – K). Additionally, wood from each type was ground in a Wiley mill to pass a size 20 mesh screen. Wood meal and distilled water were mixed together in 10 ml beakers in a 1:1 ratio. The pH of the wood meal was taken 24 hours later using a glass electrode, which had been calibrated prior to measurement with a buffer solution. Ten replicates were performed for each test. Means were tested using analysis of variance and Tukey's HSD test at $\alpha = 0.05$.

A Stanley (New Britain, CT) No. 90 FJ Bullnose plane was used to machine ten fresh strips, with target dimensions 0.25 mm x 5.0mm x 15.0 mm (r x t x l), from each of the ten miniature beams for each wood type. Five strips from each beam were randomly selected and placed into a sealed bag for DCA analysis of each wood type. The actual dimensions (width and thickness) of each DCA specimen were measured three times using calipers, averaged, and recorded at the time of DCA measurement.

Three probe liquids with known surface tensions, ethylene glycol (Fisher Scientific, Fair Lawn, NJ), formamide (Arcos Organics, Morris Plains, NJ), and deionized water were used as standards (Table 5.1) (Wu et al. 1995). Forty milliliters of each probe liquid were measured and placed serially on the moving stage of a Thermo Cahn (Newington, NH) DCA 322. Wood strips were randomly selected from a wood type, and hung perpendicular to the liquid and counterbalanced to ± 1 mg. The moving stage raised the liquid at a rate of 264 microns per second, which was above the adequate

threshold required for wood (Gardner et al. 1991). When the wood and liquid made contact, the "zero depth of immersion" was registered and force data were gathered to a depth of 4.0 mm. WinDCA software (Cahn Instruments, Inc., Madison, WI) calculated the advancing and receding dynamic contact angles using a buoyancy correction factor. The porous structure and hydrophilic nature of wood can lead to liquid absorption, which can significantly affect the receding contact angle (De Meijer et al. 2000). Therefore, only the advancing DCA was applied to the SFE calculation (Scheikl and Dunky 1998; Gindl et al. 2001; Gindl et al. 2004). Five replications were performed per wood type, each with a different wood strip, and averaged per probe liquid. Natural variation due to early/latewood ratios existed between the samples. The DCA accounts for this difference by immersing the entire sample into a probe liquid, whereas static contact angle measurements, by placement of a single droplet, cannot account for this variability.

Table 5.1 Probe liquid properties

| Probe Liquid | $\gamma_L^d$ | $\gamma_L^p$ | $\gamma_L$ |
|---|---|---|---|
| Water | 21.8 | 51.0 | 72.8 |
| Formamide | 39.0 | 19.0 | 58.0 |
| Ethylene Glycol | 29.0 | 19.0 | 48.0 |

[1]Properties of probe liquids used for the advancing contact angle measurements, mJ/ $M^2$ (Wu et al. 1995).

The geometric mean model was applied using paired liquid combinations with water to determine the dispersive and polar components of SFE for each wood type:

$$\frac{(1+\cos\theta)*\gamma_L}{2*(\gamma_L^d)^{1/2}} = (\gamma_S^d)^{1/2} + (\gamma_S^p)^{1/2} * \left(\frac{\gamma_L^p}{\gamma_L^d}\right)^{1/2} . \qquad (5.1)$$

where $\theta$ represented the mean advancing contact angle of each wood type in a liquid, $\gamma_L$ represented the total surface tension of a probe liquid, $\gamma_L^d$ and $\gamma_S^d$ represented the dispersive forces of a liquid and wood, and $\gamma_L^p$ and $\gamma_S^p$ represented the polar forces of a liquid and wood. The unknown parameters of each wood type, $\left(\gamma_s^d\right)^{1/2}$ and $\left(\gamma_s^p\right)^{1/2}$, were solved using a simple linear regression model

$$Y = \beta_0 + \beta_1 X + \varepsilon. \tag{5.2}$$

with $\frac{(1+\cos\theta)\gamma_L}{2(\gamma_L^d)^{1/2}}$ representing Y, $\left(\gamma_s^d\right)^{1/2}$ the intercept, $\left(\gamma_s^p\right)^{1/2}$ the slope, and $\left(\frac{\gamma_L^p}{\gamma_L^d}\right)^{1/2}$ X in the model respectively. The intercept, $\left(\gamma_s^d\right)^{1/2}$, and slope, $\left(\gamma_s^p\right)^{1/2}$, of each line were then squared to determine the dispersive and polar components of the wood types. Summing the two determined the total SFE of each wood type. All analyses were performed in SAS 9.1.3®.

## Results and Discussion

Mean specific gravity, porosity, and pH for each wood type are given in Table 5.2. The specific gravities and porosities of the two control treatments did not significantly differ; both were within ±0.01 (±0.6%) respectively. The blue-stained wood types differed 0.06 in specific gravity and 4.7% in porosity. Air-dried, blue-stained wood was significantly denser ($F_{3,36} = 253.33$, $p < 0.0001$) and less porous ($F_{3,36} = 130.39$, $p < 0.0001$) than all other wood types. However, the kiln-dried, blue-stained southern pine

was significantly less dense and more porous than all other wood types. The specific gravity (oven-dry weight/green volume) of unextracted southern pine is known to be affected by the extractives content (discussed later) of the wood (Koch 1972). Since the air-dried blue-stained wood was obtained from trees in the early stages of foliage chlorosis, wound response of these trees to bark beetle-attack may have led to an increase in specific gravity.

Table 5.2 Chemical and physical properties

| Wood Type | Specific Gravity | Porosity, % | pH |
|---|---|---|---|
| Air-dried blue-stain | 0.53 (0.003) A | 65.1 (0.253) C | 4.90 (0.007) B |
| Air-dried control | 0.49 (0.003) B | 68.1 (0.095) B | 5.34 (0.062) A |
| Kiln-dried control | 0.50 (0.003) B | 67.5 (0.200) B | 4.76 (0.009) C |
| Kiln-dried blue-stain | 0.47 (0.003) C | 69.8 (0.126) A | 4.73 (0.011) C |

[1]Mean (standard error) specific gravity, porosity, and pH of the four wood types used in this study.
[2]Capital letters indicate significantly different means within columns at $\alpha = 0.05$.

The average advancing DCAs of each wood type in the probe liquids are listed in Table 5.3. The dispersive forces, $\gamma_S^d$, polar forces, $\gamma_S^p$, and total SFE, $\gamma_S$, for each liquid pair of the four wood types are shown in Table 5.4. Defect-free southern pine contact angle samples typically have a greater degree of variation when obtained along the grain with hand tools versus electrically powered devices (Stehr et al. 2001). Minute imperfections along the grain due to the use of a hand tool may have contributed to some contact angle variability within this study.

Table 5.3 Advancing contact angles

| | Probe Liquid | | |
|---|---|---|---|
| | Water | Formamide | Ethylene Glycol |
| Wood Type | Mean contact angle (standard error) | | |
| Air-dried SPB blue-stain | 51.1 (1.15) | 52.7 (0.69) | 41.4 (0.70) |
| Air-dried control | 47.0 (3.72) | 42.8 (4.69) | 39.3 (1.33) |
| Kiln-dried control | 45.4 (1.85) | 45.1 (1.15) | 42.0 (0.78) |
| Kiln-dried blue-stain | 40.0 (2.26) | 45.8 (0.15) | 34.4 (2.06) |

[1]Advancing contact angles for each wood type in three probe liquids.

Table 5.4 Surface free energies

| Wood Type | Probe Liquid Pairs | $\gamma_S^d$ | $\gamma_S^p$ | $\gamma_S$ |
|---|---|---|---|---|
| Air-dried SPB blue-stain | Water-For | 9.40 | 39.6 | 49.0 |
| | Water-EG | 5.30 | 46.2 | 51.5 |
| Air-dried control | Water-For | 14.4 | 37.0 | 51.5 |
| | Water-EG | 4.20 | 52.2 | 56.4 |
| Kiln-dried control | Water-For | 11.8 | 41.4 | 53.2 |
| | Water-EG | 2.50 | 58.4 | 60.9 |
| Kiln-dried blue-stain | Water-For | 8.60 | 50.2 | 58.8 |
| | Water-EG | 3.30 | 61.2 | 64.5 |

[1]Total surface energies ($mJ/M^2$) and their components for the four wood types.
[2]For = Formamide, EG = Ethylene Glycol

Total SFE ranged from 49.0 to 58.8 $mJ/M^2$ for water-formamide and 51.5 to 64.4 $mJ/M^2$ for water-ethylene glycol. Kiln-dried blue-stained wood had the higher SFE in each liquid combination. The air-dried blue-stained wood had a lower SFE than all other wood types. The kiln-dried control and air-dried control had the second and third highest SFE in each instance. Higher SFEs were observed in the water-ethylene glycol combinations than in the water-formamide combinations.

Polar forces ranged from 37.0 to 50.2 $mJ/M^2$ in water-formamide combinations and 46.2 to 61.2 $mJ/M^2$ in water-ethylene glycol combinations. The polar forces obtained

when pairing ethylene glycol (acidic probe liquid) with water were higher than those found when comparing formamide (basic probe liquid) with water within a wood type. This may indicate an overall more basic southern pine wood surface (Gardner 1996). Kiln-dried blue-stained wood had the highest polar component within each liquid pair. The higher polar forces in water-formamide pair for kiln-dried blue-stained wood may also indicate more acidic sites were present relative to the other wood types. Across all wood types, the higher polar forces in the water-ethylene glycol combinations may have contributed to a lower dispersive component than that observed for the water-formamide dispersive component in each liquid pair. Overall, the dispersive forces contributed the least to the total SFE in this study, ranging from 2.5 to 11.8 $mJ/M^2$. There was not a consistent ordering of the wood types' dispersive forces for each liquid combination.

The wood types dried with the conventional kiln schedule had higher polar components and SFEs than the air-dried wood types. Increases in the SFE of kiln-dried wood have been reported up to the glass transition temperature of lignin, 60°C, at which point the wood structure is altered and SFE decreases (Gunnells et al. 1994). Structural degradation, however, is believed to be negligible under normal lumber drying conditions (Milota 2006). Since the SFEs were higher for both stained and unstained kiln-dried wood types in this study, excessive drying conditions did not appear to have been present.

The process of kiln-drying removes some water and extractives from southern pine, including hydrocarbons known collectively as volatile organic compounds (VOCs) (Shmulsky 2000). This occurs initially as the wood surface is heated to the wet-bulb temperature, followed by the migration and removal of bound water through internal diffusion. As the surface temperature of wood increases, vapor pressure increases, and

additional VOCs are removed (Ingram et al. 2000). As the removal of extractives increases the overall acidity of wood (Wålinder and Gardner 2000), the migration and loss of southern pine extractives, including VOCs, during the kiln-drying process may have resulted in the lower pH values observed for the kiln-dried wood types (Table 5.2). Additionally, the removal of hydrophobic extractives is known to increase wettability (Gunnells et al. 1994).

The presence of blue-stain in the air-dried wood resulted in a lower SFE than all other wood types. This is indicative of various physical, anatomical, morphological, and chemical changes taking place within the tree once fungal inoculation occurs via bark beetle attack (Barron 1979; Blanche et al. 1983; Shamoun and Levi 1985; Shigo and Marx 1977; Woo et al. 2005). These factors, among others, can affect the SFE of wood (Gindl et al. 2004). The nutrient-rich wood rays and ray parenchyma cells are the primary pathway of colonization by many microorganisms (Greaves 1971). Blue-stain fungi are initially confined to the radial parenchyma tissue of the sapwood, causing a blockage of water-conducting passages through internal wounding. This results in water at first being conducted around, but not through, fungal infected areas of the sapwood (Mathre 1964). It is likely that the wettability of the air-dried blue-stained wood was reduced in the trees following bark beetle-attack as a result of blue-stain fungal inoculation and the tree's initial wound response. This includes resin formation, which slows the rate of fungal spread but also produces abnormal levels of various extractives, including VOCs (Hodges and Lorio 1975; Tisdale et al. 2003).

Kiln-dried blue-stained wood had the highest SFE of all wood types. Wood porosity, and consequently permeability, is increased over time as defensive barriers in

the sapwood's radial, tangential, and longitudinal planes, such as occluded resin canals and pit membranes, are degraded by blue-stain fungi (Whitney 1971; Ballard et al. 1983; Tisdale et al. 2003). The increase in wood permeability due to *O. minus* infection therefore allows increased liquid movement across the grain (Greaves 1971). Mean wood porosity highly correlated with SFE in this study, though statistical significance was moderate (water-ethylene glycol- $r = 0.90$, $p = 0.1093$; water formamide- $r = 0.91$, $p = 0.0941$). Cai and Oliveira (2008) concluded that increased permeability of blue-stained lodgepole pine was due to 1) rupturing of the ray parenchyma cells, 2) rupturing of the pit membranes, 3) checking in the middle lamella, and 4) openings in the aspirated pits. Therefore, the higher SFE of kiln-dried blue-stained wood relative to other wood types can be explained by a rougher and more variable surface (Young 1976) caused by blue-stain fungal infection.

The results reported in this study for kiln-dried blue-stained wood, while promising, warrant some consideration when utilizing bark beetle-attacked timber. Due to the South's high temperatures and humidity, bark beetle-attacked timber has a relatively short period of utility. The utilization of blue-stained wood in composite products may lead to excessive tool wear, fines generation, and uneven drying during production. Over-drying can lead to excessive moisture uptake from the adhesive and affect resin flow. In addition, heating of the wood surface above safe tolerances leads to surface inactivation, reducing wettability (Christiansen 1990). The drying schedule used in this study was for lumber, which is considerably less severe than those observed in wood composite manufacturing. Any over-penetration of the adhesive can lead to irreversible thickness

swelling, first upon the press opening, and second when the composite is exposed to moisture (Byrne et al. 2005).

## Conclusions

Kelly et al. (1982) reported increases in various wood composite structural properties when utilizing SPB-killed southern pine as a portion of furnish; however, no explanations were given for the observed benefits. Our results indicate that 1) air-dried blue-stained wood from bark beetle-attacked trees had a lower surface free energy than all other wood types and 2) kiln-dried blue-stained wood had a higher surface free energy than all other wood types. These changes may be explained by increased wood porosity, and consequently surface free energy, as a result of a rougher and more variable surface caused by bark beetle blue-stain fungal infection and subsequent exposure to kiln-drying. These findings hold promising implications for the utilization of bark beetle-attacked timber in wood composite manufacturing. Further research is needed to examine the effect of drying processes on the wettability of bark beetle-attacked timber.

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# CHAPTER VI

# SUMMARY

Historically, insects and their fungal associates have been largely studied from a two-dimensional standpoint. For example, the relationship between bark beetles and their blue-stain fungal symbionts has primarily been characterized as mutualistic. However, Six and Wingfield (2011) recently re-described some of these fungal symbioses as commensalistic or antagonistic to bark beetles. In addition, some bark beetle fungal symbionts act as mediators of competitive interactions with other fungi in living, defensive trees. It is likely that knowledge of additional higher level associations between insects and fungi has been restricted because of our classic two-dimensional approach to research.

In chapters II-IV of this dissertation, the association of Eastern and Formosan subterranean termites with trees and lumber inhabited by bark beetle blue-stain fungal associates was investigated (Little et al. 2012a, 2012b, 2013b). Prior to this, practically no research had been conducted to investigate possible interactions between subterranean termites, bark beetles, and their fungal associates despite the economic and ecological importance of these organisms. The knowledge gained from this research implies that the classic one-dimensional view of subterranean termite behavior and ecology in temperate forest ecosystems is inaccurate. A myriad of complex multi-trophic ecological

interactions may occur between subterranean termites, bark beetles, and their associated fungi.

The ecology of the southern pine beetle has been extensively studied. However, seemingly basic knowledge, such as the effects of bark beetle blue-stain fungal symbionts on the surface properties of wood, remained completely undescribed at the beginning of this dissertation. In chapter V, we described how blue-stain fungal infection can improve the absorptive and the adsorptive properties of wood by altering its surface topography (Little et al. 2013a). This information will aid the forest products industry in targeting high volume outlets for bark beetle-attacked timber, which may benefit from its use as furnish for wood composite products.

Knowledge gained from investigating multi-trophic interactions between subterranean termites, bark beetles, and their fungal symbionts may lead to new technologies to control native and Formosan subterranean termites in commercial and residential structures. Additionally, it may also help us understand previously unknown interactions between the southern pine bark beetle guild, their fungal associates, and other arthropods.

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