EFFECTS OF A PINE HOST VOLATILE, 4-ALLYLANISOLE, AND OTHER SEMIOCHEMICALS ON SOUTHERN PINE BEETLE

by

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(Under the Direction of Kamal J.K. Gandhi)

ABSTRACT

Southern pine beetle, *Dendroctonus frontalis* Zimmerman (SPB), is a significant pine (*Pinus* spp.) pest in the eastern United States. When populations are high, SPB uses chemical signaling to initiate mass attacks and overwhelm healthy trees. Host tree volatiles interact with SPB's aggregation pheromone to enhance or inhibit attraction of SPB. The phenylpropanoid 4-allylanisole, a host volatile previously thought to inhibit aggregation, has recently been observed to enhance attraction of SPB. My goal was to better understand the variable responses of SPB and its predator clerid beetle, *Thanasimus dubius* F., to 4-allylanisole. Results indicated that 4-allylansiole can enhance attraction of SPB. Overall, 4-allylanisole didn't exhibit attractive effects for SPB in the absence of *endo*-brevicomin. We observed interactions between trap configuration and 4-allylanisole. No interactions were observed between *alpha*-pinene, *beta*-pinene, and 4-allylanisole. This research provides insight into the relationship between SPB and 4-allylanisole, and demonstrates the potential for more effective monitoring efforts.

INDEX WORDS: Bark beetle, conifer, semiochemical, pheromone, southern pine beetle

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B.A., University of Georgia, 2018

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2023

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DEDICATION

I would like to dedicate this thesis to my husband, Jesse, who has helped me more than he will ever realize.

ACKNOWLEDGEMENTS

I would like to thank my advisors, Dr. Kamal Gandhi and Dr. Brian Sullivan, who continuously provided professional and emotional support and without whom this project could never have been completed. I am also indebted to Brittany Barnes for her wisdom, encouragement, and help in the lab and field. I would like to thank Dr. Holly Munro for lending her time, assistance, and knowledge to this project. I would like to thank committee members Dr. Cristian Montes and Dr. Caterina Villari for their guidance and support. I am also grateful to Whit Bolado, my field companion and friend, for the many hours he dedicated to this project. I would like to thank Yu Wang and Dr. Daniel Hall for their help with statistical analyses. I would also like to thank the team at Synergy Semiochemicals for providing the lures needed to conduct these experiments. Funding for the project was provided by the USDA Forest Service, Southern Research Station and the D.B. Warnell School of Forestry and Natural Resources, University of Georgia.

I would like to thank my parents, Kim and Tommy, and my sister Emily, for their love and encouragement. I would like to thank the Forest Entomology lab for filling my time in graduate school with companionship, counsel, and levity. I would like to thank my family and friends for their continuous support of me throughout this project. I would like to thank my cat Pidge for accompanying me through the many hours spent writing this thesis. Finally, I would like to thank my husband, Jesse, for being my biggest supporter. I am so thankful for the patience, strength, and love he has shared with me through the duration of this project.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Southern Forests

Forests in the southern United States (U.S.) cover ~86 million ha and account for an estimated 60% of the Nation's timber products (Smith et al. 2009, Prestemon and Abt 2002). Despite only containing about 2% of the world's forested lands, southern forests contribute 18% of global pulpwood products. In addition to pulpwood, southern forests yield other significant economic resources such as sawtimber, biomass fuel, and a wide array of non-timber forest products including American ginseng (*Panax quinquefolius* L.), saw palmetto (*Serenoa repens* [W. Bartram] Small), and pine straw (Hanson et al. 2010). In Georgia, pine straw alone generated \$81 million in revenue in 2009 (Dickens et al. 2012). In addition to being highly productive, southern forests are also exceedingly diverse and host a wide variety of flora and fauna. Forests offer a plethora of ecosystem services, including carbon sequestration, filtration of pollutants from the water and air, stabilization of soil and erosion prevention, and climate regulation at the global and local level (Hanson et al. 2010). On private lands in Georgia, forests provide an estimated \$38 billion in ecosystem services annually (Moore et al. 2011). Hence, there is a great incentive both regionally and nationally to protect and maintain the southern forests.

The southeastern U.S. has been a major producer of timber since World War II, in part due to improved nursery practices as well as the adoption of new site preparation strategies (Fox et al. 2004). Lands that were once abandoned agricultural fields were converted into pine plantations after pines were observed to exhibit greater growth and survival rates on these sites when compared with naturally regenerated stands (Fox et al. 2004). As a result, site preparation strategies shifted to imitate the intensive mechanical methods previously used on old agricultural sites (Fox et al. 2004). This change in land management was predated by an even greater shift throughout the 18-19th centuries as European colonizers seized lands previously stewarded by Native Americans. Prior to European colonization, ~40% of the southeastern region, extending from Virginia to Texas, was dominated by the fire-adapted longleaf pine (*P. palustris* Mill.) (Zhang et al. 2010). Forests once dominated by longleaf pine, which covered an estimated 37 million ha of the southern coastal plain, have been replaced by stands of loblolly pine (*P. taeda* L.) (Frost 2006). While longleaf pines are typically more resistant to herbivorous insects and diseases, loblolly pines are more susceptible to these biotic agents of disturbances (Snow et al. 1990). Considering the high value of commercial loblolly pines planted in the southeastern region, it is important to protect them from insects and diseases to maximize productivity and sustainability of these forests.

1.2 Bark Beetles

One of the most prevalent and important groups of insects in the southeastern region are the bark beetles (Coleoptera: Curculionidae: Scolytinae), are a group of plant-dwelling insects that are comprised of ~6,000 species across 247 genera (Kirkendall et al. 2015). They are widespread, highly diverse insects that consume and reproduce in a variety of plant parts. Though bark beetles can colonize a wide range of host plants, tree-killing species are overwhelmingly associated with those in the Pinaceae family (Raffa et al. 2015). As a clade, bark beetle species share several morphological similarities, including spherical heads, shortened antennae and legs, and a cylindrical body shape (Raffa et al. 2015). These adaptations make them well-suited for tunneling and excavating egg galleries within plant tissues.

The bark beetle life cycle proceeds through three general phases (Sauvard 2004). It begins with dispersal, in which mature brood adults exit the natal host tree in search of a new host. Dispersal typically takes place in spring or summer (Raffa et al. 2015). Adults then locate a host and attempt to bore into it to begin the colonization or reproduction stage. Healthy trees will attempt to resist attack by exuding resin, so most bark beetle species utilize dead or dying hosts with weakened defenses (Raffa et al. 2015). If successful, adult bark beetles will colonize the host and begin the development stage under the bark. The development stage includes the establishment of egg galleries, oviposition, and the development of the brood (Franceschi et al. 2005). Temperature plays a key role in the bark beetle life cycle and influences initiation of emergence and dispersal, oviposition rate, brood development rate, and generation length (Bentz et al. 1991; Gaylord et al. 2014). The exact mechanism through which bark beetles kill trees is not fully understood (Krokene 2015). Bark beetles can contribute to tree death through the excavation of egg galleries, feeding on the phloem and cambium, and the introduction of symbionts such as pathogenic fungi (Franceschi et al. 2005). Bark beetles must overcome the tree's resistance threshold, which varies between hosts depending on tree vigor and site condition (Paine et al. 1997). The tree will continue to mount a defense until they are overwhelmed, and bark beetles will continue to attack the tree until they are flushed out or successfully colonize the tree (Paine et al. 1997). Fungi can benefit bark beetles by detoxifying plant defenses (Birkemoe et al. 2018). Many tree-killing bark beetles introduce bluestain pathogenic fungi to their hosts (Krokene 2015). Bluestain fungi are so named because of the characteristic blue or black stain they leave behind on infested plant tissues. They can spread beyond bark beetle tunnels and colonize sapwood, thereby disrupting water transportation (Paine et al. 1997). Inoculations of staining fungi can cause tree death in the absence of bark beetles, although mortality may be delayed when compared to natural

beetle attacks (Paine et al. 1997). Although trees killed by bark beetles often exhibit staining, it is not a requirement for tree death, and bark beetle infestations have been observed where no bluestain fungi were detected (Bridges et al. 1985, Whitney and Cobb 1972). These trees were observed to have higher densities of bark beetles than those that did not contain staining (Bridges et al. 1985).

Bark beetles can utilize monogamous or polygamous reproductive strategies (Raffa et al. 2015). In monogamous species (e.g., genus *Dendroctonus* Eichson) females are typically the pioneer sex and are the first to arrive at a host and initiate attack (Kirkendall et al. 2015). Females may release pheromones to attract secondarily-arriving males. Females of some monogamous species may mate with siblings or unrelated males in natal galleries prior to dispersal (Bleiker et al. 2013). Males typically are the pioneer sex in polygamous species (e.g., genus Ips DeGeer) and create nuptial chambers wherein they mate with multiple females (Kirkendall et al. 2015). Treekilling species are often gregarious and use aggregation pheromones to coordinate attacks on trees, but some bark beetles do not conduct mass attacks (e.g., *Dendroctonus micans* Kugelann) (Lieutier 2007). Some parasitic species have a greater tolerance of tree defense chemicals than tree-killing bark beetles, which allows them to occupy living trees without exhausted defenses (Gilbert et al. 2001). Mating for these species typically takes place within the brood gallery (Raffa et al. 2015). Though they do not use aggregation pheromones to initiate mass attacks, the larvae of some parasitic species (e.g., D. micans) use aggregation pheromones to coordinate gregarious feeding (Grégoire et al. 1981).

Bark beetle species exist along a tree-killing continuum and can be categorized by the condition of their typical host. Despite their reputation as tree-killers, most bark beetle species are saprophytic and exclusively colonize dead trees (Paine et al. 1997). Most bark beetle larvae require

dead or dying tissues to successfully develop (Raffa et al. 1993). Secondary bark beetles colonize weakened or dying trees, while primary bark beetles can colonize living trees (Paine et al. 1997). Species able to colonize living trees are mostly associated with conifer species, which have evolved formidable physical and chemical defenses to thwart colonization (Franceschi et al. 2005). Defenses can either be constitutive or induced in the event of an attack (Franceschi et al. 2005). The dead, outer bark is the first constitutive defense that bark beetles must be overcome for successful attack (Franceschi et al. 2005). Upon penetration of the periderm, bark beetles encounter preformed resin. Resin provides a physical and chemical defense against bark beetles (Franceschi et al. 2005). Resin is toxic to bark beetles and when released from damaged vascular tissues can physically force beetles out of a tree (Krokene 2015). Host trees will increase resin production in response to attacks to kill invading bark beetles (Franceschi et al. 2005).

The genus *Dendroctonus* is composed of 19 species arranged into six different species groups (Six and Bracewell 2015). The genus is named for its ability to kill trees (*Dendro-* tree, *- tonus* destroyer), although not all *Dendroctonus* do so (Six and Bracewell 2015). *Dendroctonus* species colonize trees in the family Pinaceae, with a majority restricted to trees in the genus *Pinus*. Five *Dendroctonus* species do not colonize *Pinus* and instead utilize *Larix*, *Picea*, or *Pseudotsuga* (Six and Bracewell 2015). The majority of *Dendroctonus* are generalists, although the generalist species typically favor some host species over others (Kelley and Ferrell 1998, Six and Bracewell 2015). The greatest diversity of *Dendroctonus* species occurs in Mexico, suggesting this is the geographic origin of the genus (Wood 1982). Fossilized galleries resembling those of *Dendroctonus* suggest that the genus is more than 45 million years old (Labandeira et al. 2001). Body size of bark beetles can indicate which part of the tree a species attacks: larger species feed on the lower bole and root collar where the phloem is thickest, while small and medium species

typically attack the mid- to upper bole (Six and Bracewell 2015). *Dendroctonus valens* Leconte is the largest species (mean 7.3 mm long) and feeds on lower bole-roots, whereas *Dendroctonus frontalis* Zimmerman (Southern pine beetle or SPB) is the smallest (mean 2.8 mm long) and feeds on the mid-bole (Six and Bracewell 2015, Wood 1982).

1.3 Southern Pine Beetle

Southern pine beetle, *Dendroctonus frontalis* Zimmerman (SPB), is a native pest that has caused significant destruction to pine forests in the eastern U.S. Within this region, SPB's range extends from Maine to Florida and west to Texas (Hain et al. 2011, Ray 2022). Its favored host trees are loblolly pine and shortleaf pine (*P. echinata* Mill.), though it also commonly infests longleaf pine, slash pine (*P. elliottii* Englm.), pitch pine (*P. rigida* Mill), pond pine (*P. serotina* Michx.), and Virginia pine (*P. virginiana* Mill.) (Hain et al. 2011). Its range further extends from Arizona and New Mexico southward to Nicaragua, where it primarily utilizes ponderosa pine (*P. oocarpa* Schiede.), and Caribbean pine (*P. caribaea* Morelet.) (Clarke and Nowak 2009, Hain et al. 2011).

At epidemic levels, SPB can cause catastrophic losses of pine trees. Outbreaks cost millions of dollars from lost revenue, higher harvesting costs, and expensive prevention and control measures. Between 1980 and 2010, SPB produced economic losses of an estimated \$43 million per year (Nowak et al. 2015). From 1999 to 2003, SPB outbreaks damaged ~400,000 ha of land (Nowak 2004), and in 2010, SPB damaged >5,000 ha of pine in New Jersey alone (Dodds et al. 2018). From 1973 to 2003, timber producers lost ~\$1.2 billion due to SPB outbreaks that resulted in a loss of >11 million cubic meters of timber (Pye et al. 2011). In addition to having serious economic impacts, damage from SPB can cause mortality of nest cavity trees used by red-cockaded

woodpeckers (*Leuconotopicus borealis* Vieillot), a threatened species and an important ecosystem engineer in the longleaf pine ecosystem (Conner and Rudolph 1995).

SPB populations are typically endemic or latent. During this population phase, beetle densities are low and healthy trees can resist colonization. Beetles seek susceptible trees with reduced defenses, and only trees weakened by lightning strikes, drought, disease, or other insect colonization are suitable hosts (Clarke 2012). When latent, the SPB is virtually undetected in forests, which contrasts significantly with its conspicuousness and impacts at high densities. SPB population densities can periodically increase resulting in an epidemic or outbreak. In this population phase, SPB produces mass attacks and can overwhelm defenses of healthy trees (Hain et al. 2011). After killing a tree, SPB may attack adjacent trees rather than disperse to new locations, and this leads to the formation of "spots" of contiguous infested and vacated trees (Ayres et al. 2011).

Females begin the construction of egg galleries by boring serpentine tunnels within the cambium of host trees (Hain et al. 2011). The female first constructs a "nuptial chamber" that is wide enough to provide space for mating (Fronk 1947, Wagner et al. 1981). The female then constructs branchless tunnels that extend 2-3 cm outward from the nuptial chamber (Sullivan 2011). Males arriving at attacked trees will search for entrance holes used by females. Males may randomly land in proximity to a female entrance or be drawn to pheromones emitted from them (Sullivan 2011). Fights may occur between competing males on the surface of the bark or within the gallery, sometimes resulting in the death of losing males (Yu and Tsao 1967). The male joins the female within the gallery and initiates courtship by emitting acoustic signals (stridulation) while rubbing his head against her elytra (Ryker 1988, Yu and Tsao 1967). The pair will then retreat to the nuptial chamber where they will mate (Payne 1980). The pair encounters liquid resin

within the chamber, which they must excavate to avoid being engulfed (Hopkins 1899). This expelled resin contains high concentrations of volatile compounds, including *alpha*-pinene, which is synergistic with SPB pheromones in signaling conspecifics to an attacked tree's location (Billings 1985). Following pairing, the female will extend the gallery and construct egg niches 1-2 mm deep on alternating sides (Fronk 1947). She is followed by the male who keeps the gallery portions immediately adjacent to the pair free of boring dust (Hain et al. 2011). As the gallery progresses, the boring dust accumulates and is used to block the gallery behind the pair (Sullivan 2011). The female lays a single egg into each niche and secures it in place using boring dust (Fronk 1947). The white, oblong eggs are about 1.5 mm in length and 1 mm in width (Hain et al. 2011). Newly hatched larvae are ~2 mm long and grow to 5-7 mm before they pupate (Hain et al. 2011). SPB spend 15 to 40 days as larvae at average temperatures of 15° to 25°C (Hain et al. 2011). The larvae progress through four instars, of which the first two are spent extending narrow galleries from the parental gallery (Thatcher and Pickard 1967). Larvae feed on phloem tissue as well as symbiotic fungi deposited by parent females (Hain et al. 2011). During the third instar, larvae construct a feeding chamber within the phloem, and, in the fourth instar, they move into the outer bark, excavate a pupal chamber, and form a pupa (Fronk 1947, Goldman and Franklin 1977). They spend 15 to 17 days as pupae at temperatures from 30° to 15°C (Payne 1980). After eclosion, the adults remain in the tree for approximately one week and then emerge (Hain et al. 2011).

SPB must kill their host trees to complete their life cycle; however, individual beetles are incapable of infesting vigorous trees alone, and SPB attacks must be coordinated to ensure successful host colonization (Hain et al. 2011). They accomplish this by using sophisticated chemical signaling. Females initiate colonization and elicit aggregation with the pheromone frontalin which signals other beetles to the host's location (Kinzer et al. 1969). Arriving males then

produce a second pheromone component, *endo*-brevicomin, which acts synergistically with frontalin to enhance aggregation (Sullivan 2011). Upon arrival, the beetles attempt to penetrate the tree's bark and colonize the host. Fungal associates of SPB are also introduced upon entry into a host tree. Some fungal associates, such as *Ophiostoma minus* Hedge., are transmitted on the exoskeleton while others, such as *Ceratocystiopsis ranaculosus* T.J Perry & J.R. Bridges or *Entomocorticium cobbii* T.C. Harrington, McNew & Batzer, are carried within the mycangia of females (Klepzig and Hofstetter 2011). Mycangial fungi provide an important supply of nitrogen for developing larvae (Ayres et al. 2000). *O. minus* often outcompetes mycangial fungi for available phloem and can deprive larvae of valuable nutritional resources (Klepzig and Wilkens 1997). Of the three fungi most consistently associated with SPB, *O. minus* apparently contributes the most to tree-killing by exhausting induced defenses in healthy hosts and interfering with vascular tissue (Klepzig and Hofstetter 2011). The construction of galleries also disrupts water and nutrient flow within the tree. The tree's ability to resist colonization is weakened as many SPB adults colonize the host, and the tree dies (Sullivan 2011).

Alone, the combination of frontalin and *endo*-brevicomin is attractive to SPB, but it is strongly synergized by host volatiles, although host volatiles alone are not attractive to SPB (Sullivan et al. 2007). Combinations of host volatiles and SPB pheromone components have been used in lures to monitor and detect SPB populations. Currently, the recommended lure for SPB includes frontalin, *endo*-brevicomin, and *alpha*- and *beta*-pinene (Sullivan et al. 2021). In a recent study that examined the effects of individual pine resin volatiles on SPB aggregation, only *alpha* and *beta*-pinene and 4-allylanisole (4 AA) demonstrated enhancement of the SPB aggregation pheromone (Munro et al. 2020). Although *alpha*-pinene had already been established as an attractive synergist (Staeben et al. 2015), this study was the first to document *beta*-pinene and 4-

allylansiole as enhancing aggregation (Munro et al. 2020). 4-allylanisole is a phenylpropanoid found in much smaller quantities in resin when compared to *alpha*-pinene or *beta*-pinene (Mirov 1961, Drew and Pylant 1966). Further, 4-allylansiole was observed to elicit a positive dose response and enhanced attractive effects of *alpha*-pinene but not turpentine (i.e., the volatile, liquid portion of pine resin; Sullivan et al. 2022).

SPB monitoring is important for predicting outbreaks and reducing beetle impacts on forest stands. Prior to the inclusion of the synergist *endo*-brevicomin, lures for SPB were comprised of frontalin and turpentine. Due to the limited attractiveness of this lure, it was necessary to deploy detection traps during the spring flight when airborne SPB are most abundant (Billings 1988). The lure often failed to detect beetles during the latent population phase or during seasons other than spring. Annual surveys switched to a 7:3 mixture of *alpha:beta*-pinene as the host odor component of the lure after turpentine sourced from southern pines become commercially unavailable in 2006 (Billings and Upton 2010, Sullivan et al. 2021). The addition of *endo*-brevicomin to the lure generally overcame the limitations indicated above for the earlier formulations (Clarke 2012). As SPB populations begin to expand northward due to climate change, a more effective lure is desired to detect SPB in areas that have not been previously exposed to SPB outbreaks (Lesk et al. 2017). The aforementioned trapping studies (Munro et al. 2020, Sullivan et al. 2022) suggested that 4-allylansiole has the potential to substantially increase SPB response to lures.

However, a better understanding of the relationship between SPB and 4-allylansiole is needed before the compound can be incorporated into management strategies. 4-allylansiole has been shown to inhibit attractive response of SPB in several previous studies (Hayes et al. 1994, Strom et al. 2004). 4-allylansiole reduced beetle attraction to frontalure (an attractive, 2:1 combination of *alpha*-pinene and frontalin) and repelled walking beetles (Hayes et al. 1994). These

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findings stimulated research to determine if 4-allylansiole could be used as a tree protectant against SPB attacks; however, applications of 4-allylansiole releasers did not reduce tree mortality (Strom et al. 2004). The contradictory responses of SPB to 4-allylansiole between studies leads to questions regarding the role of 4-allylansiole in the host selection of SPB. Why do SPB sometimes avoid 4-allylansiole, while at other times being drawn to it? The role of 4-allylansiole in the chemical ecology of SPB is unclear, but understanding this relationship could be key to improving SPB monitoring and elucidating the semiochemistry of host selection by this species.

1.4 Research Objectives

The overarching goal of this research project was to improve detection and monitoring technology for SPB and thereby enhance forecasting of outbreaks and, ultimately, improve pest management and forest sustainability. The knowledge gained through this study will provide valuable insight into the development of more effective lures for SPB. The objectives of my thesis were to investigate the relationships between SPB and 4-allylansiole by determining how different trapping variables (trap type, lure composition, and lure configuration) influence SPB response to 4-allylansiole. These results can then be used to develop strategies for incorporating 4-allylansiole into existing lures to be used in annual surveys. My specific research objectives were as follow:

- 1. To compare trapping methods to determine whether SPB responses to 4-allylanisole may depend on trapping methodology (Munro et al. 2020, Sullivan et al. 2022).
- 2. To investigate whether *endo*-brevicomin influences SPB behavioral response to 4-allylansiole.
- 3. To explore whether 4-allylansiole interacts with host volatiles *alpha* and *beta*-pinene in influencing SPB response.

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CHAPTER 2

FIELD RESPONSES OF SOUTHERN PINE BEETLE AND ITS PREDATOR TO HOST VOLATILE, 4-ALLYLANISOLE, AND OTHER SEMIOCHEMICALS

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Abstract

Southern pine beetle, Dendroctonus fontalis Zimmerman (SPB) is a native bark beetle and a significant pine (*Pinus* spp.) pest in the eastern United States. SPB typically persists at endemic levels and attacks weakened and dying trees, such as those suffering from drought, disease, lighting-strike, or attacks from other insects. SPB populations periodically have outbreaks, during which they use complex chemical signaling to initiate mass attacks and overwhelm the defenses of healthy trees. Attacked host trees release volatiles that can enhance or inhibit SPB's response to its aggregation pheromone. The host volatile 4-allylanisole (4 AA), previously thought to inhibit SPB aggregation, has recently been observed to enhance SPB attraction, but the factors governing these differing responses are unknown. We investigated how certain procedural differences in trapping techniques may have caused contrasting observations of SPB's and its clerid beetle, Thanasimus dubius F., responses to 4-allylanisole in previous studies. Multiple changes to trapping methodology (including trap type, isomers of brevicomin in lures, and positioning of release devices) influenced SPB response to 4-allylanisole. Additionally, 4-allylanisole did not increase attraction of SPB in the absence of *endo*-brevicomin. 4-allylanisole and host odor *alpha*pinene were additive in increasing SPB attraction, whereas the synergistic host odor beta-pinene had no effect either when added to this combination or 4-allylanisole alone. Clerid predators demonstrated a strong attraction to 4-allylanisole in the presence and absence of *endo*-brevicomin. The host volatile, 4-allylanisole has the potential to improve detection of SPB and could enhance monitoring in the newly invaded, northern reaches of its range where the species may be difficult to detect.

INDEX WORDS: Bark beetle, conifer, semiochemical, pheromone, southern pine beetle

2.1 Introduction

Southern pine beetle, *Dendroctonus frontalis* Zimmerman (SPB) is an economically significant bark beetle and natural disturbance agent in southern pine (*Pinus* spp.) forests. In the eastern U.S. its range extends from eastern Texas to New Jersey, but in recent years it has been detected in Connecticut, Main, Massachusetts, New York, and Rhode Island (Clarke and Nowak 2009, Dodds et al. 2018). Although SPB primarily attacks southern yellow pine species such as loblolly (*P. taeda* L.) and shortleaf pine (*P. echinata* Mill.), it can also attack most pine species within its range (Hain et al. 2011). SPB often cohabitate with other phloem-feeding scolytines that colonize southern pines in what is known as the southern pine bark beetle guild (Smith et al. 1993). In addition to SPB, this guild includes *Dendroctonus terebrans* Oliver (black turpentine beetle), *I. avulsus* Eichhoff, *Ips calligraphus* Germar, and *I. grandicollis* Eichhoff (Smith et al. 1993). Species within this guild occupy different portions of the tree which may occasionally overlap, and they may assist in varying degrees to contribute to tree dieback and mortality (Smith et al. 1993).

While SPB focus attacks on the lower and mid-bole, *D. terebrans* target the lower bole and roots of dying or dead trees (Six and Bracewell 2015, Smith et al. 1993). SPB also commonly overlap with *I. avulsus*, which typically occupy the upper portions of the bole and crown, and *I. calligraphus*, which occur throughout the lower- and mid-bole (Birch et al. 1980, Nebeker 2011, Smith et al. 1993). The *Ips* species of this guild are less aggressive than SPB but singly have also contributed to significant tree mortality in periods of drought or following natural disturbances such as hurricanes (Nebeker 2011, Vogt et al. 2020). Though all members of this guild have been shown to be capable of contributing to the mortality of trees, SPB is the only one considered a primary bark beetle and thus only attacks living trees (Stephen 2011).

When populations are low, SPB will attack weakened and dying trees, such as those affected by drought, disease, lightning strikes, or attacks by other insect species (Clarke 2012). At high densities, SPB are able to overwhelm healthy trees and exhaust host defenses without the aid of other disturbance agents (Hain et al. 2011). SPB adults construct egg galleries within the inner bark, girdling the phloem and eventually killing the tree. If the host tree is still capable of mounting a defense, the tree will use resin to eject or entomb the attacking SPB (Franceschi et al. 2005). An individual SPB is incapable of colonizing a healthy tree on its own and must coordinate attacks with conspecifics to overcome a tree's defenses, and they do so by using a sophisticated chemical communication system (Hain et al. 2011).

SPB has a complex and intricate chemical communication system. Once females locate a suitable host, they initiate aggregation with a pheromone component, frontalin (Kinzer et al. 1969). Although it is only weakly attractive on its own, it is synergized by resin monoterpenes that enhance attraction (Payne et al. 1978). Frontalin is also the only compound attractive to SPB in the absence of other semiochemicals, and no other semiochemicals can attract SPB without frontalin (Smith et al. 1993; Skillen et al. 1997). Frontalin functions as both an aggregation pheromone and a sex pheromone, as it is essential for mass attack and is also used by males to locate females for pairing. Male-produced pheromone components, *endo*-brevicomin and verbenone, are released gradually prior to entrance into the bark and rapidly thereafter (Sullivan 2011). Early research indicated that *endo*-brevicomin functioned as an antiaggregation pheromone of SPB due to its ability to reduce SPB attraction to baited traps and reduce landings on host trees. Since males arrive at trees after females, it was hypothesized that *endo*-brevicomin helped terminate mass-attack, regulated attack densities, and promoted the attack of nearby, uncolonized trees (Payne 1980, Renwick and Vité 1969, Smith et al.1993). Although *endo*-brevicomin can

inhibit aggregation, low concentrations act synergistically with frontalin and host monoterpenes to enhance attraction of SPB (Sullivan 2011).

Some tree host odors interact synergistically with SPB pheromones to enhance attraction. In a recent study on the effect of individual pine volatiles on SPB aggregation, *alpha*-pinene, *beta*pinene, and 4-allylanisole were observed to significantly enhance attraction of SPB (Munro et al. 2020). Alpha-pinene was previously established as a synergist of the SPB aggregation pheromone (Staeben et al. 2015), but response to 4-allylanisole has been variable (Munro et al. 2020, Sullivan et al. 2022). 4-allylanisole was observed to increase average SPB catches by 50-fold in the absence of other host odors (Munro et al. 2020). In a different study, 4-allylanisole failed to enhance attraction in the absence of other host odors but increased trap catches when included with *alpha*and beta-pinene (Sullivan et al. 2022). Differences in experimental methods between trapping studies could explain the contrasting responses of SPB to 4-allylanisole. The experiments where 4-allylanisole was an attractant synergist when alone (i.e., was the only host odor) employed crossvane panel traps rather than funnel traps (Lindgren 1983), included the D. terebrans pheromone component exo-brevicomin in the lure, and utilized a different spatial arrangement for semiochemical release devices on the trap. We believe that exploration of the behavioral significance of these and other experimental variables may provide insight into the ecological significance of 4-allylanisole for SPB and allow optimization of the compound in an operational lure.

The pheromone, *exo*-brevicomin is produced by many *Dendroctonus* species, including *D*. *terebrans* and *D. brevicomis* LeConte (Symonds and Elgar 2004). *exo*-Brevicomin, though not believed to be a component of the SPB pheromone, stimulates SPB olfactory receptors and can enhance attraction of SPB (Pureswaran et al. 2014). *exo*-Brevicomin has also been observed to

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inhibit or have no effect on SPB aggregation (Payne et al. 1978, Vité and Renwick 1971). Since *D. terebrans* are strongly attracted to host odors in the absence of their pheromone, they are often the first species of bark beetle to attack pines that have been struck by lightning (Hodges and Pickard 1971). It is hypothesized that SPB have adapted to use *exo*-brevicomin from attacking *D. terebrans* to identify potential host trees (Sullivan 2011).

Thanasimus dubius F. (Coleoptera: Cleridae) is a major predator of SPB and may influence SPB population cycles (Turchin et al. 1999). Because their population dynamics are closely linked, *T. dubius* catches in traps baited with SPB aggregation attractant are one component of the data set used to predict SPB outbreaks (Billings and Upton 2010). *Thansimus dubius* responds to chemical cues from numerous prey species and the host trees of their prey (Billings 1985, Costa and Reeve 2011, Herms et al. 1991). They are attracted to the *D. frontalis* pheromone component frontalin and the synergists *alpha*-pinene, *beta*-pinene, and 4-allylanisole (Staeben et al. 2015, Munro et al. 2020). A better understanding of which chemical cues are being used by this clerid predator may assist with enhanced monitoring efforts for its SPB prey as well.

To elucidate the interactions between SPB and host volatiles, we conducted three different experiments to assess how variables such as trapping methodologies, host volatiles, and beetle pheromones interact with 4-allylanisole to influence SPB attraction. Experiment 1 examined whether trapping methodology influences SPB response to 4-allylanisole. We duplicated and compared two trapping methodologies [experiment 1 in Munro et al. (2020) and experiment 3 in Sullivan et al. (2022)] that resulted in either strong or no synergistic effects for 4-allylanisole, respectively. These experiments differed in placement of release devices, trap type, and isomers of brevicomin in lures. Before exploring these variables individually, we wanted to confirm that the contrasting SPB responses were not due to the differing times and locations of the experiments.

Experiment 2 investigated interactions between *endo*-brevicomin and 4-allylanisole as lure components. The objective of this experiment was to determine if the presence of *endo*-brevicomin changes SPB response to 4-allylanisole. Experiment 3 examined the interactions between 4-allylanisole, *alpha*- and *beta*- pinene. *Alpha*-pinene and *beta*-pinene have been observed to attract SPB and are often used in SPB trapping. The objective of this experiment was to investigate how *alpha*- and *beta*-pinene influence SPB response to 4-allylanisole and determine which combination of these host odors is most attractive to SPB. Identification of procedures that reliably produce desired effects from 4-allylanisole are essential for incorporating this compound into management technologies, especially detection of cryptic populations in the northeastern United States where SPB has extended its range due to climate change.

2.2 Methods

2.2.1 Site Description

During the spring of 2021, three consecutive trapping experiments were conducted at the Oconee Ranger District, Oconee National Forest, Greene County, Georgia (within 5 km of 33° 39.9072' N, -83° 20.6530' W). The Oconee Ranger District spans eight counties across the Piedmont ecoregion of Georgia and covers 10,788 ha in Greene County. This area receives an average 119.2 ± 4.6 cm of rainfall each year (PRISM Climate Group). Annual temperatures average 17.2 ± 0.15 °C (PRISM Climate Group 2020). Soils on these sites were characterized by gravelly loam and gravelly sandy loam Ultisols (Soil Survey Staff). In general, the sites were characterized by a mix of loblolly pine (*P. taeda*) and hardwood trees, including various oaks (*Quercus* spp.), sweetgum (*Liquidambar styraciflua* L.), and flowering dogwood (*Cornus florida*)

L.). This site has served as a trapping location for several previous SPB field studies (Staeben et al. 2015, Munro et al. 2020).

2.2.2 Experiment 1: Interactions between 4-allylanisole and trapping methods.

This experiment investigated whether trapping procedure (methods differing simultaneously in trap construction, isomers of brevicomin in the lure, and positioning of release devices) influenced SPB response to 4-allylanisole. Two trapping procedures used in past studies produced different results with respect to responses by SPB to 4-allylanisole (Munro et al. 2020, Sullivan et al. 2022); however, this apparent difference had not been investigated in a single experiment (i.e., with all treatments tested at the same location and time of year). The treatments included in the study were: 1) panel trap (IPM Technologies, Portland, Oregon, U.S.) baited with frontalin and *endo-* and *exo-* brevicomin devices placed at trap center ("trap/lure arrangement A"); 2) as treatment 1 but with a 4-allylanisole release device at trap center; 3) funnel trap (Lindgren 1982; 12-unit; Synergy Semiochemicals Corp., Delta, British Columbia, Canada) with frontalin devices at trap center, an *endo-*brevicomin device ("trap/lure arrangement B"), and 4) as treatment 3 but with a 4-allylanisole release device at track to the top of the trap (Table 1).

Four transects with one trap of each of the four treatments (16 traps total) were maintained from 23 March to 4 April 2021, during peak SPB flight. To account for the influence of both trap location and date on trap catches, the experiment was established as a multiple Latin squares, factorial design, with rows of each square represented by trap location within each transect and columns represented by collection dates. Following each collection (3-4 d intervals) treatments were randomly re-positioned without replacement among the four trap locations within each transect. For each transect, every treatment was present in every trap location for one collection interval. Traps were placed >9 m away from pine trees to avoid attracting SPB to them. Trap locations were not randomly selected and were instead selected to ensure adequate distance between traps and nearby pines. To avoid interference between lures, traps were established >150 m from other traps within the same transect and ~250 m from traps in different transects. Metal poles were used to suspend traps with the top of the trap ~2 m above the ground. Dilute propylene glycol (SuperTech RV & Marine Antifreeze, Wal-Mart, Bentonville, Arkansas, U.S.) was used to fill collection cups and preserve captured insects.

Catches were collected every four days into paper paint filters and stored at 8°C. Adult SPB and the clerid bark beetle predator, *T. dubius* were counted (Wood 1982). Voucher specimens were deposited at the Georgia Museum of Natural History, University of Georgia, Athens, Georgia, U.S.

2.2.3 Experiment 2: Interaction of 4-allylanisole and *endo*-brevicomin lures

This experiment examined interactions between *endo*-brevicomin and 4-allylanisole lures. Four transects with four funnel traps each (16 traps total) were established. We used funnel traps for all treatments to duplicate trapping protocols used in annual SPB monitoring surveys. Samples were collected every three days during 8 - 17 April 2021. All traps included frontalin and *alpha*pinene in addition to variable treatments per trap: 1) control; 2) *endo*-brevicomin; 3) 4-allylanisole; 4) *endo*-brevicomin and 4-allylanisole. All devices were positioned near the center funnel of each trap. The experiment was otherwise conducted as Experiment 1.

2.2.4 Experiment 3: Synergistic effects of 4-allylanisole, *alpha*- and *beta*-pinene alone and in combination

This experiment investigated how trap captures might be influenced by different combinations of host volatiles in order to identify redundant, additive, or synergistic effects. In addition to a host odor-free control, the treatments were *alpha*-pinene, *beta*-pinene, and 4-allylanisole presented individually, in all possible binary combinations, and as the ternary combination (eight total treatments). Frontalin and *endo*-brevicomin lures were always present. The *endo*-brevicomin lure was tied at 1-2 m height to the branch of a nearby hardwood located 4-6 m from the trap. All other devices were affixed near the center funnel. Three transects of eight funnel traps (24 traps total) were established, and the experiment occurred 26 April - 17 May 2021. The experiment was otherwise conducted as Experiment 1.

2.2.5 Statistical analyses

The response variables were adult SPB (male and female combined) and clerid trap catches. Negative binomial Generalized linear mixed-effects models (NB GLMM) with fixed effects for treatment and random effects for date, transect, and trap location were used to determine the effect of treatment on trap catch. Each experiment was analyzed as a factorial design with tests for main effects and interactions of each factor. Presence of 4-allylanisole was considered a factor (all experiments) as was trap/lure arrangement (2×2 factorial, Experiment 1), presence of *endo*-brevicomin (2×2 factorial, Experiment 2), and presence of *alpha*- or *beta*-pinene (2×2×2 factorial, Experiment 3). For all tests, $\alpha = 0.05$. Data were analyzed using the statistical software R, version 4.2.2. (R Core Team 2022) and RStudio (R Studio Team 2022) using the packages lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), emmeans (Lenth 2022), car (Fox and Weisburg

2019), DescTools (Andri et al. 2022), glmmTMB (Brooks et al. 2017), dplyr (Wickham et al. 2022), and ggplot2 (Wickham 2016).

2.3 Results

2.3.1 Experiment 1: Response to 4-allylanisole and trapping methods

A total of 2,771 SPB and 1,174 clerid beetles were collected during Experiment 1. Trap catches ranged from 0 to 376 SPB per collection, while clerid catches ranged from 0 to 111 per collection. The addition of 4-allylanisole had a significant effect on trap catches for both SPB (z = 10.290, p < 0.001) and clerids (z = 8.561, p < 0.001). The addition of 4-allylanisole increased mean SPB trap catch by 16-fold with trap/lure arrangement A and 4-fold with trap/lure arrangement B (Figure 2.1). When ignoring trap/lure arrangement, 4-allylanisole increased SPB trap catch overall by 8-fold (z = 10.290, p < 0.001). Factor trap/lure arrangement had a significant effect on SPB trap catch (z = 4.750, p < 0.001), but not on the trap catch of clerids. Overall, trap/lure arrangement A caught twice as many SPB as trap/lure arrangement B (z = 4.750, p < 0.001). There also was a significant interaction between the presence of 4-allylanisole and trap/lure arrangement for SPB (z = -3.302, p = 0.001) but not clerids. Trap/lure arrangement A with 4-allylanisole yielded the greatest SPB trap catch (Figure 2.1).

2.3.2 Experiment 2: Response to 4-allylanisole and *endo*-brevicomin lures

A total of 4,285 SPB and 3,200 clerids were collected during Experiment 2. A range of 0 to 904 SPB were captured per collection, while the total number of clerids ranged from 3 to 227 per collection. Both *endo*-brevicomin (z = -17.108, p < 0.001) and 4-allylanisole (z = 8.983, p < 0.001) had a significant effect on SPB trap catch (Figure 2.3). The interaction between these two

factors was also significant, indicating synergism between semiochemicals (z = 3.931, p = 0.001). Traps with both 4-allylanisole and *endo*-brevicomin had a 17-fold greater mean trap catch than those with *endo*-brevicomin alone (Figure 2.3). Only 4-allylanisole had a significant effect on clerid trap catch (z = 8.734, p < 0.001), and no interaction was detected between *endo*-brevicomin and 4-allylanisole for this species (Figure 2.4).

2.3.3 Experiment 3: Synergistic effects of 4-allylanisole, *alpha-* and *beta-*pinene alone and in combination

A total of 18,488 SPB and 2,825 clerids were collected during Experiment 3. Trap catches for SPB ranged from 0 to 1,763 beetles per collection, while clerids ranged from 0 to 150 beetles per collection. *Alpha*-pinene (z = 12.131, p < 0.001) and 4-allylanisole (z = 12.702, p < 0.001) had a significant effect on SPB trap catch, but the effect of *beta*-pinene was non-significant (Figure 2.5). There was no interaction between any of the three semiochemicals. The combination of *alpha*-pinene, *beta*-pinene, and 4-allylanisole yielded the greatest mean trap catch for SPB (Figure 2.5). *Alpha*-pinene (z = 10.234, p < 0.001), *beta*-pinene (z = 7.007, p < 0.001), and 4-allylanisole (z = 10.089, p < 0.001) all had a significant effect on clerid trap catch. The interaction between these semiochemicals was also significant (z = 4.556, p < 0.001). We did not further conduct pairwise comparisons.

2.4 Discussion

Our trapping study revealed several key findings regarding the relationship between 4allylanisole and SPB: 1) Some previously observed variation in synergistic effects of 4-allylanisole may be attributable to trapping methodology alone; 2) 4-allylanisole enhances SPB attraction only when accompanied by *endo*-brevicomin; 3) the effects of *alpha*-pinene and 4-allylanisole on SPB trap catches are additive rather than synergistic. Similarly, we found that the clerid catches were higher with the addition of 4-allylanisole, but their response was not influenced by trapping methodology or presence of *endo*-brevicomin.

In Experiment 1, 4-allylanisole enhanced attraction of SPB in the absence of *alpha*-pinene. This trend has been observed in some, but not all, recent studies on 4-allylanisole and SPB (Munro et al. 2020, Sullivan et al. 2022). However, the effect of 4-allylanisole on SPB catches was not the same in either trapping configuration. 4-allylanisole demonstrated a 16-fold increase of SPB catch with trap/lure arrangement A, which utilized panel traps baited with both *endo-* and *exo*-brevicomin. These traps also had all lure components at trap center. Because multiple details of the trapping procedure distinguished trap/lure configurations A and B, it is not possible to attribute the difference in SPB responses to any one variable.

However, past research indicates that these variables individually can affect *Dendroctonus* spp. responses to traps. Panel traps have been shown to be more effective than other trap types at catching *D. terebrans* (Miller et al. 2011), however, no studies have investigated trap suitability for SPB. It is unknown which differences between trap-types contributed to this difference in responses in *T. terebrans*, but panel traps have a larger capturing surface area and may provide a more representative tree silhouette than the funnel traps. Follow-up experiments exploring these variables separately are needed. *Thanasimus dubius* did not show a preference for trap-type, although it responded in higher numbers to the presence of 4-allylanisole.

In Experiment 2, the addition of *endo*-brevicomin resulted in increased SPB catch in traps with and without 4-allylanisole. The pheromone component *endo*-brevicomin demonstrated a strong synergism with 4-allylanisole and the combination of these two semiochemicals resulted in

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17-fold increase of SPB trap catches when compared with endo-brevicomin alone and a 100-fold increase when compared with 4-allylanisole alone. Interestingly, 4-allylanisole did not enhance attraction of SPB in the absence of *endo*-brevicomin despite being accompanied by *alpha*-pinene. Earlier studies have found 4-allylanisole to inhibit SPB aggregation (Hayes et al. 1994, Strom et al. 1999). Several differences exist between our study and these earlier experiments. Both Hayes et al. (1994) and Strom et al (1999) trapped within active SPB infestations and utilized frontalure (a 2:1 combination of *alpha*-pinene and frontalin) rather than frontalin alone or the combination of frontalin and a high release rate of *alpha*-pinene. These experiments also did not use *endo*brevicomin as a lure adjuvant, as *endo*-brevicomin enhances aggregation outside of SPB spots but inhibits aggregation within SPB spots (Sullivan et al. 2011). Although the effects of endobrevicomin were not understood at the time and was not included in the lure itself in these past experiments (Hayes et al. 1994, Strom et al. 1999), it would have been within the environment since those experiments took place within active SPB infestations. It is possible that, like *endo*brevicomin, 4-allylanisole inhibits SPB aggregation within infestations but enhances aggregation outside of them. This aspect merits further investigation and suggests that like *endo*-brevicomin, 4-allylanisole is also a biphasic chemical for SPB.

We did not find similar results in Experiment 2 for the clerid beetle *T. dubius*, which responded positively to 4-allylanisole but not to *endo*-brevicomin. Past experiments that observed an inhibitory effect of 4-allylanisole on SPB did not see the same inhibition of clerids (Hayes et al. 1994). Our results suggest that these two species may use different combinations of chemicals to find their resources at low SPB population levels. SPB are more sensitive to conspecific-produced *endo*-brevicomin, but *T. dubius* are more sensitive to 4-allylanisole being produced by the trees. However, both species are highly attracted to frontalin produced by SPB adults (Sullivan

2011). Considering that *T. dubius* also preys on other members of the southern pine bark beetle guild (Billings and Cameron 1984, Reeve et al. 2009), 4-allylanisole may indicate the presence of appropriate host trees for their prey species.

Both 4-allylanisole and *alpha*-pinene increased SPB trap catch in experiment 3, while *beta*pinene did not affect SPB trap catch. The combination of all three host odors yielded the greatest mean SPB trap catch, although this was not significantly greater than catch with 4-allylanisole and *alpha*-pinene. Though no interaction between 4-allylanisole and *alpha*-pinene was detected, traps baited with a combination of 4-allylanisole and *alpha*-pinene caught more SPB on average than the treatment mimicking the current operational lure, which is composed of 70% *alpha*- and 30% *beta*-pinene. This experiment also revealed that trapping with *alpha*- and *beta*-pinene is equally effective as trapping with just *alpha*-pinene, and removal of *beta*-pinene may reduce the cost of the lure.

2.5 Conclusions

Our results indicate that the host volatile, 4-allylanisole acted synergistically with SPB pheromone to enhance the attraction of SPB. Prior to this study, the interactions between *endo*-brevicomin and 4-allylanisole had not been examined. The 4-allylanisole produced attractive responses in SPB when in the presence of *endo*-brevicomin but failed to attract SPB in its absence. Future studies may focus on isomers of brevicomin in lures, positioning of release devices, and the influence of SPB population densities on their response to 4-allylanisole. Our findings suggest that 4-allylanisole can be used to reliably increase SPB trap catches. This is especially important as climate continues to warm and SPB's range continues to expand into the northern U.S. Early detection of SPB can aid landowners in managing outbreaks and mitigating impacts.

2.6 Acknowledgements

We thank the Oconee Ranger District, Chattahoochee National Forest for providing access to sampling sites. Whit Bolado (University of Georgia) assisted in the field and laboratory, and Yu Wang and Dr. Daniel Hall (Department of Statistics, University of Georgia) assisted with the statistical analyses. Funding for the project was provided by the USDA Forest Service, Southern Research Station and the D.B. Warnell School of Forestry and Natural Resources, University of Georgia.

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Compound	Source	Purity	Release device	Device load	Release rate at mean 21 °C
alpha-pinene	Sigma-Aldrich	≥97	Experiment 2: Polyethylene transfer pipette	3 ml	60 mg/d
	Synergy	99	Experiment 3: "Sirex sleeve" device	75 ml	800 mg/d
beta-pinene	Synergy	99	"Sirex sleeve" device	32 ml	425 mg/d
endo-brevicomin	Synergy	≥95%	Flexlure	11.2 mg	0.12 mg/day
exo-brevicomin	Synergy	≥95%	Flexlure	11.2 mg	0.12 mg/day
frontalin	Synergy	≥95%	Microcentrifuge tube (x2)	550 mg	10-12 mg/d
4-allylanisole	Sigma-Aldrich	98	Experiment 2: Bottle with cotton dental wick extending 1.3 cm through the cap	50 ml	1 g/d
	Sigma-Aldrich	98	Experiment 1 & 3: Polyethylene transfer pipette (x2)	2.9 g	48 mg/d

Table 2.1 Composition of lures used in the trapping study.

List of Figures

- **Figure 2.1:** Mean number (±SE) of southern pine beetle (*Dendroctonus frontalis*, SPB) adults trapped with different types of traps and lure arrangements and with/without 4allylansisole. Presence of 4-allylanisole is indicated with differing colors. "Trap/lure arrangement A" = panel traps baited with frontalin, *endo*-, and *exo*- brevicomin devices placed at trap center. "Trap/lure arrangement B" = funnel trap with frontalin devices at trap center, and an *endo*-brevicomin device tied to the branch of a nearby sapling (4-6 m away at 1-2 m height). Means associated with the same lower-case letter were not significantly different ($\alpha = 0.05$)
- **Figure 2.2:** Mean number (±SE) of clerids (*Thanasimus dubius*) trapped with different types of traps and lure arrangements and with/without 4-allylansisole. "Trap/lure arrangement A" = panel traps baited frontalin, *endo-*, and *exo-* brevicomin devices placed at trap center. "Trap/lure arrangement B" = funnel trap with frontalin devices at trap center, an *endo-* brevicomin device tied to the branch of a nearby sapling (4-6 m away at 1-2 m height). Means associated with the same lower-case letter were not significantly different (α = 0.05).
- **Figure 2.3:** Mean number (±SE) of southern pine beetle (*Dendroctonus frontalis*, SPB) trapped with funnel traps treated with *endo*-brevicomin and 4-allynalisole (4AA) individually and in combination. All traps were identically baited with frontalin and *alpha*-pinene, and the control had no additional semiochemicals. Means associated with the same lowercase letter were not significantly different ($\alpha = 0.05$).
- **Figure 2.4:** Mean number (±SE) of clerids (*Thanasimus dubius*) trapped with funnel traps baited with *endo*-brevicomin and 4-allynalisole (4AA) individually and in combination. All

traps were identically baited with frontalin and *alpha*-pinene, and the control had no additional semiochemicals. Means associated with the same lower-case letter were not significantly different ($\alpha = 0.05$).

- **Figure 2.5:** Mean number (±SE) of southern pine beetle (*Dendroctonus frontalis*, SPB) trapped with funnel traps treated with *alpha*-pinene (α -Pn), *beta*-pinene (β -Pn), and 4-allylanisole (4AA) presented individually, in all possible binary combinations, and as the ternary combination. All traps were also baited with frontalin and *endo*-brevicomin, and the control had no additional semiochemicals. Means associated with the same lower-case letter were not significantly different ($\alpha = 0.05$).
- **Figure 2.6:** Mean number (\pm SE) of clerids (*Thanasimus dubius*) trapped with funnel traps treated with *alpha*-pinene (α -Pn), *beta*-pinene (β -Pn), and 4-allylanisole (4 AA) presented individually, in all possible binary combinations, and as the ternary combination. All traps were also baited with frontalin and *endo*-brevicomin, and the control had no additional semiochemicals. Means associated with the same lower-case letter were not significantly different ($\alpha = 0.05$).



Figure 2.1



Figure 2.2



Figure 2.3



Figure 2.4



Figure 2.5





CHAPTER 3

THESIS CONCLUSIONS

3.1 Conclusion

Our objective in experiment 1 was to better understand the variable responses of southern pine beetle (*Dendroctonus frontalis* Zimmerman, SPB) to 4-allylanisole. For that purpose, we compared trapping methodologies between Munro et al. (2020) and Sullivan et al. (2022) to determine which factors influenced SPB response to 4-allylanisole. We measured the trap catches of SPB across four different treatments. These treatments included trapping configurations resembling those used in both Munro et al. (2020) and Sullivan et al. (2022), with and without the addition of 4-allylanisole. Results indicated that traps with 4-allylanisole caught more SPB than those without it. Panel traps also caught more SPB than funnel traps. Interactions between trapping configurations and 4-allylanisole also impacted trap catches. Trap/lure arrangement A captured five times as many SPB than trap/lure arrangement B when both were baited with 4-allylanisole. Future studies will be needed to isolate the effects that trap type, isomers of brevicomin in lures, and positioning of release devices have on SPB response to 4-allylanisole.

Our study was the first to examine the interactions between *endo*-brevicomin and 4allylanisole. The objective of experiment 2 was to determine the effects of *endo*-brevicomin on SPB response to 4-allylanisole. We compared SPB trap catches in four different treatments that included *endo*-brevicomin and 4-allylanisole presented individually and in combination as well as a control with neither semiochemical. Our results revealed that the combination of 4-allylanisole and endo-brevicomin greatly increased SPB trap catch. Our results also indicated that 4allylanisole was not attractive without endo-brevicomin suggesting a strong synergism between these two chemicals.

Annual surveys utilize a combination of frontalin, *endo*-brevicomin, *alpha*-pinene, and *beta*-pinene to trap for SPB. The objective of experiment 3 was to examine the interactions between the *alpha*- and *beta*-pinene with 4-allylanisole to determine if 4-allylanisole could be incorporated into SPB monitoring. We measured SPB trap catches in response to *alpha*-pinene, *beta*-pinene, and 4-allylanisole presented individually and in combination. Results indicated that *alpha*-pinene and 4-allylanisole increased SPB trap catches while *beta*-pinene did not. No interactions between any semiochemicals were detected.

Our results suggest that 4-allylanisole has the potential to reliably increase SPB trap catch in annual monitoring efforts. Monitoring of SPB is vital for helping landowners prepare management strategies and mitigate impacts. Early detection is becoming increasingly important as SPB expand their range northward as a result of warming temperatures. Future work using 4allylanisole in SPB trapping studies in the northern reaches of its range could determine if this semiochemical will prove effective for early detection. Future research could examine the effects of 4-allylanisole on SPB behavior within infestations. Understanding the variability in responses to 4-allylanisole can help better reproduce desired responses in annual surveys and ultimately improve forest sustainability.