ROLE OF FUNGAL AND HOST-ASSOCIATED VOLATILES IN THE CHEMICAL ECOLOGY OF SCOLYTINE BEETLES AFFECTING HARDWOOD TREES

by

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ABSTRACT

Native and invasive bark and ambrosia beetles threaten the health and productivity of natural and planted forests worldwide. Management of these pests relies on semiochemical-based tactics, but these are often ineffective at monitoring for incipient populations or decreasing pest populations. The role of fungal and non-host volatiles in colonization behavior remains unknown for many important bark and ambrosia beetle species, thereby hindering their control. In this dissertation, I tested the hypothesis that fungal and tree-associated volatiles influence the host colonization behavior of bark and ambrosia beetles that affect hardwood trees. This work describes the identification of novel fungal and host-associated semiochemicals that may aid in future management of these important pests.

In Chapter 1, I review the current literature describing the volatile chemical ecology of bark and ambrosia beetles that inhabit hardwood trees. A review of groups with numerous identified semiochemicals, as well as considerations for future research is included.

In Chapter 2, I test the hypothesis that host colonization by the peach bark beetle (*Phloeotribus liminaris*) is chemically mediated by compounds associated with infested hosts. I found that benzaldehyde mediates colonization by the peach bark beetle, and that that benzaldehyde lures are effective attractants in field-trapping studies.

In Chapter 3, I test the hypothesis that ambrosia beetle attraction to host stress compounds can be modified by symbiotic fungal volatiles. I found that for three species of invasive ambrosia beetles individual fungal volatiles act as repellents, with species-specific differences in response to different compounds.

In Chapter 4, I test the hypothesis that attraction of the walnut twig beetle (*Pityophthorus juglandis*) to its pheromone lure can be enhanced by symbiotic fungal volatiles. I found that symbiotic fungal volatiles consistently enhance attraction of the beetles to their fungus, while one symbiotic fungal volatile of ambrosia beetle species repelled the walnut twig borer.

In Chapter 5, I summarize results from each of the chapters and discuss patterns observed in the response to fungal and host-associated volatiles among the focal bark and ambrosia beetle species. I also discuss future research needs and directions to continue development of the knowledge surrounding scolytine chemical ecology and management of these pest beetle species.

CHAPTER 1. A REVIEW OF THE CHEMICAL ECOLOGY OF BARK AND AMBROSIA BEETLES THAT INFEST HARDWOOD TREES

1.1 Introduction

Understanding the biology and ecology of bark and ambrosia beetles has become increasingly important, as numerous invasive species continue to threaten the health and productivity of forests, which are predicted to undergo rising levels of stress due to global climate change. These beetles are among the most prevalent species captured at ports of quarantine (Haack and Rabaglia 2013, Rabaglia et al. 2019) and often carry pathogenic microorganisms that cause widespread diseases in naïve tree hosts (Hulcr and Dunn 2011, Ploetz et al. 2013). Detecting and monitoring invasive beetle species, in addition to managing established pest populations, has led to the development of control tactics that rely heavily on manipulating responses to olfactory cues and signals. While many decades of research have expanded our understanding of the chemical ecology of scolytine pests of conifers in North America, there remains a scarcity of knowledge regarding semiochemicals of bark and ambrosia beetles that colonize hardwood trees. In this chapter, I present a broad review of known volatile semiochemicals of hardwood-infesting bark and ambrosia beetles.

There are approximately 6,000 species of bark and ambrosia beetles (Coleoptera: Scolytinae, Platypodinae) worldwide, although most species breed in angiosperm trees found in tropical climates (Kirkendall et al. 2015, Raffa et al. 2015). While both bark and ambrosia beetles spend most of their lives concealed beneath the bark of host trees, their nutrient sources and host range can drastically differ. Most bark beetle larvae feed on relatively nitrogen-rich phloem tissue and, due to nutritional reliance on specific hosts, adults only colonize from one to a small number of tree species (Hulcr et al. 2007, Raffa et al. 2015). Ambrosia beetle larvae, in contrast, feed solely on symbiotic fungi that colonizing adult beetles transport in specialized mycetangia and inoculate into gallery walls as they bore deep into host wood. Due to this nutritional independence from host tissues, ambrosia beetles typically colonize a wide range of hosts, with some beetle species utilizing more than 100 species of trees (Hulcr et al. 2007, Gohli et al. 2017). For ambrosia beetles, host physiological condition is often more important than host species, because symbiotic fungi grow and outcompete co-occurring fungi under suitable environmental conditions. (Ranger et al. 2015, 2018).

In addition to differences in nutrient sources and host breadth, the damage caused to host trees often differs between bark and ambrosia beetles. Damage caused by bark beetles is most often limited to phloem. Adult beetles preferentially attack stressed or moribund hosts, but mass attack by some bark beetles on living trees, such as the southern pine beetle or mountain pine beetle, can lead to widespread tree mortality (Harvey et al. 2014, Clarke et al. 2016). In contrast, ambrosia beetles can cause direct damage to host wood as they create galleries for oviposition (Alfaro et al. 2007, Boland 2016), but more troublesome is the recent advent of novel tree diseases caused by invasive beetle species that vector pathogenic fungi (Hulcr and Dunn 2011, Ploetz et al. 2013). For example, recent invasions by the redbay ambrosia beetle (*Xyleborus glabratus* Eichoff) and Euwallacea shot-hole borers (*Euwallacea* nr. *fornicatus* Eichoff) have caused extensive death of hardwood trees to laurel and fusarium wilt, respectively (Fraedrich et al. 2008, O'Donnell et al. 2016).

The cryptic life cycle of bark and ambrosia beetles complicates management of incipient and existing pest populations. Because beetles spend little time outside of host trees, they are rarely affected by prophylactic insecticide applications, and degree-day models and calendar-driven sprays are unreliable. Signs of beetle colonization can also be difficult to observe, creating a lag time between establishment of invasive populations and detection by visual surveys of trees. For these reasons, management of these pests has focused on exploiting chemically mediated colonization behaviors to detect, monitor, and control scolytine populations (Rabaglia et al. 2008, 2019). Conspecific pheromones, volatiles associated with stressed or moribund hosts, and compounds produced by mutualistic organisms, attract adult beetles to suitable trees or prospective mates (Borden 1989, Byers 1989, Ranger et al. 2015, Ranger et al. 2021). On the other hand, volatiles associated with competing beetles, non-host trees, and competing fungi can lead to repulsion (Byers and Zhang 2011, Audley et al. 2020, Martini et al. 2020). Behaviorally active compounds are then exploited in tactics such as mass kill traps, trap trees, mating disruption, repellent lures, and push-pull programs (Borden 1989, Gitau et al. 2013, Ranger et al. 2021).

Although the chemical ecology of bark and ambrosia beetle pests of conifer trees has been studied for much of the last six decades, semiochemicals of beetles that colonize hardwood trees has remained, until recently, relatively unexplored. The economic importance of conifer pests in North America, such as the southern pine beetle (*Dendroctonus frontalis* Zimmerman) and mountain pine beetle (*Dendroctonus ponderosae* (Hopkins)), has driven research into their

management and semiochemicals. In addition to primary research, numerous reviews of the chemical ecology of coniferophagous bark beetles have been published, focused on such areas as compound identification and behavioral responses (Birch 1978, Borden 1989, Byers 1989, Byers and Zhang 2011); pheromone production (Blomquist et al. 2010, Tittiger and Blomquist 2016, Keeling et al. 2021); and their use in management (Schlyter 2012, Gitau et al. 2013). Many reviews have claimed to discuss the chemical ecology of bark beetles at large, yet a comprehensive review of semiochemicals that affect hardwood-colonizing bark and ambrosia beetles is lacking from the literature. There exists a critical need for such a synthesis, in light of continued invasion and establishment of exotic beetle species, as well as recent outbreaks of beetle-vectored fungal tree diseases in hardwood species.

In this chapter, I review the current state of knowledge regarding the identity and behavioral response of bark and ambrosia beetles that colonize hardwood trees to identified semiochemicals. Because this review is focused on semiochemicals, compounds that lead to a behavioral change in the receiver, volatiles that generated no response when tested will rarely be discussed. Also, when possible, the discussion will focus on compounds that have been identified, as mixtures of compounds, such as "host volatiles" may contain components that may or may not play a role in modifying the behavioral response. Because general trends in chemical ecology often correlate with taxonomic relatedness, major groups of beetles within each subfamily of bark and ambrosia beetles will be discussed separately. This will allow for comparisons of semiochemicals both within and without related groups.

To find available literature, a broad search was conducted using the Google Scholar database using a large list of search terms. In addition to general search terms (e.g. "ambrosia beetle semiochemicals", "bark beetle attractant", "ambrosia beetle repellent"), specific genera of more well-known beetles were explored by searching for more specific terms (e.g. "Scolytus attractant", "Xylosandrus repellent"). The first 150 results of each search were processed by eliminating studies conducted in conifer systems and then gathering data related to the species, compounds, volatile sources, and responses as described in each publication. The information is summarized in separate tables for ambrosia beetles (Table 1.1) and bark beetles (Table 1.2) below.

1.2 Ambrosia Beetles

For ambrosia beetles, research has focused primarily on species that are either pests of plantation and nursery trees or act as vectors for fungal pathogens that cause widespread tree wilt and death in North America (see Table 1.1). Most of the species are attracted to a variety of host volatiles, and some utilize pheromones to find mates, with several cases of host volatiles synergizing with pheromones. Because many ambrosia beetle species are attracted to ethanol, a volatile produced by many stressed trees, widespread trapping surveys utilizing this compound have also increased our knowledge of non-pest species. Pheromones of conifer-infesting beetles, namely verbenone, and non-host volatiles, such as limonene and α-pinene, were generally repellent.

1.2.1 Platypodinae

Beetles in the subfamily Platypodinae are obligate fungal feeders, as are other ambrosia beetles, but differ morphologically from beetles in the Scolytinae. Interest in the semiochemicals of platypodine species has occurred in response to severe damage caused several global pests of *Populus* spp. plantations, as well as recent invasions by several vectors of oak wilt diseases (Alfaro et al. 2007, Nakajima 2019). Unlike almost all scolytine ambrosia beetles, there is strong evidence that pheromones play an important role in platypodine host-colonization behavior. Male platypodines are the pioneer sex, colonizing trees as they are attracted by volatiles such as α-copaene and ethanol associated with susceptible hosts (Lucia et al. 2014, Rainho et al. 2021). As males bore into trees, they appear to produce volatile compounds that attract female beetles (Milligan et al. 1988, Ytsma 1989). These compounds have not been specifically identified for some platypodine species, but in others blends of volatile compounds have been identified as pheromones. In *Euplatypus parallelus* (Fabricius), the pheromone blend consists of five components: 1-hexanol, 1-octanol, hexyl acetate, isoamyl alcohol, and trans-geraniol (Rainho et al. 2021). In *Megaplatypus mutatus* Chapuis, a worldwide pest of poplar in plantations, the pheromone consists of three components: retusol (an enantiomer of sulcatol), sulcatone, and 3-pentanol (Audino et al. 2005, Gatti Liguori et al. 2008, Gonzalez-Audino et al. 2013). The pheromone of the Korean oak wilt vector, *Platypus koryoensis* (Murayama), has been identified from whole-body washes of male beetles as a blend of five components: citronellol, geranial, geraniol, neral, and nerol (Kim et al. 2009). Interestingly, the vector of Japanese oak wilt, *Platypus*

quercivorus (Murayama), uses quercivorol as an aggregation pheromone, while all other species utilize male-produced blends as sex pheromones (Kashiwagi et al. 2006, Kamata et al. 2008).

1.2.2 Scolytinae

Euwallacea

Our knowledge of *Euwallacea* spp. semiochemicals has recently expanded in response to the recent establishment of the invasive shot-hole borer species complex in California, Hawaii, and Florida. Although the taxonomy of *Euwallacea nr. fornicatus* Eichoff remains in question, it has been demonstrated that all the species vector fungal pathogens that cause wilt diseases in susceptible hardwood trees (O'Donnell et al. 2016). Compounds that are used for host selection in this species complex remain poorly understood, but there is evidence for a pheromone blend composed of 2-heneicosanone and 2-tricosanone that differs in the ratios of each component by species (Cooperband et al. 2017). To date, this is the only group of scolytine beetles where there is evidence for production of a long-distance pheromone. The shot-hole borers are somewhat unique in their lack of attraction to the ubiquitous host stress volatile ethanol, although the host compound α-copaene enhances their attraction to the putative pheromone (Kendra et al. 2017). All species in the complex are also attracted to quercivorol, a compound identified as the pheromone of a platypodine species, but also produced by a symbiotic fungus of *Euwallacea* nr. *fornicatus* (Byers et al. 2017, Cooperband et al. 2017, Dodge et al. 2017). Two enantiomers of verbenone, as well as the non-host volatile piperitone, repel all species in the *Euwallacea* nr. *fornicatus* complex, as well as *E. validus* Eichoff to varying degrees in the field (Ranger et al. 2013, Dodge et al. 2017, Byers et al. 2018). Attempts at developing a "push-pull" system using these repellents and the attractant quercivorol have been unsuccessful thus far (Byers et al. 2020).

Xyloborini

Species within this tribe of Scolytinae are among the most abundant of invasive ambrosia beetle species. For almost all species in this group, reproductive behavior includes extreme inbreeding and haplodiploid development of progeny (Kirkendall 1983, Kirkendall et al. 2015). The sex ratio of these species is extremely female-biased and the rare males are smaller and flightless. Because sexual mating occurs with sibling males before females leave their natal host,

there appears to be little need for conspecific pheromones to promote mate finding; indeed, to date there is no evidence of pheromone use among the Xyloborini (Ranger et al. 2021).

Xyleborus

The redbay ambrosia beetle (*Xyleborus glabratus* Eichoff) vectors the fungal pathogen responsible for laurel wilt (*Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva) (Hanula et al. 2008). Since its recent establishment in the southeastern U.S., studies have focused on developing semiochemical-based tactics for its management in avocado orchards and natural forest stands (Ploetz et al. 2017). Similar to the *Euwallacea nr. fornicatus* complex, the redbay ambrosia beetle is not attracted to ethanol, but is attracted to the host volatile α-copaene (Johnson et al. 2014, Carrillo et al. 2016). Additional volatiles from other deciduous trees, including cubeb, lychee, manuka, and phoebe oils, as well as eucalyptol, are attractive to colonizing beetles, with cubeb lures being the most efficacious (Hanula and Sullivan 2008, Kendra et al. 2011, 2014, Kuhns et al. 2014). Laboratory experiments have also shown that *X. glabratus* is attracted to volatiles of its symbiotic fungus *R. lauricola* (Hulcr et al. 2011) and field experiments have shown that these volatiles enhance attraction of the manuka-oil lure (Kuhns et al. 2014). Verbenone is a strong repellent for this species (Hughes et al. 2017, Martini et al. 2020), and the host-stress compound methyl salicylate is somewhat repellent (Hughes et al. 2017, Martini et al. 2020).

Other species of Xyleborus, including *X. affinis* Eichoff, *X. bispinatus* Eichoff, *X. dispar* (Fabricius), and *X. ferrugineus* (Fabricius), were all attracted to ethanol when tested in trapping surveys (Montgomery and Wargo 1983, Miller and Rabaglia 2009a, Rivera et al. 2020). Similar to *X. glabratus*, its congener *X. bispinatus* is also repelled by the host-stress compound methyl salicylate (Rivera et al. 2020). Additionally, *X. ferrugineus* exhibited attraction towards volatiles of its symbiotic fungus, as well as the symbiotic fungus of *Xylosandrus crassiusculus*, in laboratory experiments (Hulcr et al. 2011).

Xylosandrus

Three *Xylosandrus* species are among the most common invasive bark and ambrosia beetle species detected in North America. *Xylosandrus crassiusculus* (Motschulsky), *X. germanus* (Blandford), and *X. compactus* (Eichoff) are each strongly attracted to ethanol (Miller and Rabaglia

2009a, Ranger et al. 2010, 2015, Ranger et al. 2013). Conophthorin, a compound associated with a variety of deciduous trees, has enhanced attraction to ethanol in both *X. crassiusculus* and *X. germanus*, although this result varied among sites, years, and experiments (VanDerLaan and Ginzel 2013, Ranger et al. 2014, Miller et al. 2015). All three of the species were weakly attracted to volatiles of their symbiotic fungus in laboratory assays (Hulcr et al. 2011), but when tested in a field setting, *X. compactus* was only weakly attracted to the fungal volatiles, and *X. germanus* was repelled by individual components of the fungal volatiles (Egonyu and Torto 2018, Ranger et al. 2021). Verbenone is repellent to all three of the species, and the non-host volatile limonene repels *X. crassiusculus* and *X. compactus* (Burbano et al. 2012, Ranger et al. 2013, VanDerLaan and Ginzel 2013).

1.3 Bark Beetles

For bark beetles, research has again centered on species of economic concern, especially those that vector pathogenic fungi. Although fewer bark beetle species have identified semiochemicals, pheromones were more common than in ambrosia beetles (see Table 1.2). Similar to findings among ambrosia beetles, non-host and non-conspecific compounds, such as verbenone and α-pinene, are repellent.

Hypothenemus

The coffee berry borer (*Hypothenemus hampei* (Ferrari)) is a worldwide pest of coffee production and development of semiochemical tactics for this pest has occurred over the last two decades. A 1:1 mixture of ethanol and methanol has proven effective in capturing *H. hampei* for monitoring purposes, but its use remains ineffective for properly managing beetle populations below economic injury levels (Dufour and Frrot 2008, Ranger et al. 2010). Behavioral assays of the response of adult berry borer have demonstrated attraction to ripening coffee berries; therefore, many experiments have focused on collections of the volatile host compounds. Some of the more behaviorally active attractants include methyl salicylate and linalool, as well as conophthorin, were found to be associated with damaged hosts (Njihia et al. 2014, Cruz-López et al. 2016). In fact, volatiles from berries may operate similar to a “push-pull” system, where conophthorin attracts beetles for several days, and then as beetle density increases, the repellent frontalin is produced,

effectively reducing possible competition among young feeding in the berries (Njihia et al. 2014). Both β-caryophyllene and verbenone have also repelled coffee berry borer in separate field experiments (Ranger et al. 2013, Góngora et al. 2020).

Pityophthorus

The walnut twig beetle (*Pityophthorus juglandis* Blackman) vectors the pathogenic fungus *Geosmithia morbida* M. Kolarik, E. Freeland, C. Utley, and N. Tisserat, which can cause Thousand Cankers Disease (TCD) in susceptible *Juglans* and *Pterocera* species (Tisserat et al. 2009). The male-produced pheromone for this species has been identified as 3-methyl-2-buten-1-ol (Seybold et al. 2015). In laboratory assays both sexes were also attracted to volatiles from black walnut, its preferred host (Blood 2016, Blood et al. 2018), as well as volatiles from its symbiotic fungus, *G. morbida* (Blood et al. 2018). Although other attractive compounds have yet to be discovered, several strongly repellent compounds have been identified. Enantiomers of verbenone, limonene, and α-pinene, as well as racemic chalcogran and trans-conophthorin, strongly repel walnut twig beetle from pheromone sources and baited traps (Blood et al. 2018, Audley et al. 2020, Audley et al. 2020). With both a strong attractant and numerous repellents in hand, development of a viable "push-pull" system to manage TCD may be within reach.

Scolytus

Scolytus multistriatus (Marsham) and *S. scolytus* Geoffroy vector the pathogens responsible for Dutch Elm Disease in the United States and Europe, respectively (Millar et al. 1986), which provided the impetus for identifying semiochemicals of these species. A pheromone blend has been identified for many *Scolytus* spp., along with host volatiles that enhance attraction of the pheromone. The main component shared amongst *Scolytus* spp. is (-)-4-methyl-3-heptanol, with varying rates of (-)-α-multistraitin and (-)-4-methyl-3-hexanol present in different species (Pearce et al. 1975, Blight et al. 1977, 1978, Lanier et al. 1977). Enantiomers are used to maintain reproductive isolation among the species: for example, the pheromone blends of *S. laevis* (Chapuis) and *S. amygdali* Geurin-Meneville vary by a single enantiomer (Zada et al. 2004). In addition to pheromone components, unidentified host volatiles, 4-methyl-3-heptanone, vanillin, and

syringaldehyde were all attractive when tested in a lab setting (Meyer and Norris 1967, Blight et al. 1983).

1.4 Summary

Our understanding of the chemical ecology of bark and ambrosia beetles that inhabit hardwood trees has greatly increased over the last two decades. Although this expansion in knowledge has, unfortunately, been in response to widespread outbreaks of beetle attack and novel tree diseases, the information that we have gained has increased our ability to detect and manage destructive beetle populations. Host volatiles, such as ethanol, conophthorin, and α-copaene, are important attractants, and in many cases may enhance attraction to conspecific pheromones. Verbenone is a nearly universal repellent when tested against beetles that inhabit hardwood trees, but other compounds, such as limonene and α-pinene, can also strongly repel beetles. In addition to general trends of attractants and repellents, the current literature also demonstrates that responses to semiochemicals are not always similar among closely related species, even congeners, and assumptions regarding chemical ecology should not be based on taxonomic relatedness.

This review not only demonstrates that our knowledge of the chemical ecology of hardwood-infesting bark and ambrosia beetles has increased during the last two decades, but also that few species have been adequately studied and many factors related to semiochemicals remain poorly understood. For instance, semiochemicals of most bark and ambrosia beetles have yet to be identified, and, for many studied species, only single compounds have been tested for behavioral responses, when blends of compounds are more biologically relevant. Also, although studies have elucidated the mechanisms of pheromone production in conifer-infesting bark beetles (Blomquist et al. 2010, Tittiger and Blomquist 2016, Keeling et al. 2021), no studies, to date, have explored the same mechanisms in the pheromone-producing beetles that inhabit deciduous trees. The release rate of semiochemical lures appears to play an important role in species-specific responses, but is poorly understood for almost all lures (Ranger et al. 2011, Reding et al. 2011). For example, the walnut twig beetle was repelled to a greater degree as the release rate of repellent lures was increased (Audley et al. 2020), but this remains one of the few combinations of release rate and beetle species studied. Lastly, determining the active range of lures for bark and ambrosia beetles is imperative to increase efficacy of trapping and control tactics, yet this remains unknown for the majority of semiochemical products. Increasing such research will aid in developing sustainable

management tactics for the ever-increasing number of invasive species which affect forests and nursery stock worldwide.

Table 1.1: Semiochemicals of ambrosia beetle species (Coleoptera: Platypodinae, Scolytinae). Asterisks indicate: *attraction of males only, **attraction of females only, or ***symbiotic fungal species.

	Compound	Type/Source	Response	Setting	Reference(s)
Platypodinae					
Euplatypus parallelus	1-hexanol	sex pheromone	attraction	field	Rainho et al. 2021
	1-octanol	sex pheromone	attraction	field	Rainho et al. 2021
	hexyl acetate	sex pheromone	attraction	field	Rainho et al. 2021
	isoamyl alcohol	sex pheromone	attraction	field	Rainho et al. 2021
	trans-geraniol	sex pheromone	attraction	field	Rainho et al. 2021
	ethanol	stressed host	attraction*	field	Rainho et al. 2021
Megaplatypus mutatus	3-pentanol	sex pheromone	attraction	field	Gatti Liguori et al. 2008, Gonzalez-Audino et al. 2013
	retusol	sex pheromone	attraction	field	Audino et al. 2005, Gonzalez-Audino et al. 2013
	sulcatone	sex pheromone	attraction	field	Audino et al. 2005, Gonzalez-Audino et al. 2013
	α-copaene	host volatiles	attraction*	field	Lucia et al. 2014
Platypus apicalis	host volatiles	infested host volatiles	attraction	lab	Milligan et al. 1988
Platypus caviceps	host volatiles	uninfested host	attraction*	field	Ytsma 1989
	host volatiles	infested host	attraction**	field	Ytsma 1989
Platypus koryoensis	citronellol	male body extracts	attraction	field	Kim et al. 2009
	geranial	male body extracts	attraction	field	Kim et al. 2009
	geraniol	male body extracts	attraction	field	Kim et al. 2009

Table 1.1 Continued

	neral	male body extracts	attraction	field	Kim et al. 2009
	nerol	male body extracts	attraction	field	Kim et al. 2009
Platypus quercivorus	quercivorol	aggregation pheromone	attraction	field	Kashiwagi et al. 2006, Kamata et al. 2008
	host volatiles	fresh host leaves	attraction	lab	Pham et al. 2019
Platypus subgranosus	ethanol	stressed host	attraction	field	Elliott et al. 1983
Scolytinae					
Ambrosiodmus tachygraphus	ethanol	stressed host	attraction	field	Miller and Rabaglia 2009
Anisandrus sayi	ethanol	stressed host	attraction	field	Miller and Rabaglia 2009
	(-)-α-pinene	non-host volatiles	repellent	field	Ranger et al. 2011
	verbenone	pine bark beetle pheromone	repellent	field	Ranger et al. 2013
Cnestus mutilatus	ethanol	stressed host	attraction	field	Klingeman et al. 2017
	conophthorin	tree/insect/fungal volatile	repellent	field	Miller et al. 2015
Cyclorhipidion bodoanum	ethanol	stressed host	attraction	field	Miller et al. 2015
	conophthorin	tree/insect/fungal volatile	repellent	field	Miller et al. 2015
Cyclorhipidion pelliculosum	ethanol	stressed host	attraction	field	Ranger et al. 2014, Miller et al. 2015
	conophthorin	tree/insect/fungal volatile	enhanced attraction to ethanol	field	Ranger et al. 2014, Miller et al. 2015
Dryoxylon onoharaensum	ethanol	stressed host	attraction	field	Miller and Rabaglia 2009
	(-)-α-pinene	non-host volatiles	repellent	field	Miller and Rabaglia 2009

Table 1.1 Continued

	conophthorin	tree/insect/fungal volatile	repellent	field	Miller et al. 2015
Euwallacea nr. *fornicatus*	ethanol	stressed hosts	no response, repellent	field	Dodge et al. 2017, Chen et al. 2021
	2-heneicosanone	pheromone	attraction	lab	Cooperband et al. 2017
	2-tricosanone	pheromone	attraction	lab	Cooperband et al. 2017
	host volatiles	infested host volatiles	attraction	field	Byers et al. 2018
	quercivorol	symbiotic fungus	attraction	field	Carrillo et al. 2015, Byers et al. 2017, Cooperband et al. 2017, Dodge et al. 2017
	(-)-α-copaene	host volatiles	enhanced attraction to pheromone	field	Kendra et al. 2017
	(R)-verbenone	pine bark beetle pheromone	repellent	field	Dodge et al. 2017, Byers et al. 2018
	(S)-verbenone	pine bark beetle pheromone	repellent	field	Dodge et al. 2017, Byers et al. 2018
	piperitone	non-host volatiles	repellent	field	Dodge et al. 2017, Byers et al. 2018
Euwallacea validus	ethanol	stressed host	attraction	field	Ranger et al. 2014
	conophthorin	tree/insect/fungal volatile	enhanced attraction to ethanol	field	Ranger et al. 2014
	verbenone	pine bark beetle pheromone	repellent	field	Ranger et al. 2013
Monarthrum mali	ethanol	stressed hosts	attraction	field	Montgomery and Wargo 1983, Miller and Rabaglia 2009
	(R)-(-)-sulcatol	pine bark beetle pheromone	enhanced attraction to ethanol	field	Miller and Crowe 2020
Monarthrum scutellare	ethanol	stressed hosts	attraction	field	Noseworthy et al. 2012

Table 1.1 Continued

	3-hydroxy-octan-2-one	cerambycid pheromone	enhanced attraction to ethanol	field	Noseworthy et al. 2012
Xyleborinus saxesenii	ethanol	stressed hosts	attraction	field	Miller and Rabaglia 2009, Chen et al. 2021
	benzaldehyde	host tree	enhanced attraction to ethanol	field	Yang et al. 2018
	conophthorin	tree/insect/fungal volatile	enhanced attraction to ethanol	field	Miller et al. 2015
	lineatin	pine bark beetle pheromone	enhanced attraction to ethanol	field	Ranger et al. 2014
	(-)-α-pinene	non-host volatiles	repellent	field	Ranger et al. 2011
	fungal volatiles	*R. lauricola* volatiles	repellent	lab	Hulcr et al. 2011
	verbenone	pine bark beetle pheromone	repellent	field	Ranger et al. 2013
Xyleborus affinis	ethanol	stressed hosts	attraction	field	Miller and Rabaglia 2009
Xyleborus bispinatus	ethanol	stressed hosts	attraction	field	Rivera et al. 2020
	methyl salicylate	host volatile	repellent	lab	Rivera et al. 2020
Xyleborus dispar	ethanol	stressed hosts	attraction	field	Montgomery and Wargo 1983
Xyleborus ferrugineus	ethanol	stressed hosts	attraction	field	Miller and Rabaglia 2009, Hulcr et al. 2011
	fungal volatiles	*A. xylebori* volatiles	attraction	lab	Hulcr et al. 2011
	fungal volatiles	*Ambrosiozyma* sp. volatiles***	attraction	lab	Hulcr et al. 2011
Xyleborus glabratus	ethanol	stressed hosts	no attraction	field	Johnson et al. 2014
	α-copaene	host volatiles	attraction	field	Kendra et al. 2016
	cubeb oil	non-host tree volatiles	attraction	field	Kendra et al. 2014, 2015

Table 1.1 Continued

	eucalyptol	non-host tree volatiles	attraction	field	Kuhns et al. 2014
	lychee oil	non-host tree volatiles	attraction	field	Kendra et al. 2011
	manuka oil	non-host tree volatiles	attraction	field	Hanula and Sullivan 2008
	phoebe oil	non-host tree volatiles	attraction	field	Hanula and Sullivan 2008
	host volatiles	host leaf volatiles	attraction	field	Martini et al. 2015
	fungal volatiles	*R. lauricola* volatiles***	enhanced attraction to manuka oil lure	field	Hulcr et al. 2011, Kuhns, et al. 2014
	fungal volatiles	*Ambrosiozyma* sp. volatiles	attraction	lab	Hulcr et al. 2011
	fungal volatiles	*A. xylebori* volatiles	repellent	lab	Hulcr et al. 2011
	methyl salicylate	host volatile	repellent	field	Hughes et al. 2017, Martini et al. 2017
	verbenone	pine bark beetle pheromone	repellent	field	Martini et al. 2017, 2020
Xylosandrus compactus	ethanol	stressed hosts	attraction	field	Miller and Rabaglia 2009
	2,3-butanediol	*F. solani* volatiles***	weak attractant	field	Egonyu and Torto 2018
	methyl isovalerate	*F. solani* volatiles***	weak attractant	field	Egonyu and Torto 2018
	(-)-verbenone	pine bark beetle pheromone	repellent	field	Burbano et al. 2012
	R-(+)-limonene	non-host volatiles	repellent	field	Burbano et al. 2012
Xylosandrus crassiusculus	ethanol	stressed hosts	attraction	field	Miller and Rabaglia 2009, Hulcr et al. 2011, Ranger et al. 2015
	conophthorin	tree/insect/fungal volatile	enhanced attraction to ethanol	field	VanDerLaan and Ginzel 2013, Miller et al. 2015
	fungal volatiles	*A. xylebori* volatiles***	attraction	lab	Hulcr et al. 2011
	fungal volatiles	*R. lauricola* volatiles	repellent	lab	Hulcr et al. 2011
	(-)-verbenone	other bark beetle pheromone	repellent	field	Burbano et al. 2012
	R-(+)-limonene	non-host deciduous	repellent	field	Burbano et al. 2012
	verbenone	pine bark beetle pheromone	repellent	field	VanDerLaan and Ginzel 2013

Table 1.1 Continued

Xylosandrus germanus	ethanol	stressed hosts	attraction	field	Miller 2009; Ranger 2010, 2013b, 2015
	conophthorin	tree/insect/fungal volatile	enhanced attraction	field	Ranger et al. 2014
	fungal volatiles	*A. grosmanniae* volatiles***	arrestant	lab	Ranger, et al. 2021
	2-phenylethanol	*A. grosmanniae* volatiles***	repellent	field	Ranger et al. 2021
	3-methyl-1-butanol	*A. grosmanniae* volatiles***	repellent	field	Ranger et al. 2021
	conophthorin	non-host deciduous	repellent	field	Miller et al. 2015
	verbenone	pine bark beetle pheromone	repellent	field	Ranger et al. 2013, VanDerLaan and Ginzel 2013

Table 1.2: Semiochemicals of bark beetle species (Coleoptera: Scolytinae). Asterisks indicate: *attraction of males only, **attraction of females only, or ***symbiotic fungal species.

	Compound	Type/Source	Response	Setting	Reference(s)
Hypothenemus dissimilis	Ethanol	stressed hosts	attraction	field	Ranger et al. 2010
	Verbenone	pine bark beetle pheromone	repellent	field	Ranger et al. 2013
Hypothenemus hampei	Ethanol	stressed hosts	attraction	field	Dufour and Frrot 2008, Ranger et al. 2010
	Methanol	stressed hosts	attraction	field	Dufour and Frrot 2008
	methyl salicylate	damaged host	attraction**	lab	Cruz-López et al. 2016
	Linalool	damaged host	attraction	lab	Cruz-López et al. 2016
	3-ethyl-4-methylpentanol	host volatiles	attraction	lab	Mendesil et al. 2009
	Methylcyclohexane	host volatiles	attraction	lab	Mendesil et al. 2009
	Nonane	host volatiles	attraction	lab	Mendesil et al. 2009
	Ethylbenzene	host volatiles	attraction	lab	Mendesil et al. 2009
	Conophthorin	host volatiles	attraction	field	Njihia et al. 2014
	Frontalin	host volatiles	repellent	field	Njihia et al. 2014
	β-caryophyllene	non-host volatiles	repellent	field	Góngora et al. 2020
	Verbenone	other bark beetle pheromone	repellent	field	Ranger et al. 2013
Phloeotribus liminaris	Benzaldehyde	infested host	attraction	field	Ethington et al. 2021
Phloeotribus scarabaeiodes	Ethylene	stressed hosts	attraction	field	Campos and Peña 1995
Pityophthorus juglandis	host volatiles	black walnut	attraction	lab	Blood et al. 2018
	fungal volatiles	*G. morbida* volatiles***	attraction	lab	Blood et al. 2018
	3-methyl-2-buten-1-ol	pheromone	attraction	field	Seybold et al. 2015
	S-(-)-verbenone	pine bark beetle pheromone	repellent	field	Audley et al. 2020
	R-(+)-verbenone	pine bark beetle pheromone	repellent	field	Audley et al. 2020

Table 1.2 Continued

	racemic chalcogran	pine bark beetle pheromone	repellent	field	Audley et al. 2020
	trans-conophthorin	tree/insect/fungal volatile	repellent	field	Audley et al. 2020
	R-(+)-limonene	non-host volatiles	repellent	field	Blood et al. 2018, Audley et al. 2020
	S-(-)-limonene	non-host volatiles	repellent	field	Audley et al. 2020
	(±)-α-pinene	non-host volatiles	repellent	field	Audley et al. 2020
Scolytus amygdali	(3S,4S)-4-methyl-3-heptanol	pheromone	attraction	field	Ben-Yehuda et al. 2002, Zada et al. 2004
	(3S,4S)-4-methyl-3-hexanol	pheromone	attraction	field	Ben-Yehuda et al. 2002, Zada et al. 2004
Scolytus intricatus	host volatiles	uninfested host	attraction	field	Hovorka et al. 2005
Scolytus laevis	(3R,4S)-4-methyl-3-heptanol	pheromone	attraction	field	Anderbrant et al. 2010
	(3S,4S)-4-methyl-3-heptanol	congeneric pheromone	repelled	field	Anderbrant et al. 2010
Scolytus mediterraneus	infested host volatiles	pheromone	attraction	field	Gurevitz and Ishaaya 1972
Scolytus multistriatus	(-)-4-methyl-3-heptanol	pheromone	attraction	field	Pearce et al. 1975, Lanier et al. 1977
	(-)-α-multistriatin	pheromone	attraction	field	Pearce et al. 1975, Lanier et al. 1977
	(-)-α-cubebene	host volatile	attraction	field	Pearce et al. 1975, Lanier et al. 1977
	(-)-4-methyl-3-heptanone	host volatile	attraction	lab	Blight et al. 1983
	vanillin	host volatile	attraction	lab	Meyer and Norris 1967
	syringaldehyde	host volatile	attraction	lab	Meyer and Norris 1967
Scolytus schevyrewi	host volatiles	uninfested host	attraction	field	Lee et al. 2010
Scolytus scolytus	(-)-4-methyl-3-heptanol	pheromone	attraction	field	Blight et al. 1977, 1978

Table 1.2 Continued

	(-)-α-multistriatin	pheromone	attraction	field	Blight et al. 1977, 1978
	(-)-α-cubebene	host volatile	attraction	field	Blight et al. 1977, 1978
	(-)-4-methyl-3-heptanone	host volatile	attraction	lab	Blight et al. 1983
Trypophloeus klimeschi	methyl benzoate	host volatiles	attraction	field	Gao et al. 2019

CHAPTER 2. CHEMICALLY-MEDIATED COLONIZATION OF BLACK CHERRY BY THE PEACH BARK BEETLE, PHLOEOTRIBUS LIMINARIS

2.1 Introduction

The peach bark beetle (*Phloeotribus liminaris* Harris, PBB) is an economic pest of black cherry (*Prunus serotina* Ehrh.), which is among the most valuable hardwood timber species in the eastern U.S. (Rexrode 1982, Cassens 2004). Adult beetles colonize the branches and stems of mature trees, where they bore into the bark to mate. In response to PBB colonization attempts, the tree produces a resinous gum to push out colonizing beetles as they chew through the bark. This resin causes a dark-colored stain in the wood which is eventually incorporated into the growth ring of the tree, leading to a defect referred to as a "gum spot" (Rexrode and Baumgras 1984, Rexrode and Smith 1990). Gum spots make harvested lumber unsuitable for veneer and reduce its value by as much as 90 percent (Rexrode and Baumgras 1984, Cassens 2004). Attack by adult PBB is the primary cause of gum spots, which are the most important defect limiting cherry veneer production in the Central Hardwood Forest Region (CHFR) of North America (Rexrode and Baumgras 1984, Rexrode and Smith 1990, Cassens 2004, Wiedenbeck et al. 2004).

The peach bark beetle is a small (1.8-2.1 mm) insect native to eastern North America, where it colonizes many *Prunus* species, but preferentially attacks black cherry (Rexrode 1982, Wood 1982). Adults overwinter under the bark and emerge during spring to colonize suitable hosts. Adult PBB prefer to colonize stressed or wounded trees, but at high beetle densities healthy trees may also be attacked (Rexrode 1981). Adults mate within host trees and females lay eggs in galleries they excavate below the bark (Kulman 1964, Rexrode 1981). Developing larvae also form galleries in the inner bark, as they feed on nutrient-rich phloem. In repeatedly or heavily attacked trees, these extensive feeding galleries can girdle the main stem, interrupting root to shoot conductivity, which weakens host trees and can lead to death (Rexrode 1982, Rexrode and Baumgras 1984). The peach bark beetle produces two generations per year in the northern part of its range and as many as three generations per year under favorable conditions further south. Colonizing adult beetles and developing larvae also attract predators, including woodpeckers, whose feeding can cause considerable damage to infested trees (Kenis et al. 2004, Wiedenbeck et al. 2004).

The destructive nature of *P. liminaris* is exacerbated by difficulty in controlling its populations in natural and managed stands. Current management of PBB relies on cultural controls that attempt to reduce PBB pressure on valuable black cherry trees. These tactics include thinning of black cherry to low densities and removing post-harvest slash (Rexrode and Smith 1990). These practices require considerable time and effort and, in much of the eastern U.S., have proven insufficient for maintaining high-quality black cherry free of gum spots. Chemical controls, such as sprayed and systemic insecticides, are generally ineffective at managing bark beetles because the majority of their life cycle is spent below the bark, and trunk-injected insecticides are ineffective at preventing gum spots caused by PBB colonization. Although cultural controls can occasionally decrease local PBB populations, private and public land managers need additional methods to manage this pest and reduce gum spots in valuable cherry wood in the eastern U.S.

The detection, monitoring, and mass trapping of woodboring pests in urban and natural forest systems often exploit their chemically mediated behavior as part of an integrated pest management (IPM) strategy (Witzgall et al. 2010). However, few species-specific semiochemicals have been identified for bark beetles affecting hardwood trees. In fact, detection and monitoring efforts rely almost solely on the use of ethanol, a volatile produced from stressed trees, as an attractant (Kimmerer and Kozlowski 1982, Miller and Rabaglia 2009b), and the use of other host-associated compounds has been met with varying success (Miller and Rabaglia 2009b, VanDerLaan and Ginzel 2013, Miller et al. 2015, Ranger et al. 2016). Currently, little is known about the chemically mediated host colonization behavior of PBB, but the identification of host and conspecific attractants may hold promise in the development of effective management strategies.

In this study we test the hypothesis that adult PBB utilize volatile compounds associated with black cherry and pioneering conspecifics to locate susceptible hosts. Our objective is to determine the extent to which adult PBB are attracted to odors associated with injured and conspecific-infested black cherry, and to identify attractive compounds that can be used to manage beetle colonization and reduce damage. We used both olfactometer and field bioassays to determine the behavioral response of adult PBB to wounded and infested host material. We then used dynamic headspace sampling and gas chromatography-mass spectrometry (GC-MS) analysis to identify compounds associated with attractive odor sources, and trapping assays to test the bioactivity of these compounds in the field.

2.2 Methods and Materials

2.2.1 Source of Beetles

Adult PBB were reared from naturally infested black cherry material collected from the Richard G. Lugar Forestry Farm, Tippecanoe Co., IN, USA. Insects used for laboratory experiments were reared to adults in a greenhouse according to Browne (1972), and emerging adults were collected daily. Adult beetles were separated by sex according to Rexrode (1981) and stored for up to two weeks at 4 °C in moist paper towels until used in experiments. Only vigorous and active beetles were utilized in bioassays, and individual beetles were used in olfactometer bioassays no more than once per day.

2.2.2 Response of PBB to Volatiles of Black Cherry and Infested Host Material

We conducted behavioral assays to determine the extent to which adult PBB were attracted to volatile compounds associated with bolts of black cherry that were damaged and those infested with either male or female conspecifics. Only actively moving beetles were used for assays, which were conducted between 1000-1700 h in a greenhouse under ambient conditions. Assays were performed 28 July to 10 August 2012 within a glass-tube olfactometer (27.5 cm length x 3 cm dia.) attached to a separate glass chamber (24 cm x 60 mm) where odor sources were introduced. An attached vacuum pulled filtered air over each odor source and into the olfactometer at a constant rate of 1 L/min as measured using a flowmeter. Five adult PBB of the same sex were introduced to the downwind end of the olfactometer (section no. 0) opposite the odor source and their position in the olfactometer was observed for 10 min. The walking response of individual beetles was calculated by dividing the olfactometer length into five sequentially numbered sections of 55 mm and recording the location of beetles at the end of each 10-min trial. Each trial was conducted using one of the following four odor sources:

1) cotton wick moistened with deionized water (control),

2) wounded black cherry bolt (approximately 90 mm length x 20 mm dia.),

3) black cherry bolt infested with 20 female PBB, or

4) black cherry bolt infested with 20 male PBB.

Bolts were infested by drilling 20 holes (2 mm x 2 mm) into the bark and placing individual beetles into each hole. Beetles were allowed 24 h to colonize bolts before they were used in

bioassays. The 24-h delay allowed time for the beetles to ingest plant tissue, as pheromone production in many bark beetles is induced by feeding (Blomquist et al. 2010, Gitau et al. 2013). To account for volatile compounds that may be released from holes drilled in the bark, we also drilled 20 holes in the uninfested control bolts. Based on individual beetle walking response within the olfactometer, an attraction index was calculated for each odor using the equation below (per Zagatti et al. 1987):

$$X/D_{max}*100$$

Where,

$$X = \sum_{i=0}^{4} in_i$$

n_i is the number of beetles in section i at 10 min;

$D_{max} = 4n$ is the value if all beetles were in the most upwind section (no. 4) at the end of the assay.

The assay was repeated 25-27 times for each odor source. The walking response of males and females to each odor source were used as the response variable and analyzed separately using a one-way analysis of variance (ANOVA). The response index for each odor source was then compared to the control using a Dunnett's test (Sokal and Rohlf 1995).

2.2.3 Response of PBB to Infested Bolts in Field Bioassays

To determine the extent to which adult PBB respond to volatiles emanating from infested black cherry in a natural setting, we conducted a field assay using traps baited with odor sources similar to those used in olfactometer experiments. This experiment was conducted 21 May–6 July 2015, using a transect of four, 12-unit Lindgren funnel traps placed in a mixed-age hardwood stand, dominated by black cherry, at the Purdue University Martell Forest, Tippecanoe Co., IN. Traps were placed at least 10 m apart on stands (approx. 1.5 m tall) constructed of polyvinyl chloride (PVC) pipe. A highly concentrated saltwater solution was placed in trap collection cups to kill and preserve captured insects. Captured beetles were collected daily and specimens were placed in 70% ethanol until identified to species and sex. Traps were rotated one position each day to control for possible location effects. Each trap was baited with one of the following odor sources:

1) cherry bolt (approx. 9 cm length x 4 cm dia.) with 45 drilled holes (2 mm x 2 mm),

2) cherry bolt infested with 45 female PBB,

3) cherry bolt infested with 45 male PBB, or

4) blank trap (control).

Each bolt was infested in the same manner as above and allowed 24 h for colonization. Cut ends of the bolts were sealed with a thin layer of paraffin wax (Gulf Wax®, Roswell, GA) to minimize desiccation in the field. To account for host-associated volatiles of injured bolts, 45 holes were drilled through the bark of control bolts. The bolts were then placed in a mesh bag (20 cm x 28 cm) to prevent further colonization by beetles in the field and each bag was secured to the top of an individual trap using nylon cord. Differences in the number of beetles captured by each treatment were analyzed using a non-parametric Kruskal-Wallis rank sum test followed by post-hoc Dunn's Test using the statistical program R (Kruskal and Wallis 1952).

2.2.4 Identification of Volatiles Associated with Colonized Bolts

To identify compounds emanating from odor sources used in olfactometer and field bioassays, we collected volatiles from injured and infested cherry bolts and analyzed samples using GC-MS. Volatiles were collected from individual black cherry bolts placed in a glass vacuum trap (55/50 trap bottle, Chemglass Life Sciences, Vineland, NJ) connected to a laboratory vacuum system. Ambient air was pulled into the chamber through an activated carbon filter, passing over the odor source and then through an absorbent filter at a rate of 1 L/min. The absorbent filter consisted of a disposable glass pipette containing 100 mg of 80/100 HayeSep-Q® (Ohio Valley Specialty Company, Marietta, OH) connected to the vacuum trap and vacuum using 5 cm Tygon® tubing. The chamber was maintained under full-spectrum light on a 16:8 light:dark cycle. Odor sources consisted of:

1) black cherry bolt with 40 drilled holes (2 mm x 2 mm),

2) black cherry bolt infested with 40 male PBB, or

3) black cherry bolt infested with 40 female PBB.

Because adult PBB actively fly from dusk to dawn and may respond to volatiles produced during that time, volatiles were collected for 12 h from 8:00 p.m. to 8:00 a.m. the following day. Our field assays also suggested that there is a lag between the time of colonization and peak attraction to colonized branches; therefore, volatiles were collected daily for 20 days after bolts were infested, for a total of 20 samples for each bolt treatment. Filters were eluted each day using three successive 0.5-mL aliquots of methylene chloride and the resulting extracts were stored in

glass vials at -4 °C. Extracts were analyzed using a Hewlett-Packard 6890N gas chromatograph in splitless mode, coupled to a Hewlett-Packard 5973 mass spectrometer with electron impact ionization (EI, 70 eV). The GC was equipped with a DB5 column (30 m x 0.25 mm x 0.25 μm; J&W Scientific, Folsom, CA, USA) with helium carrier gas. Immediately prior to injection, samples were sonicated (30 s) and vortexed (30 s) to prevent compounds from adhering to the sides of the glass vial. The injection port was maintained at 100 °C, and the oven temperature was programmed to start at 40°C for 1 min, increase by 10°C to 250 °C, then held for 15 min. Individual compounds were identified by comparing their mass spectra to those in the National Institute of Standards and Technology (NIST) mass spectral library (ca. 120,000 spectra; ChemStation Version D.05.01; Hewlett Packard Corp.; Palo Alto, CA, USA), and matching their retention time and mass spectra to those of authentic standards.

2.2.5 Response of PBB to Synthetic Compounds in Field

Experiments to assess the attraction of adult PBB to compounds identified from infested bolts were conducted at three field sites in 2016 and one site in 2017. In 2016, trapping assays were conducted May 27–July 6 at three sites with high black cherry density: Purdue Wildlife Area, Martell Forest, and ArborAmerica, Inc. (all Tippecanoe Co. IN). Field trials in 2017 were conducted May 9–August 11 at Purdue Wildlife Area. At each site, 4-unit Lindgren funnel traps were hung individually at 3 m from a metal conduit pole and baited with a semi-permeable polyethylene sachet containing 1 mL of an odor treatment. Traps were placed at least 15 m from one another.

In 2016, we assessed the extent to which adult PBB are attracted to benzaldehyde, the dominant compound associated with female-infested bolts. Due to the propensity of pure benzaldehyde to spontaneously oxidize to yield benzoic acid when exposed to air (Sankar et al. 2014) and to aid in volatile dispersion, we combined benzaldehyde with two different chemical "carriers": ethanol and isopropyl alcohol. These combinations, as well as the carriers, were each tested separately to determine if adult PBB were attracted to lures containing the compounds. Therefore, traps were baited with one of the following treatments: (1) blank (negative control); (2) isopropyl alcohol (4.0 mg/day, IPA); (3) ethanol (10 mg/day, EtOH); (4) benzaldehyde + EtOH (22 mg/day, 1:4 blend); and (5) benzaldehyde + IPA (14 mg/day, 1:4 blend). Benzaldehyde and

IPA were purchased from Sigma-Aldrich (St. Louis, MO) and EtOH was purchased from Thermo Fisher Scientific (Waltham, MA).

In 2017, we assessed the extent to which adult PBB are attracted to lures containing benzaldehyde or α-longipinene, a minor component of many of the volatile collections from infested cherry bolts. Minor components often play an important role in behavioral responses to combinations of volatile compounds and may play a role in modifying the response of PBB to benzaldehyde. Once again benzaldehyde was combined with a chemical carrier (EtOH) and α-longipinene was combined with methanol as a carrier. Each of these combinations were tested, as well as testing the carriers individually. Therefore, traps were baited with one of the following treatments: (1) blank; (2) EtOH; (3) methanol (8 mg/day); (4) benzaldehyde + EtOH (1:4 blend); (5) α-longipinene + methanol (14 mg/day, 1:4 blend); or (6) a benzaldehyde blend lure, along with a separate lure of the α-longipinene blend. Compounds were procured as before, and α-longipinene and methanol were purchased from Sigma-Aldrich.

In each field assay, collection cups were filled with a concentrated saltwater solution to kill and preserve captured insects. Beetles were collected three times per week and traps were rotated one position along the transect each collection period to control for location effects. Differences in the number of beetles captured by each treatment were analyzed using a non-parametric Kruskal-Wallis rank sum test followed by a post-hoc Dunn's Test using the statistical program R (Kruskal and Wallis 1952).

2.3 Results

2.3.1 Response of Beetles to Volatiles from Injured and Infested Bolts

In olfactometer assays using volatiles associated with infested black cherry and injured cherry bolts, male PBB were more attracted to female-infested bolts than all other odor sources (Fig. 2.1; $F_{7,194} = 6.35$, p-value < 0.001, Dunnett's test p-value < 0.001). No other odor sources were more attractive to either sex than ambient air.

In subsequent field assays using infested bolts, a total of 1,076 PBB were captured with an overall sex ratio of 1.45:1 (female:male). Both sexes of adult PBB were more attracted to female-infested bolts than male-infested or control bolts (Fig. 2.2; Kruskal-Wallis $\chi^2 = 42.22$, df = 3, p-value < 0.001). The sex ratio of PBB captured by female-infested bolts was more balanced (1.38:1)

than the male-infested (4:1) and injured cherry (4.89:1). Trap capture remained low until six days after bolts were placed on traps, with the majority of beetles captured six to ten days after bolt colonization and peak capture at day eight (Fig. 2.3).

2.3.2 Identification of Volatiles Associated with Colonized Bolts

The major component present in most samples was identified and verified as benzaldehyde. The relative abundance of benzaldehyde differed between treatments and over the time course following beetle colonization. Benzaldehyde was present in greater abundance in female-colonized bolts than male-infested (Fig. 2.4) and injured control bolts. Benzaldehyde abundance increased over time in all treatments, but particularly in female-colonized bolts, with a dramatic increase starting 13 days after colonization and continuing through day 20 (Fig. 2.5). Another compound, α-longipinene, was identified using the same method and detected in low relative abundance in all treatments prior to period of high benzaldehyde emission.

2.3.3 Response of PBB to Synthetic Compounds in Field Conditions

In both field experiments, adult PBB were attracted to traps baited with lures that included benzaldehyde. In the 2016 field bioassay, more PBB were captured in traps baited with lures that included benzaldehyde, irrespective of other compounds included in the mixture (Fig. 2.6; Kruskal-Wallis $\chi^2 = 80.997$, df = 4, p-value < 0.001). There was no statistical difference in the number of beetles captured between the two benzaldehyde treatments. The chemicals used as carriers in the benzaldehyde mixtures, ethanol and isopropyl alcohol, were themselves no more attractive than blank traps. Similarly, in the 2017 field bioassay both treatments that included benzaldehyde captured more PBB than all other treatments (Fig. 2.7; Kruskal-Wallis $\chi^2 = 44.085$, df = 6, p-value < 0.001). A total of 855 adult PBB were captured in 2017. Traps baited with lures containing α-longipinene, or its carrier (methanol), were no more attractive than blank traps.

2.4 Discussion

Woodboring insects are commonly attracted to volatile compounds associated with hosts, especially those in a weakened or stressed physiological state, as well as pheromones produced by sexually mature conspecifics (Reddy and Guerrero 2004, Gitau et al. 2013, Ranger et al. 2016). In

our study, adult PBB of both sexes were attracted to female-infested host material, and female colonization dramatically increased the amount of benzaldehyde collected from infested bolts. Field bioassays confirmed that benzaldehyde, irrespective of what chemical carrier was used, is attractive to adult PBB. Low-molecular-weight alcohols, especially ethanol, are commonly associated with stress or wounds in many genera of deciduous trees (Graham 1968, Kimmerer and Kozlowski 1982), but benzaldehyde is more strongly associated with injured *Prunus* species (Power and Moore 1909). Our field bioassays demonstrated that PBB are not attracted to ethanol alone, but that benzaldehyde is required to attract colonizing adults. Sensitivity to volatiles associated with a wounded host tree may allow PBB to identify *Prunus* hosts among many non-hosts and find physiologically susceptible trees to ensure successful colonization and larval development.

Benzaldehyde plays an important role in the behavior of diverse groups of insects but has not previously been recorded as a long-range attractant for bark beetle species. It is a biproduct of cyanogenesis and included in defensive secretions of adult tiger beetles (Blum et al. 1981) and chrysomelid larvae (Moore 1967). As an olfactory stimulant, many lepidopteran species are attracted to benzaldehyde produced by host plants and conspecific males (Dickens et al. 1993, Deng et al. 2004). Several pests of *Prunus* fruits, including plum curculio and the coffee berry borer, use host-produced benzaldehyde in combination with conspecific pheromones to find food and oviposition sites (Piñero et al. 2001, Pereira et al. 2012). In perhaps the most closely related example among wood-boring insects, an ambrosia beetle species (*Xyleborinus saxesenii* Ratzeburg) was attracted to a combination of ethanol and benzaldehyde in Chinese peach orchards during a portion of the growing season (Yang et al. 2018). Finally, benzaldehyde has been isolated from adult *Phloeotribus scarabaeoides* Bern., a congener of *P. liminaris*, but no behavioral role has been identified for this compound (Plaza et al. 2000). The functional role of benzaldehyde appears to be diverse among insects but, to our knowledge, this represents the first report of benzaldehyde acting as a long-range attractant of a wood-boring bark beetle.

Although benzaldehyde production increases when female PBB colonize black cherry, the proximate source of benzaldehyde in this system remains unclear. Possible sources may include: (1) endogenous synthesis by female PBB, (2) sequestration and subsequent emission of benzaldehyde ingested from host tissue, or (3) increased cyanogenesis from host tissue as females colonize black cherry. Our results provide substantial evidence for *de novo* synthesis of

benzaldehyde by female PBB. Colonization of black cherry bolts by females, the pioneer sex, increased benzaldehyde emission several times over that of male-infested and control bolts. Dynamic headspace collections also demonstrated a definite lag time between colonization and benzaldehyde emission. Although the lag time for benzaldehyde detection in experimental bolts was greater than the time observed for peak attraction to traps baited with infested logs, this may be due to somewhat cooler and unnatural lab conditions, thereby reducing the activity of colonizing adult beetles. Similar lag times have been noted in the pheromone production of other bark beetles that require some host feeding in order to produce pheromone, including the conifer-infesting bark beetles *Ips pini* (Say) and *Dendroctonus ponderosae* Hopkins (Tillman et al. 1998, Pureswaran et al. 2000). Although *de novo* synthesis is accepted as the predominant form of pheromone biosynthesis in bark beetles that infest conifers, sequestration of host precursors or oxidation of bioactive compounds could still play a role in benzaldehyde production. Examples of host precursors involved in pheromone production include α-pinene and n-heptane derivatives, which are used by conifer-infesting beetles (Blomquist et al. 2010). In addition, increased benzaldehyde in female-infested material may result from greater tissue disruption by beetle tunneling and feeding. Damaged *Prunus* stem and bark tissue exposes cyanogenic precursors (prunasin and mandelonitrile) to oxygen, resulting in the production of benzaldehyde and hydrogen cyanide (Power and Moore 1909). Although some tunneling occurs when males infest black cherry in the absence of females, most of these beetles appear to cease tunneling after a short time (MWE pers. obs.). Several biosynthetic possibilities exist for the production of benzaldehyde, but incorporation of a host defensive compound for aggregation and mating may be an example of a sexual kairomone (*sensu* Ruther 2004), similar to the use of myrcene and α-pinene by conifer-infesting beetles (Miller and Lindgren 2000, Hofstetter et al. 2012).

Current PBB management relies on cultural controls that require significant labor and time (Rexrode and Smith 1990), but our results suggest that benzaldehyde, with ethanol or isopropyl alcohol as a carrier, could be used as a lure in mass trapping or trap-tree tactics to manipulate beetle populations. Mass trapping tactics for bark beetles often include multi-funnel traps placed in forest and plantation stands to reduce the number of colonizing and reproducing adults. Mass trapping tactics have been employed to control pest bark beetle species, such as *Ips duplicatus*, *Ips typographus*, and *Dendroctonus pseudotsugae* (Ross and Daterman 1997, Schlyter et al. 2001, Wermelinger 2004). Attractive compounds can also be used to attract beetles to host trees with

little value (i.e., trap trees) to monitor population levels or concentrate colonizing beetles for insecticide applications to reduce bark-beetle pressure (Raty et al. 1995, Prokopy et al. 2003). While the primary focus of this research has been management of PBB to protect veneer-quality black cherry, *Prunus* species are also heavily represented in many fruit orchards and lures that include benzaldehyde may offer an alternative to pesticide applications for pest management. For example, benzaldehyde has been paired with the pheromone of the plum curculio (*Conotrachelus nenuphar* (Herbst) to monitor flight activity to properly time insecticidal sprays in commercial production of *Prunus* spp. stone-fruits (Leskey and Wright 2004).

Identifying novel semiochemicals is increasingly important as forest and plantation managers search for sustainable methods to monitor and manage insect pests, as a changing climate increases biotic and abiotic stress (Bentz et al. 2010, Weed et al. 2013). Utilizing semiochemicals within IPM programs can reduce the development of pesticide resistance and aid in long-term management of both native and exotic pest species. Our results support the use of benzaldehyde to exploit the chemically mediated colonization behavior of PBB and manage this important pest of black cherry within the CHFR. In addition, our results imply that the use of additional compounds, in concert with ethanol, may increase the efficacy of native and invasive bark-beetle management.

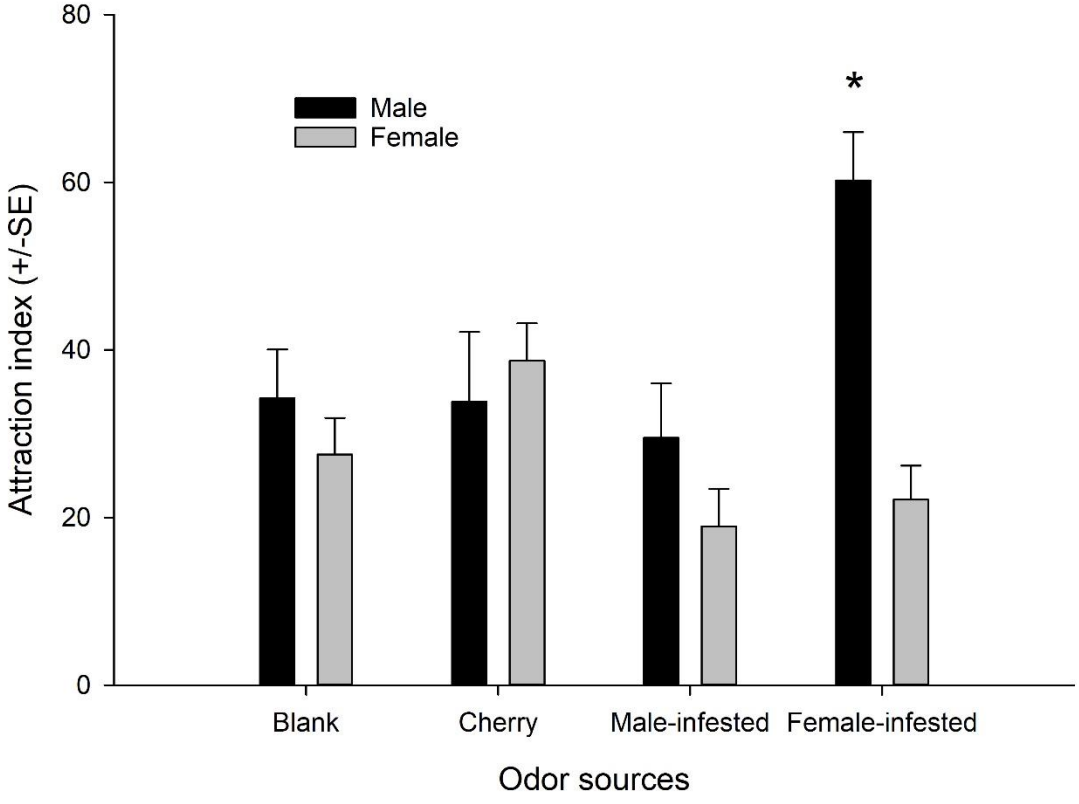

Fig. 2.1: Mean attraction index (+SE) of adult PBB to different odor sources in a straight-tube olfactometer. Statistically different response from the control treatment by sex indicated by * ($F_{7,194} = 6.35$, $P < 0.001$, Dunnett's test $P < 0.001$).

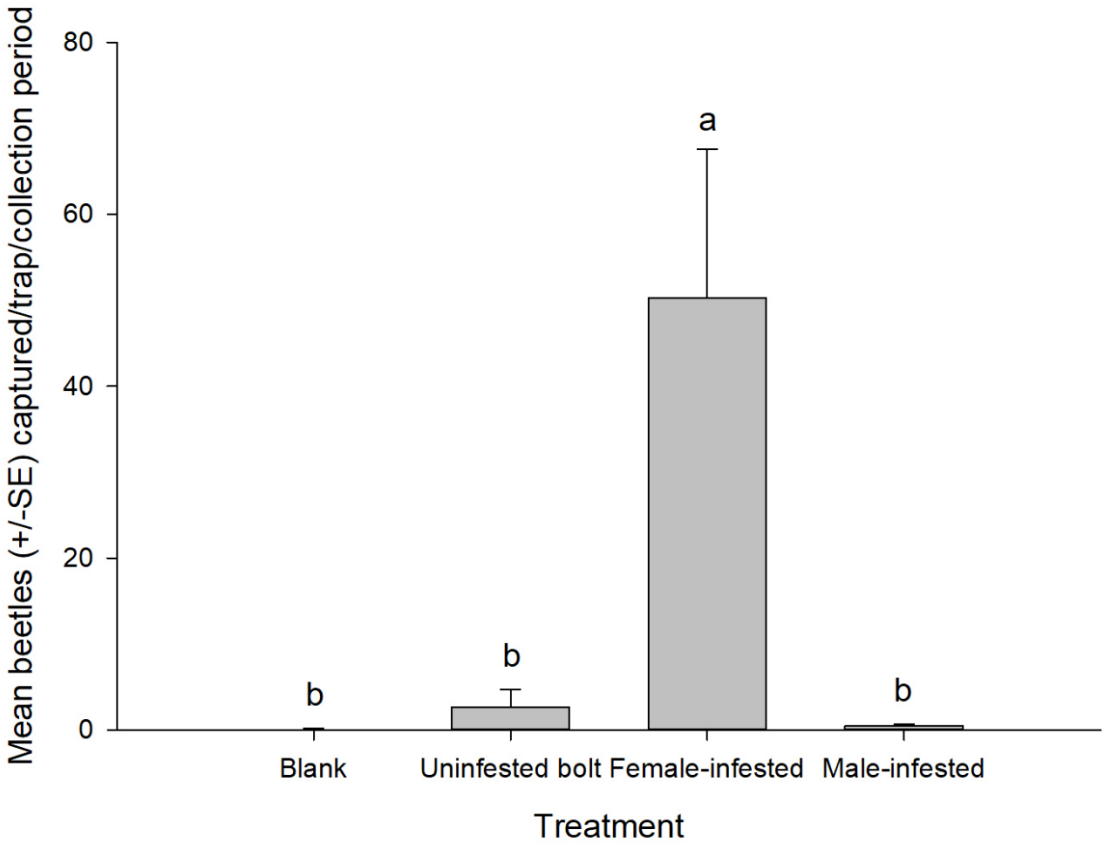

Fig. 2.2: Mean PBB captured (+SE) in 2015 during field assay using cherry bolts infested with different sexes or artificially wounded with drill holes. Different letters indicate statistically different treatments (P = 0.05).

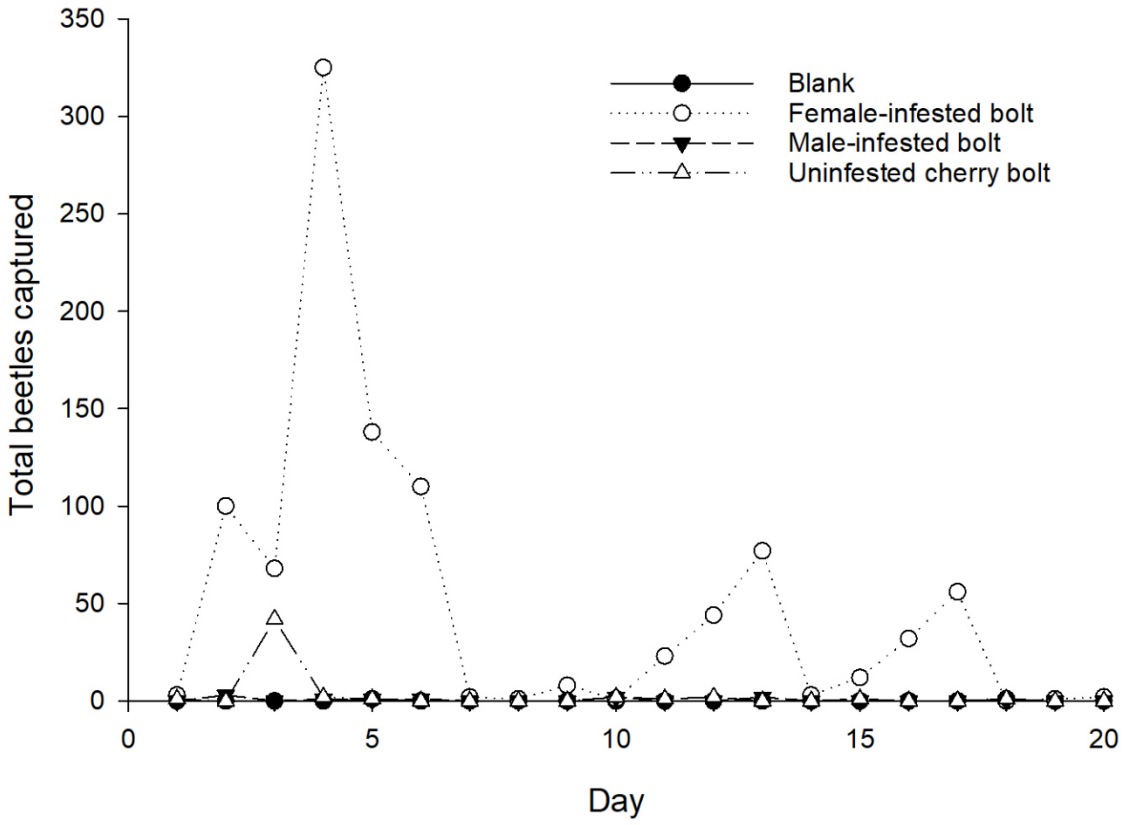

Fig. 2.3: Total number of PBB captured each day following placement of infested bolts on multi-funnel traps at one site in 2015. Traps were baited with bolts infested with female or male PBB, an artificially wounded cherry bolt, or no bolt (blank control). Beetles were collected daily.

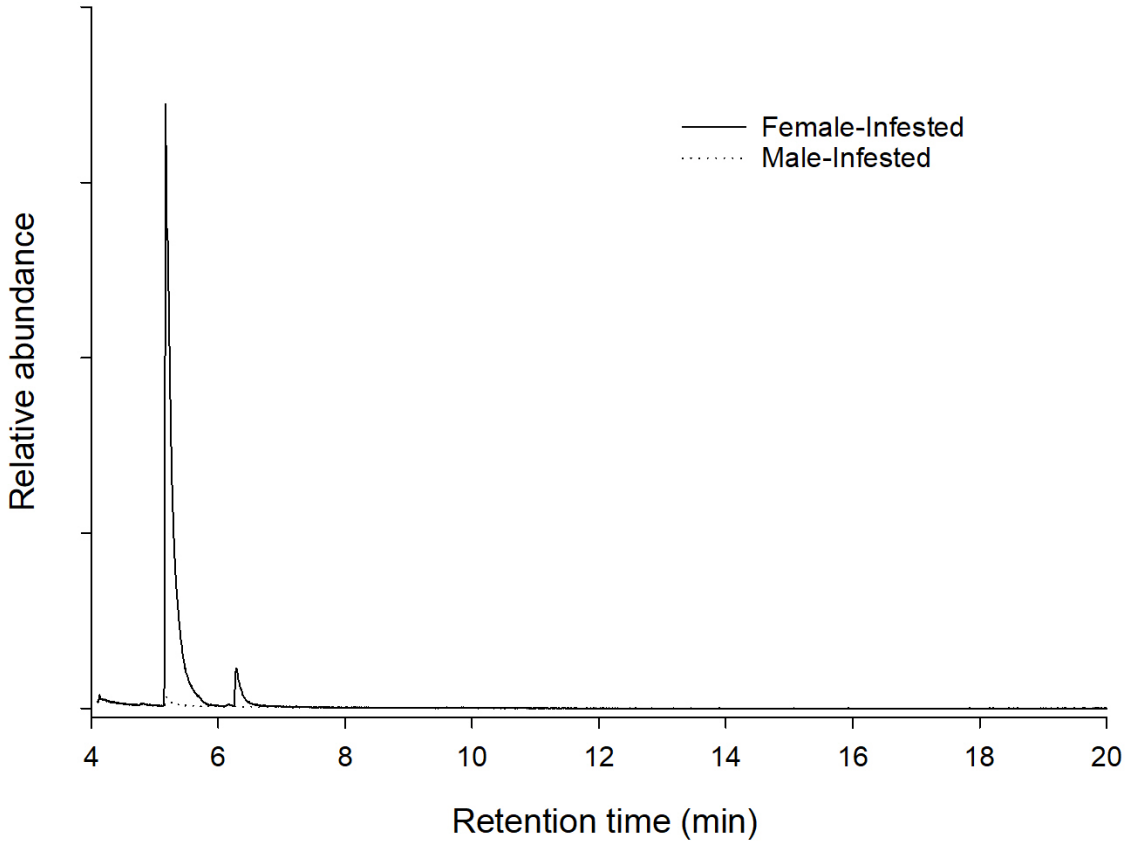

Fig. 2.4: Representative chromatogram of volatile collections from male- and female-infested cherry bolts 18 days after colonization. Major (~5 min) and minor (~6.5 min) peaks were identified as benzaldehyde and α-longipinene, respectively.

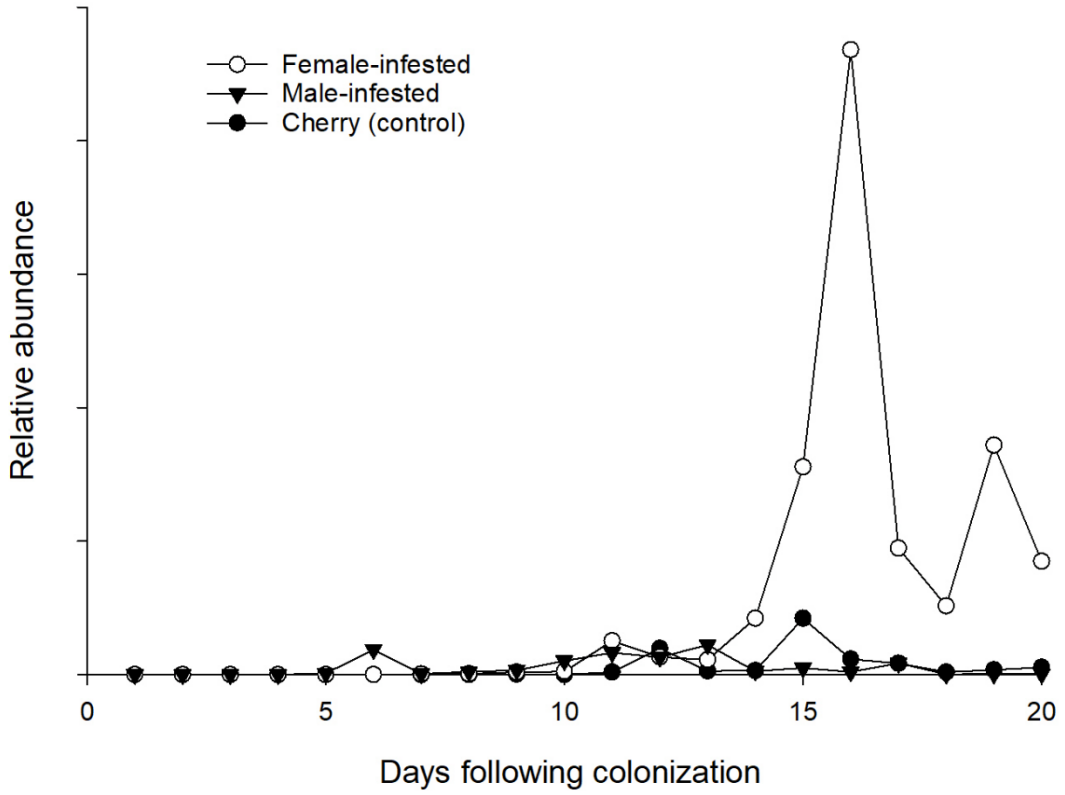

Fig. 2.5: Relative abundance of benzaldehyde in PBB-infested and artificially wounded black cherry bolts. Dynamic headspace samples were collected over 12 hours (20:00–8:00 the following day) each day for 20 days following beetle colonization and artificial wounding.

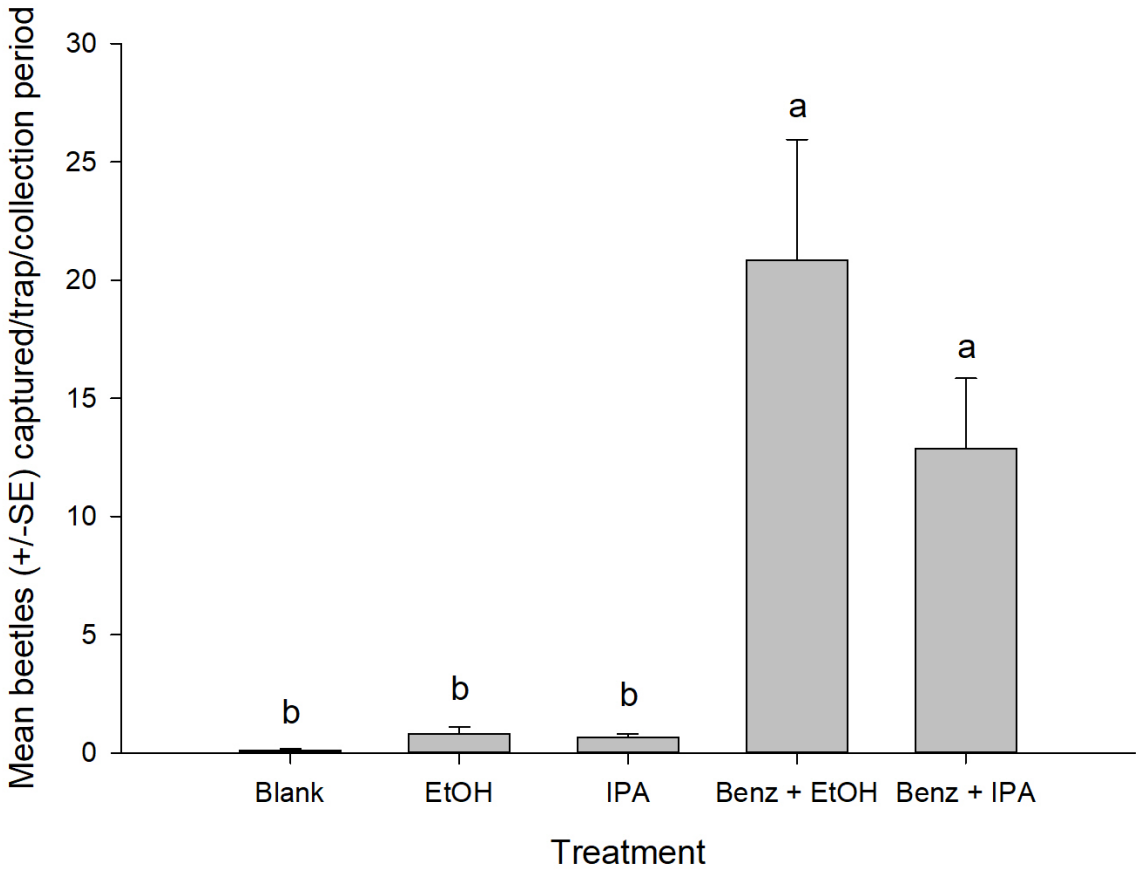

Fig. 2.6: Mean PBB (+SE) collected at three field sites in 2016. Multi-funnel traps were baited with one of the following treatments: no lure (blank), ethanol (EtOH), isopropyl alcohol (IPA), or blends of 1:4 benzaldehyde:EtOH (Benz + EtOH), and 1:4 benzaldehyde:IPA (Benz + IPA). Different letters represent statistical differences among means (P = 0.05).

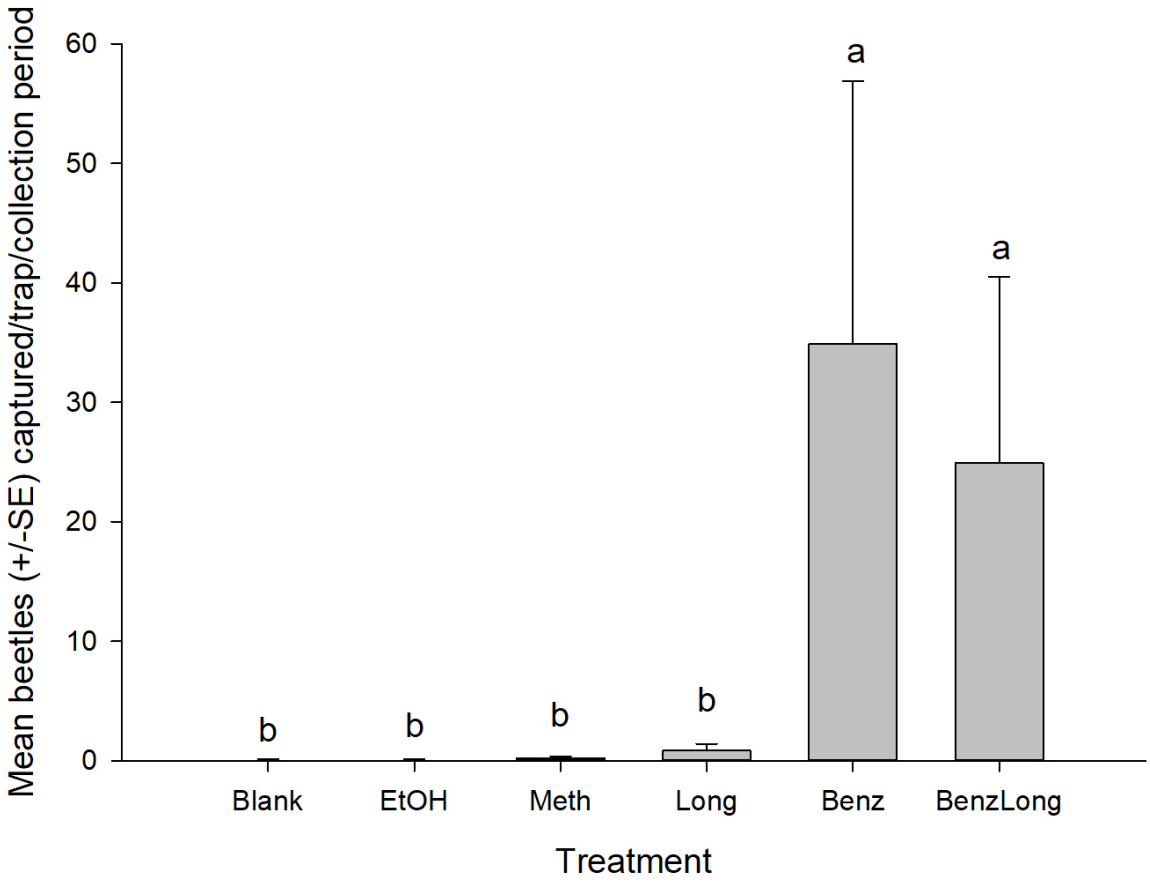

Fig. 2.7: Mean PBB (+SE) collected at one field site in 2017. Multi-funnel traps were baited with one of the following treatments: no lure (blank), ethanol (EtOH), methanol (Meth), blends of 1:4 α-longipinene:methanol (Long), 1:4 benzaldehyde:EtOH (Benz), or combination of Benz and Long lures (BenzLong). Different letters represent differences among means (P = 0.05).

CHAPTER 3. FUNGAL VOLATILES MODIFY THE RESPONSE OF INVASIVE AMBROSIA BEETLE SPECIES TO ETHANOL LURES

3.1 Introduction

Ambrosia beetles (Coleoptera: Scolytinae, Platypodinae) are among the most invasive insects in the world. Their cryptic lifecycle, unique reproductive behavior, and broad host range has allowed them to increasingly invade and establish in new environments that affect the ecology and economics of forests worldwide. Within the United States, exotic ambrosia beetles damage a broad range of hardwood tree species and vector pathogenic fungi to naïve host trees. The ability of exotic ambrosia beetles to rapidly establish in novel environments and cause extensive economic and ecological damage threatens the health and productivity of forests worldwide (Hulcr and Dunn 2011, Ploetz et al. 2013).

Many ambrosia beetles are highly proficient at establishing incipient populations due to their cryptic lifecycle, haplodiploid reproduction, and broad host range. These beetles are also obligate fungal feeders, carrying fungal spores that they inoculate into gallery walls, which will serve as the sole source of nutrients for larvae and adults (Farrell et al. 2001, Raffa et al. 2015). Ambrosia beetle colonization can result in damage due to reduced growth and degradation of host xylem, making wood unsuitable for harvest as lumber and weakening the structure of trees, leading to breaks in branches and main stems (Orbay et al. 1994, Alfaro et al. 2007, Boland 2016). Perhaps even more alarming is the rising incidence of ambrosia beetle-vectored pathogenic fungi that can cause widespread tree mortality due to a lack of co-evolved defenses (Hulcr and Dunn 2011, Ploetz et al. 2013). For example, recent establishment of the exotic ambrosia beetle *Xyleborus glabratus* Eichoff and its symbiotic fungus *Raffaela lauricola* has led to widespread laurel wilt in the southeastern U.S. and exotic *Euwallacea* sp. transmit Fusarium fungi that are causing increased mortality in hardwood trees in California and Florida (Fraedrich et al. 2008, O'Donnell et al. 2016).

Current management of invasive ambrosia beetles relies on using semiochemical-based tactics to monitor and manipulate flight behavior. A large number of ambrosia beetles are attracted to ethanol, a volatile compound associated with stressed or injured hardwood trees, but other compounds have shown variable increases in attraction or repellency (Ranger et al. 2010). Due to the attractive nature of ethanol, it is utilized on multi-funnel traps as part of a standard monitoring protocol throughout the United States (Rabaglia et al. 2008, Ranger et al. 2016). Conophthorin, a

compound produced by various deciduous trees, has enhanced attraction of ambrosia beetles to ethanol in some studies (VanDerLaan and Ginzel 2013, Ranger et al. 2014), whereas in others the results are highly variable (Miller et al. 2015, 2018). On the other hand, verbenone, produced by coniferous bark beetles and trees alike, has repelled several ambrosia beetle species from attractive lures in field experiments. As incursions by exotic ambrosia beetles continue to increase, there is a growing need to increase the efficacy of monitoring lures and semiochemical-based management tactics.

Recent studies have demonstrated that volatile compounds produced by symbiotic and co-occurring fungi of ambrosia beetles are behaviorally active and may enhance or reduce attraction to other attractants (Cale et al. 2019, Kandasamy et al. 2019). For example, in laboratory assays three species of invasive ambrosia beetles were attracted to volatiles of their symbiotic fungi, with varied responses to the fungi of other species (Hulcr et al. 2011). Additional laboratory experiments have also demonstrated attraction of other highly invasive ambrosia beetles to volatiles of their symbiotic fungus (Kuhns et al. 2014, Egonyu and Torto 2018). Fewer experiments have been conducted in field settings, but some have also shown varying rates of attraction, and even repellency, in response to compounds produced by symbiotic fungi (Kuhns et al. 2014, Ranger, Dzurenko, et al. 2021). Recent findings by (Ranger et al. 2021) have shown that several volatile components of volatiles produced by the symbiotic fungi of *Xylosandrus germanus* (Blandford) were mild arrestants in olfactometer studies, yet repelled beetles when utilized in field-trapping experiments. Although the current laboratory and field results are currently inconclusive, fungal volatiles may play a role in the long-range host selection of invasive ambrosia beetles and could be used to increase the efficacy of monitoring and management tactics.

The objective of this study is to determine the extent to which a suite of fungal volatiles modify attraction of invasive ambrosia beetles to attractive ethanol lures. We test the hypothesis that fungal volatiles enhance the attraction of ambrosia beetles to ethanol, especially those species that are symbiotic with the volatile-producing fungi. To test this hypothesis, trapping studies were conducted over three years at a variety of field sites throughout central and southern Indiana. Although many exotic ambrosia beetle species are established in Indiana, this study focuses on the three most common and abundant species throughout the Central Hardwood Forest: *Xylosandrus crassiusculus* (Motschulsky), *Xylosandrus germanus* (Blandford), and *Xyleborinus saxesenii* (Ratzeburg). These species overlap in distribution and their host range encompasses more than 100

hardwood tree species present in the Central Hardwood Forest; therefore, the semiochemicals of each may significantly overlap.

3.2 Methods and Materials

To determine the extent to which fungal compounds modify attraction of the focal species to ethanol lures, trapping studies were conducted in plantations and hardwood forests of mixed species in central Indiana. Information for all ten sites used during the experiments is included as Table 3.1. At each site, transects of traps were positioned approximately linearly through plots of mixed hardwood trees. Traps consisted of four-unit Lindgren multi-funnel traps (Synergy Semiochemical, Burnaby, B.C., Canada) or bottle traps made of a 2-L plastic container attached to a smaller plastic collection container, as described in Ranger et al. (2010). The traps were attached by a 30-cm polyvinyl chloride (PVC) crossbar to a 2 m tall PVC held upright by 0.5 m of construction rebar. To avoid mixing lure plumes, traps within a transect were placed >15 m from one another and transects were established >25 m apart. Traps were moved weekly to the next position in the transect to avoid any positional bias. Individual traps were baited as described below and concentrated salt water was placed in the collection container, to preserve captured insects, which were collected weekly or bi-weekly and identified to species.

Lure treatments included a negative control (trap without lure), a positive control (ethanol lure), and fungal volatile lures in addition to an ethanol lure (indicated by "+"). Fungal volatiles were identified from *Ambrosiella grosmanniae* and *Ambrosiella roeperi*, the symbiotic fungi of *X. germanus* and *X. crassiusculus*, respectively (Ranger et al. 2021, unpublished data, Ranger et. al). The fungal volatiles included a number of low-molecular weight alcohols (e.g. isoamyl alcohol, isobutyl alcohol, benzyl alcohol) as well as methyl benzoate and methyl phenylacetate. All lures, excluding ethanol, were purchased as bubble lures from Synergy Semiochemical. Ethanol lures were made by placing 1 mL of 70 percent ethanol (70:30 ethanol:water) in a semi-permeable polyethylene sachet (press-seal bags, Bagette model 14,770; 5.1 x 7.6 cm, 0.05-mm wall thickness; Cousin Corp., Largo, FL). Lures were hung from a short wire attached to the middle of each trap; fungal volatile and ethanol lures, when present together, were attached next to one another. Information regarding lure treatments is summarized in Table 3.2.

3.2.1 Field Experiments

Experiment 1 (Exp. 1)

This experiment was conducted in 2018 to test the extent to which volatiles produced by symbiotic fungi enhanced attraction of invasive ambrosia beetles to ethanol lures. A total of four transects were used, two at the Lugar site, and two at the Martell site, with eight traps in each transect. All transects utilized bottle traps and individual traps were treated with one of the following treatments: (1) ethanol (EtOH [+]), (2) benzyl alcohol (Benz Alc +), (3) 2-Ethyl-1-hexanol (Hex +), (4) isoamyl alcohol (IAA +), (5) methyl benzoate (Meth Benz +), (6) methyl phenylacetate (Methyl Phen +), (7) phenethyl alcohol (Phen Alc +), or (8) an unbaited trap (Blank). Insects were collected weekly from 11 May–27 July, corresponding with the peak flight of ambrosia beetles in Indiana.

Experiment 2 (Exp. 2)

This experiment was conducted in 2019 to test the extent to which a subset of fungal lures used in Exp. 1 modify the attraction of invasive ambrosia beetles to ethanol lures. A total of four transects were used in the experiment, with two placed at the Nelson site, and one placed at each of the Finley and Lugar sites. To determine if trap type influenced ambrosia beetle capture, all transects used multi-funnel traps, unlike the bottle traps used in Exp. 1. In addition, piperitone, a compound isolated from several general of tropical grasses that has previously repelled *Euwallacea* spp. ambrosia beetles in field studies (Byers et al. 2018) was added as a lure treatment in each of the transects. Individual traps were treated with one of the following treatments: (1) EtOH [+], (2) Benz Alc +, (3) Hex +, (4) IAA +, (5) IBA +, (6) Meth Benz +, (7) piperitone (Piper +), or (8) the blank trap. Insects were collected weekly from 30 April–29 July and processed as previously described.

Experiment 3 (Exp. 3)

This experiment was conducted in 2020 at additional sites to test the extent to which fungal volatiles and piperitone modify attraction to ethanol using both trap types. Paired transects of ten traps were placed at each site; bottle traps were used for one transect, while multi-funnel traps

were utilized in the other. A total of 20 transects were used, with one pair placed at each of the Darlington, DPAC I, DPAC II, Finley, Martell, Miller, Nelson, Lugar, SEPAC I, and SEPAC II sites (see Table 3.2 for a summary of treatments used in each experiment). Individual traps were baited with one of the following treatments: (1) EtOH [+], (2) Benz Alc +, (3) Hex +, (4) IAA +, (5) IBA +, (6) Meth Benz +, (7) Methyl Phen +, (8) Phen Alc +, (9) Piper +, or (10) the blank trap. Beetles were collected weekly during 14 April–4 August and processed as previously described, except at the DPAC I, DPAC II, Miller, SEPAC I, and SEPAC II sites, where insects were collected bi-weekly.

3.2.2 Statistical Analyses

Data for each beetle species within each of the experiments were analyzed independently. Collection periods were used for analysis only if it met a standard threshold (mean number of beetles was greater than one per trap) for the species being analyzed. To determine if populations of beetles differed among sites, mean number of beetles captured in positive control traps (WTB [+]) from each site were compared using a non-parametric Kruskal-Wallis rank sum test, followed by a post-hoc Dunn test to determine pair-wise differences (Sokal and Rohlf 1995). In 2020, differences between multi-funnel and bottle traps were tested in a similar fashion. Those sites and trap types that did not yield significantly different results were combined for analysis and presentation.

To compare the response of beetles to lure treatments, the mean number of beetles captured by each treatment among similar sites was used as the response variable for analysis. Because mean count data failed the assumption of normality, a non-parametric Kruskal-Wallis rank sum test was used to determine statistical significance among treatments (Kruskal and Wallis 1952). A post-hoc Dunn's test was used to determine pair-wise differences among the treatments within each experiment (Dunn 1964).

3.3 Results

Exp. 1

A total of 729 beetles were captured during 2018, including 582 *X. crassiusculus*, 124 *X. germanus*, and 23 *X. saxesenii*. Due to low captures of *X. germanus* and *X. saxesenii*, these species

were not analyzed for significant differences. Population densities of *X. crassiusculus* were similar among all sites (Kruskal-Wallis $\chi^2 = 4.8381$, df = 3, p-value = 0.184). Both benzyl and isoamyl alcohol lures reduced the number of *X. crassiusculus* captured when paired with ethanol lures, although each treatment captured more beetles than blank traps (Fig. 3.1; Kruskal-Wallis $\chi^2 = 77.909$, df = 7, p-value < .001). While phenethyl alcohol showed an intermediate level of repellency, all other lure treatments captured a similar number of beetles to the ethanol lures.

Exp. 2

A total of 4,665 beetles were captured during 2019, including 284 *X. crassiusculus*, 324 *X. germanus*, and 4,057 *X. saxesenii*. The population density of beetles was similar among all sites for *X. crassiusculus* (Kruskal-Wallis $\chi^2 = 4.7035$, df = 3, p-value = 0.194), *X. germanus* (Kruskal-Wallis $\chi^2 = 6.2633$, df = 3, p-value = 0.099), and *X. saxesenii* (Kruskal-Wallis $\chi^2 = 11.513$, df = 3, p-value = 0.092). Both isoamyl and isobutyl alcohol repelled *X. crassiusculus* from traps baited with attractive lures, while all other treatments were similar to those baited solely with ethanol lures (Kruskal-Wallis $\chi^2 = 32.853$, df = 7, p-value < .001; Fig. 3.2A). In contrast, *X. germanus* were repelled by benzyl alcohol and piperitone, and isobutyl alcohol showed intermediate repellency (Kruskal-Wallis $\chi^2 = 50.682$, df = 7, p-value < .001; Fig. 3.2B). There were no differences in the response of *X. saxesenii* to lure treatments, although all traps baited with lures captured more beetles than blank traps (Kruskal-Wallis $\chi^2 = 19.972$, df = 7, p-value < .01; Fig. 3.2C).

Exp. 3

A total of 92,095 beetles were captured during 2020, including 39,664 *X. crassiusculus*, 34,766 *X. germanus*, and 17,595 *X. saxesenii*. SEPAC 1 had a much higher density of *X. crassiusculus* than all other sites (Kruskal-Wallis $\chi^2 = 193.96$, df = 9, p-value < 0.001); therefore, means from SEPAC 1 and all other sites were analyzed separately. Lure treatments did not influence trap capture at SEPAC 1, but benzyl alcohol, isoamyl alcohol, and isobutyl alcohol all reduced capture of *X. crassiusculus* at other sites, the in both multi-funnel (Kruskal-Wallis $\chi^2 = 117.1$, df = 9, p-value < 0.001; Fig. 3.3A) and bottle (Kruskal-Wallis $\chi^2 = 193.98$, df = 9, p-value

< 0.001; Fig. 3.3B) traps, respectively. Phenethyl alcohol was also mildly repellent to *X. crassiusculus*, but all other lure treatments were comparable to the positive control.

SEPAC 1 also had a much higher density of *X. germanus*than all other sites (Kruskal-Wallis χ^2= 243.37, df = 9, p-value < 0.001), but there were no differences by trap type within site. No differences were detected in the response to lure treatments at SEPAC 1, but isobutyl and phenethyl alcohol repelled *X. germanus* in multi-funnel traps (Kruskal-Wallis χ^2 = 49.773, df = 9, p-value < 0.001; Fig. 3.4A) and benzyl, isobutyl, and phenethyl alcohols, as well as piperitone, were repellent in bottle traps (Kruskal-Wallis χ^2 = 161.31, df = 9, p-value < 0.001; Fig. 3.4B) at all other sites.

The density of *X. saxesenii* was greater at the Darlington and Lugar sites than at all others (Kruskal-Wallis χ^2 = 34.195, df = 9, p-value < 0.001), but mean capture between trap type did not differ. Isobutyl alcohol repelled *X. saxesenii* at the Darlington and Lugar sites, while all other lures were similar to the positive control (Kruskal-Wallis χ^2 = 28.388, df = 9, p-value < 0.001; Fig. 3.5B). Within all the other sites, phenethyl alcohol captured less *X. saxesenii* than all other lure treatments (Kruskal-Wallis χ^2= 55.374, df = 9, p-value < 0.001; Fig. 3.5A).

3.4 Discussion

The results of this study demonstrate that fungal alcohols modify the attraction of invasive ambrosia beetles to ethanol in a field setting, and that such responses can be genera- or species-specific. Contrary to our original hypothesis, individual volatile compounds associated with the symbiotic fungi of *X. crassiusculus* and *X. germanus* did not enhance attraction to ethanol for any of the target species, but rather acted as repellents in several cases. In general, the two *Xylosandrus* species were both strongly repelled by benzyl alcohol and isobutyl alcohol. Both species were also repelled by phenyethyl alcohol in some experiments, but the two also differed, as *X. crassiusculus* was repelled by isoamyl alcohol while *X. germanus* found piperitone to be repellent. These results differ somewhat from the findings of field experiments reported by Ranger et al. (2021). In that study, *X. germanus* was repelled by both phenethyl and isoamyl alcohol, while this study did not reveal that isoamyl alcohol repelled *X. germanus*. The response of *X. saxesenii* differed from that of the *Xylosandrus* species, only showing strong inhibition to isobutyl and benzyl alcohol in one experiment.

Ambrosia beetles are intimately associated with fungal partners and may use the presence of volatiles produced by competing fungi in host selection and oviposition (Cale et al. 2016, Kandasamy et al. 2016). Although the compounds used in the current study were identified from symbiotic fungi of *X. crassiusculus* and *X. germanus*, most of these volatile components have also been identified from other biological sources that these ambrosia beetles may attempt to avoid. For example, isobutyl alcohol and benzyl alcohol, the most repellent compounds across all experiments reported here, have been shown to be emitted by ophiostomatoid fungi that inhabit non-host conifer trees with *Ips* bark beetles, as well as from *Rafaella lauricola*, the symbiotic fungus of *Xyleborus glabratus* (Kuhns et al. 2014, Cale et al. 2019). In fact, the presence of isobutyl alcohol and benzyl alcohol may explain *X. crassiusculus* avoidance of *R. lauricola* volatiles in previous laboratory experiments (Hulcr et al. 2011). To *Xylosandrus* species, these compounds, especially as individual components, may act as cues of hosts that are unsuitable for colonization. Although host species appears to be less important for these invasive species, the presence of volatiles associated with non-hosts and competing fungi may repel beetles from landing on chemical sources. Much of the colonization behavior of these beetles appears to be guided by finding the appropriate host physiological status for growth of symbiotic fungi and larval development (Ranger et al. 2010, 2018), and these compounds may be associated with environments that negatively affect mutualists. Additional compounds that were somewhat repellent in this work, such as phenethyl alcohol and piperitone, are similarly produced by non-mutualist fungi and non-host plants (Byers et al. 2018, Cale et al. 2019).

These findings also showed substantial overlap in response to fungal volatiles, especially between the *Xylosandrus* congeners. Similar results were obtained in laboratory experiments. For example, *Xyleborus glabratus* was attracted not only to volatiles of its symbiotic fungus, but also to that of the symbiotic fungus of *Xyleborus compactus* (Hulcr et al. 2011). Although not always true, response to semiochemicals often follows taxonomic lines, hinting at evolutionary conservation of certain compounds as important sources of behavioral information (Symonds and Gitau 2016, Chapter 1 herein). This is not altogether surprising as these compounds are integral to processes that are intimately linked to evolutionary fitness, such as locating mates and hosts. Not only does this suggest that more closely related species may use similar semiochemicals, but also that it may be possible to develop more generic repellents that affect several pest species simultaneously.

Although results of the current research provide no evidence of increased attraction to symbiotic fungal volatiles, this study was limited to testing single volatile components with a low release rate in combination with ethanol. Volatile compounds produced by fungi in natural circumstances are most likely to be combinations of compounds rather than solitary components. Indeed, experiments identifying fungal volatiles have always found a suite of compounds produced even on artificial media (Kuhns et al. 2014, Cale et al. 2019, Ranger et al. 2021). Therefore, although this study tests the response of beetles to individual components, blends of volatiles are more likely to be biologically and behaviorally relevant to responding beetles. Laboratory studies that have shown attraction of ambrosia beetles to fungal volatiles have often relied on all volatiles produced by growing fungi rather than synthetic compounds (Hulcr et al. 2011, Kuhns et al. 2014, Ranger et al. 2021). Despite the paucity of field experiments with fungal volatiles, Kuhns et al. (2014) found evidence for attraction to a suite of compounds produced by symbiotic fungi, when tested as a blend. Further testing of synthetic compounds in biologically relevant blends may demonstrate long-range attraction to symbiotic ambrosia beetles. In addition to combining components, the concentration and release rate of the lure compounds may play an important role in the behavioral response of ambrosia beetles. For example, the attraction of *X. glabratus* to manuka oil lures is only enhanced by fungal volatiles at high release rates, while low release rates produce no response (Kuhns et al. 2014). The importance of ethanol lure release rate has also been demonstrated for *X. germanus* (Reding et al. 2011), one of the focal species of this study, and release rates of fungal volatiles may be similarly critical for behavioral responses. Conducting further studies with biologically relevant fungal volatile blends over a variety of release rates may elucidate the role of these factors in beetle responses.

The identification and use of repellents may increase the efficacy of semiochemical-based management tactics for invasive ambrosia beetles. Repellent lures may be used to protect specific valuable trees or provide area-wide protection by placing lures along plot edges. Previous results have shown decreased landing on hosts in response to repellent compounds in many cases, such as with the redbay ambrosia beetle (Hughes et al. 2017, Martini et al. 2020). Also, repellents can play an essential role in "push-pull" tactics. This tactic uses a repellent compound (e.g. verbenone) as the "push" to drive beetles away from forest edges or valuable trees, and uses an attractive compound (e.g. ethanol) to pull beetles into kill-traps or trap trees (Cook et al. 2007). Identification of attractants and repellents for invasive ambrosia beetle species has increased recent development

of many such systems. Results in various systems have been mixed, with repellents enhancing capture rates of attractive traps, in some cases (Byers et al. 2020, Rivera et al. 2020), while others have had little effect on reducing tree damage (Werle et al. 2019). Repellent fungal volatiles may play an important role in increasing the efficacy of repellent tactics designed to reduce attacks and colonization of susceptible host trees, but further testing is needed.

Although our results complement recent findings from field studies showing that ambrosia beetle chemo-attraction can be modified by fungal volatiles (Kuhns et al. 2014, Ranger et al. 2021), additional studies are needed to increase our understanding of these semiochemicals and how best to utilize these compounds in beneficial management tactics. Field experiments using fungal volatiles are still rare and different environmental conditions may play a role in responses to these compounds. It also remains unknown if these compounds are used as long-range identification of hosts and non-hosts or are solely short-range feeding and oviposition promoters. For practical application, determining the importance of compound concentration, lure release rate, and active range of lures will be directly related to the efficacy of using these compounds for management. With continued research, fungal volatiles may aid in addressing the ever-increasing need for additional tools to monitor and manage exotic ambrosia beetles.

Table 3.1: Site information for ten experimental sites located throughout Indiana.

Site	Abbreviation	County	Plot size	Species	Exp. 1	Exp. 2	Exp. 3
Darlington Woods	Darlington	Montgomery	57 acres	Oak, maple, black cherry			x
Davis Purdue Ag Center	DPAC I	Randolph	126 acres	Oak, maple			x
Davis Purdue Ag Center	DPAC II	Randolph	126 acres	Oak, maple			x
Finley Woods	Finley	Clay	40 acres	Oak, maple		x	x
Martell Forest	Martell	Tippecanoe	477 acres	Chestnut, maple	x		x
Miller Woodlands	Miller	Grant	191 acres	Oak, maple			x
Nelson-Stokes-Lewman	Nelson	Putnam	162 acres	Oak, hickory, maple		x	x
Richard G. Lugar Forestry Farm	Lugar	Tippecanoe	96 acres	Oak, black cherry	x	x	x
Southeastern Indiana Purdue Ag Center	SEPAC I	Jennings	230 acres	Oak, maple, hickory			x
Southeastern Indiana Purdue Ag Center	SEPAC II	Jennings	72 acres	Swamp oak			x

Table 3.2: Lure treatments for a series of three trapping experiments conducted over three years (2018-2020).

Treatment	Abbreviation	Release rate	Exp. 1	Exp. 2	Exp. 3
Ethanol	EtOH [+]	25 mg/day	x	x	x
Benzyl alcohol	Benz Alc +	4-6 mg/day	x	x	x
2-Ethyl-1-hexanol	Hex +	2-3 mg/day	x	x	x
Isoamyl alcohol	IAA +	4-5 mg/day	x	x	x
Isobutyl alcohol	IBA +	4-5 mg/day		x	x
Methyl benzoate	Meth Benz +	10 mg/day	x	x	x
Methyl phenylacetate	Methyl Phen +	7-8 mg/day	x		x
Phenethyl alcohol	Phen Alc +	2.5-3.5 mg/day	x		x
Piperitone	Piper +	6-8 mg/day		x	x
No trap	Blank		x	x	x

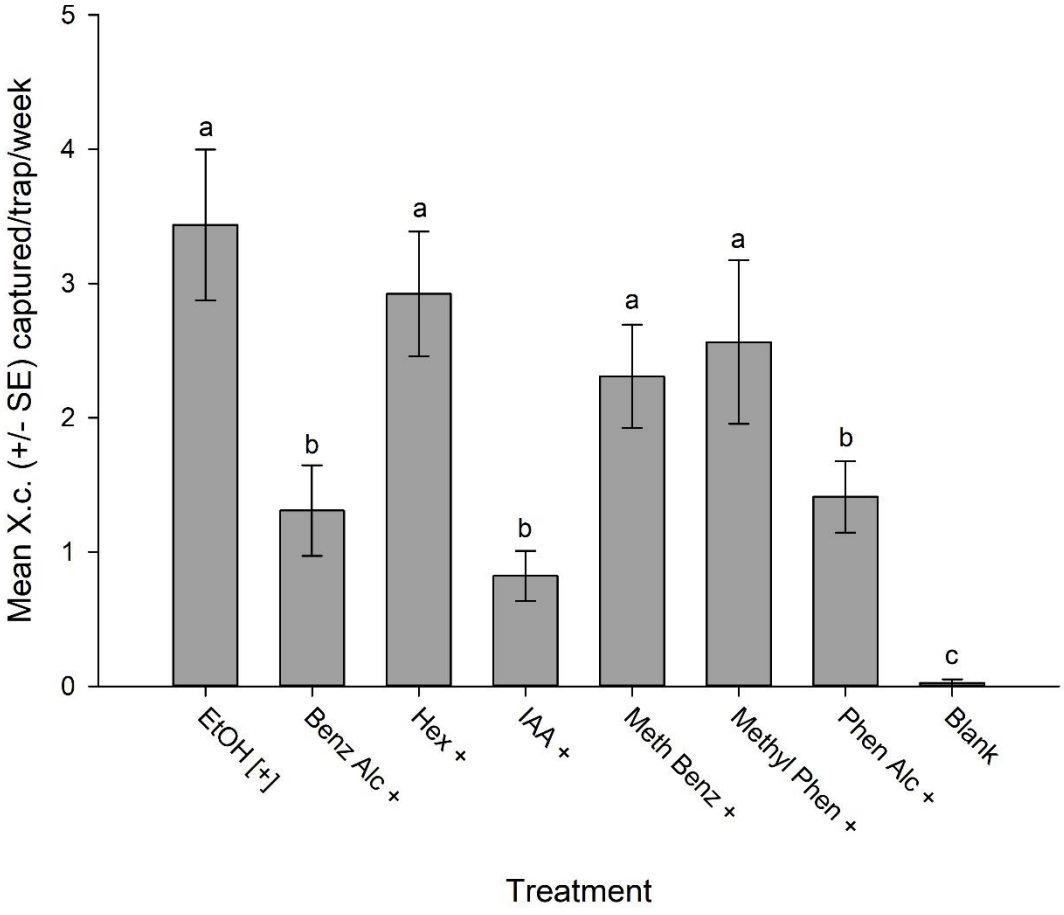

Fig. 3.1: Mean number (+/- standard error) *Xylosandrus crassiusculus* captured weekly in four transects of bottle traps baited with fungal volatile lures during 2018. Different letters represent significant differences when tested at $\alpha = 0.05$.

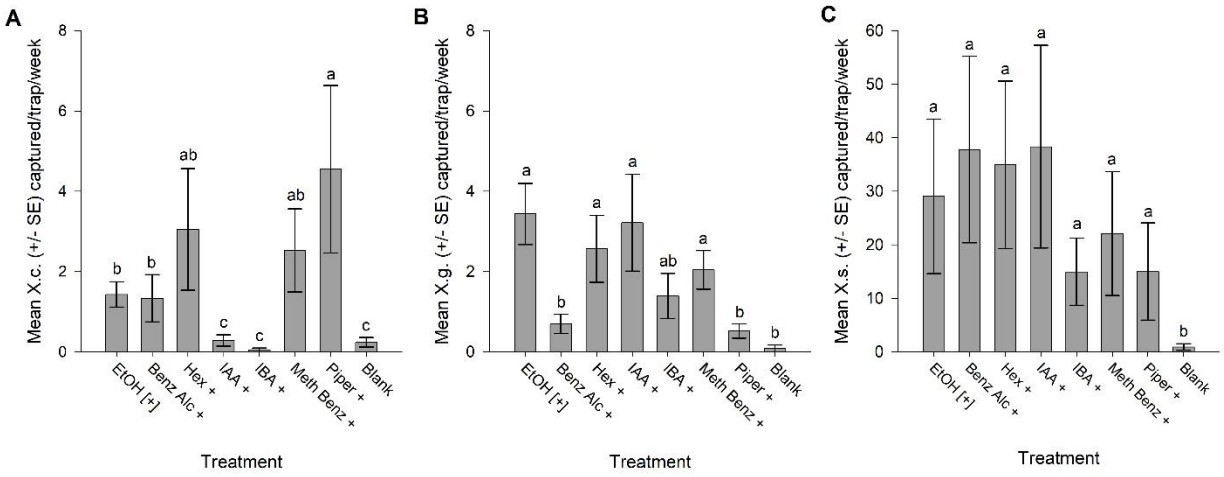

Fig. 3.2: Mean number (+/- standard error) of (A) *Xylosandrus crassiusculus*, (B) *Xylosandrus germanus*, and (C) *Xyleborinus saxesenii* captured weekly in four transects of multi-funnel traps during 2019. Different letters represent significant differences when tested at $\alpha = 0.05$.

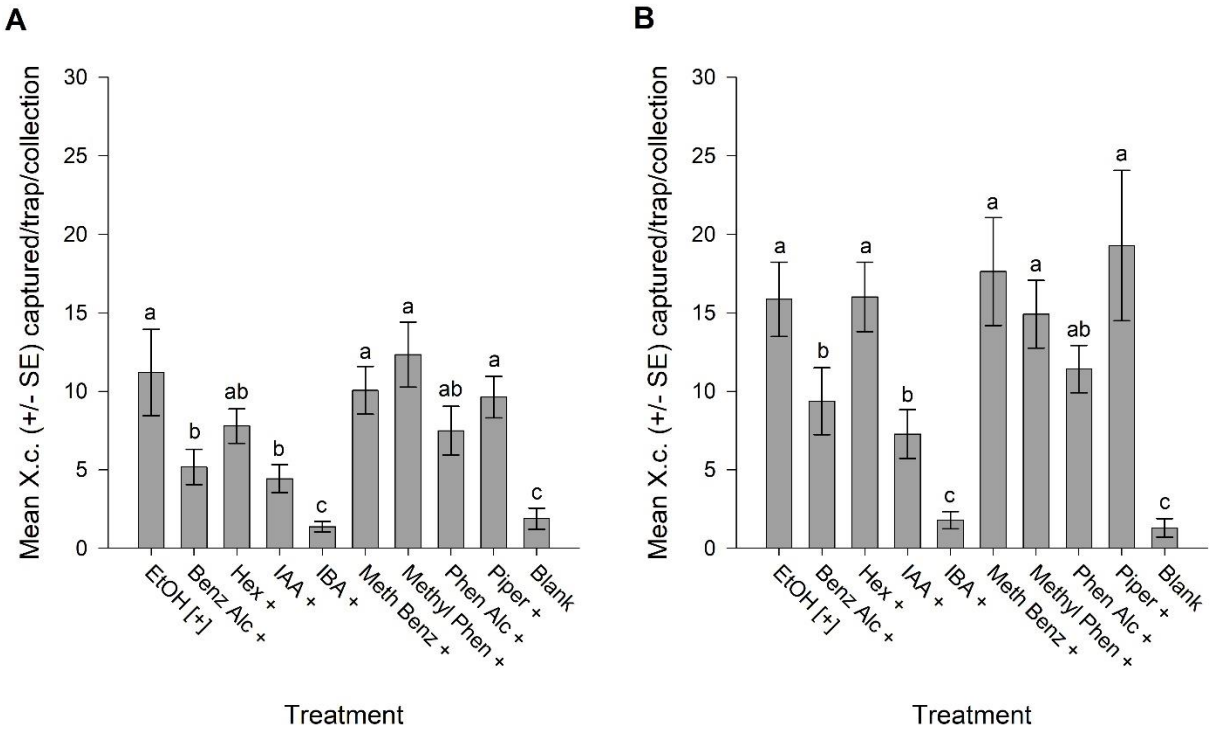

Fig. 3.3: Mean number (+/- standard error) of *Xylosandrus crassiusculus* captured during 2020 in (A) nine transects of multi-funnel traps in various sites, and (B) nine transects of bottle traps at various sites. Different letters represent significant differences among treatments when tested at $\alpha = 0.05$.

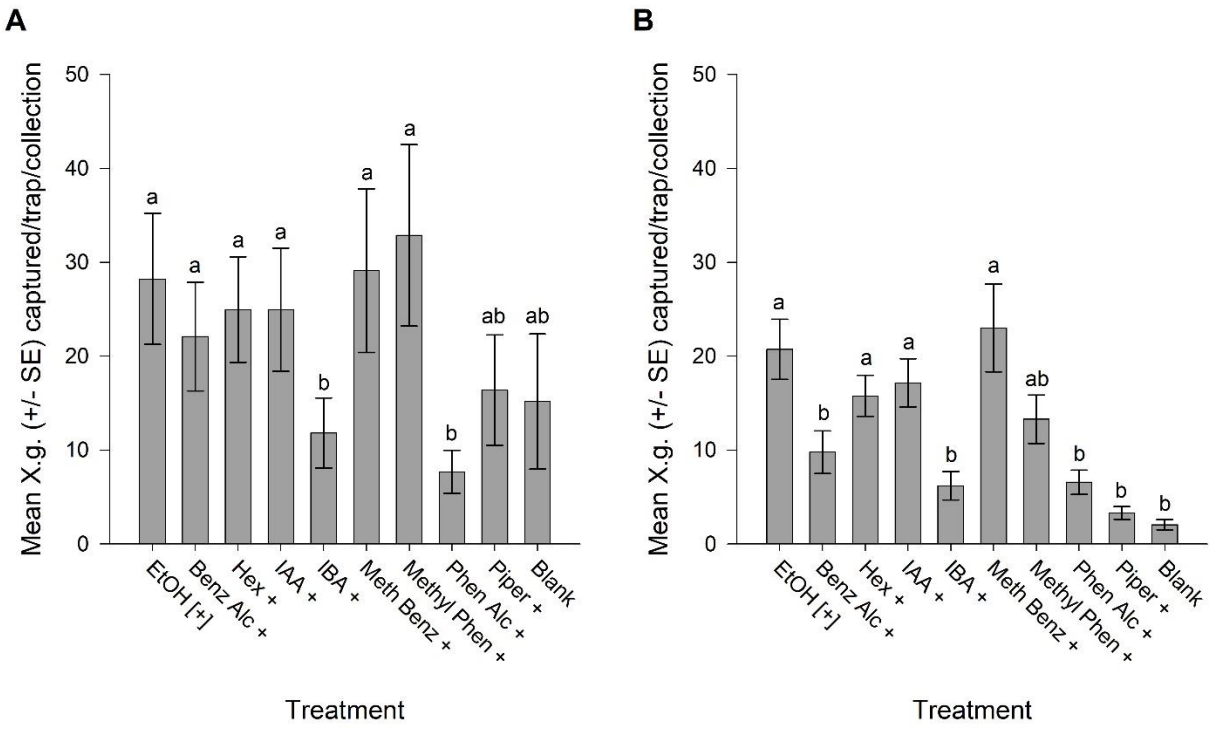

Fig. 3.4: Mean number (+/- standard error) of *Xylosandrus germanus* captured at nine sites during 2020 in (A) nine transects of multi-funnel traps, and (B) nine transects of bottle traps. Different letters represent significant differences among treatments when tested at $\alpha = 0.05$.

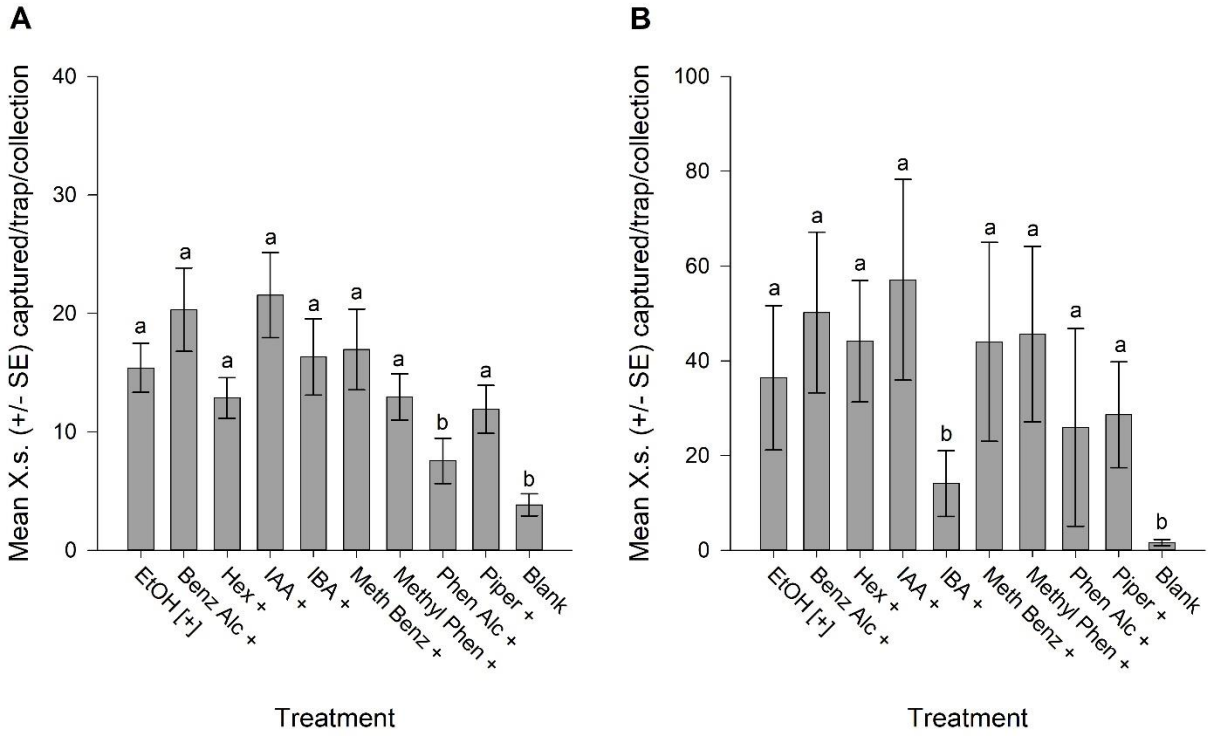

Fig. 3.5: Mean number (+/- standard error) of *Xyleborinus saxesenii* captured during 2020 in (A) multi-funnel and bottle traps at eight sites, and (B) multi-funnel and bottle traps at the Darlington and Lugar sites. Different letters represent significant differences among treatments when tested at $\alpha = 0.05$.

CHAPTER 4. FUNGAL AND NON-HOST VOLATILES MODIFY ATTRACTION OF THE WALNUT TWIG BEETLE, PITYOPHTHORUS JUGLANDIS, TO PHEROMONE LURES

4.1 Introduction

Recent invasions by exotic bark and ambrosia beetles and their novel associations with pathogenic fungi have increased outbreaks of tree diseases worldwide (Kuhnholz et al. 2001, Ploetz et al. 2013). Thousand Cankers Disease (TCD) is one such pest complex that threatens black walnut (*Juglans nigra* L.) which is among the most valuable hardwood tree species in North America. Thousand Cankers Disease is caused by mass inoculation of a pathogenic fungus to susceptible trees (primarily *Juglans* spp.) by the walnut twig beetle (*Pityophthorus juglandis* Blackman; WTB) (Tisserat et al. 2009). The rapid spread of TCD has caused the widespread death of landscape and urban walnut trees throughout the western United States, and now threatens black walnut within its natural range in the eastern United States (Utley et al. 2013, Wiggins et al. 2014). Current TCD management relies on monitoring WTB populations using pheromone-baited traps, but lures remain ineffective beyond short distances and often fail to capture beetles when populations are present at low levels. With the current spread of TCD, now even beyond U.S. borders, there is a critical need to increase the efficacy of WTB monitoring and subsequent management tactics to mitigate damage in high-value plantings and reduce the future spread of the disease.

Thousand Cankers Disease occurs when a susceptible host, primarily black walnut, is colonized by high numbers of WTB which vector the pathogenic fungus *Geosmithia morbida* M. Kolarik, E. Freeland, C. Utley, and N. Tisserat (Ascomycota: Hypocreales: Bionectriaceae) (Tisserat et al. 2009, Kolařík et al. 2011). Inoculation of the fungus causes necrotic cankers to form in the phloem, which can coalesce and girdle trees, reducing their ability to transport water and nutrients to the crown. Symptoms of the disease include yellowing of leaves, twig and branch dieback, epicormic sprouts, and in many cases, tree mortality within two years (Tisserat et al 2009, Utley et al. 2013). Although TCD was originally observed in the western U.S., over the last decade it has been detected in seven eastern states within the native range of black walnut, and was recently detected in Italy (Grant et al. 2011, Wiggins et al. 2014, Montecchio et al. 2016).

This new pest complex follows a pattern consistent with many recent global tree diseases: it is caused by a novel association between a naïve host (black walnut), a bark or ambrosia beetle vector (WTB), and a pathogenic fungus (*G. morbida*) (Hulcr and Dunn 2011, Kolařík et al. 2011). Black walnut is a deciduous tree found scattered in riparian areas, as well as planted in high-density plantations throughout its native range in the eastern U.S. (Burns and Honkala 1990, Michler et al. 2006). This species is among the most valuable hardwood trees in eastern U.S and is especially prized for use in making gunstocks, furniture, and other fine-hardwood products (Burns and Honkala 1990). The WTB is native to the southwestern U.S. where it commonly colonizes Arizona walnut (*Juglans major*) (Cranshaw 2011). The cause of the recent range expansion by WTB that has precipitated the emergence of TCD remains in question, although human-mediated dispersal of colonized wood may be a key factor (Rugman-Jones et al. 2015). While *Geosmithia* species have been previously identified inhabiting a variety of conifer and hardwood tree species, *G. morbida* is a newly described pathogenic fungus found only associated with *Juglans* and *Petrocarya* spp. throughout the U.S. (Kolařík et al. 2011). Single inoculations of *G. morbida* appear to be only mildly pathogenic, with isolated cankers forming at the inoculation site (Juzwik et al. 2020), but mass attack by WTB causes numerous infection points, thereby causing TCD (Utley et al. 2013).

Because the pathogenicity of TCD depends on extensive fungal inoculation by mass beetle attack, current management of this disease relies on monitoring WTB populations and subsequent removal of symptomatic trees. Monitoring protocols consist of placing multi-funnel traps baited with synthetic pheromone lures, previously identified as 3-methyl-2-buten-1-ol (Seybold et al. 2015), near walnut trees and collecting captured beetles weekly (Seybold et al. 2013). When WTB are discovered in traps, visual surveys are conducted to determine if nearby walnut trees are symptomatic; infested trees are most often removed and the wood destroyed (Seybold et al. 2013). This monitoring program can reliably detect WTB populations in new regions of the U.S., but the efficacy of the pheromone lures sharply decreases at distances greater than 3 m from traps, and when population densities are low (Seybold et al. 2013).

Although the male-produced WTB pheromone has been identified, other compounds have been evaluated as possible semiochemicals and may be exploited to manage beetles in high-value plantings of walnut. Several experiments have demonstrated preferential attraction of WTB to host walnut trees and their associated volatiles (Audley et al., 2020b; Blood et al., 2018), providing

evidence for chemically mediated host colonization. In addition to various attractants, several non-host volatiles have also shown repellent activity. Both enantiomers of limonene, both enantiomers of verbenone, racemic trans-conophthorin, racemic chalcogran, and α- and β-pinene have all repelled beetles from traps baited with WTB pheromone in different experiments (Audley et al., 2020; Audley et al., 2020a; Blood et al., 2018). The concentration and release rate of the compounds also appears to play an important role in behavioral activity, as most of the repellent compounds were more active at higher concentrations, and α- and β-pinene were actually attractive at low concentrations (Audley et al. 2020a, Blood et al. 2018).

Another possible source of WTB semiochemicals is volatiles produced by symbiotic and associated fungi (Davis et al. 2013). Bark beetles are commonly associated with one to many fungal species below the bark of trees, where they rely on fungi to concentrate sparse nutrients and aid in overcoming host defenses (Ayres et al. 2000, Hammerbacher et al. 2013, Raffa et al. 2015, Zhao et al. 2019). These fungi produce various compounds, most of which are low-molecular-weight alcohols and polyketides, which may act as cues for colonizing beetles. For instance, the conifer-infesting *Ips typographus* is attracted to volatiles produced by its symbiotic fungus, both as larvae and as young adults (Kandasamy et al. 2019). Previous experiments have demonstrated that WTB are attracted to volatiles produced by *G. morbida*; the fungal volatiles subsequently identified as isoamyl alcohol and isobutyl alcohol (Blood 2016, Blood et al. 2018). Although the results of laboratory trials have been promising, these fungal volatiles have yet to be tested to see if they are behaviorally active in a field setting.

The objective of this study was to test the hypothesis that fungal volatiles are behaviorally active in the WTB and modify their attraction to pheromone lures in the field. Specifically, a three-year trapping study was conducted in black walnut plantations with varying levels of TCD incidence to determine the extent to which fungal volatiles: (1) enhance attraction to WTB pheromone lures, (2) repel beetles from pheromone lures, and (3) could be used in a push-pull system utilizing both attractants and repellents. We tested compounds previously identified from *G. morbida* (Blood 2016) as well as a suite of volatiles identified from symbiotic fungi of ambrosia beetles commonly found in deciduous forests. In addition to testing the activity of fungal volatiles, the repellency of several non-host compounds was tested in combination with pheromone-baited traps. The identification of additional WTB semiochemicals may enhance our ability to monitor and control this pest in natural and planted forests.

4.2 Methods and Materials

To study the effects of fungal volatiles on WTB attraction to their own pheromone, a series of trapping studies were conducted over three years (2018–2020) in three black walnut plantations with active WTB populations (referred to as Cottonwood, Russell, and Yellowhawk) located near Walla Walla, Washington. Site characteristics for each plantation is summarized in Table 4.1. Within each site, traps were placed along linear transects following recommended monitoring guidelines (Seybold et al. 2013, 2015). Traps consisted of four-unit Lindgren funnel traps hung from ~3-m aluminum conduit placed >15 m from other traps in the transect and transects were placed >25 m from one another. Traps were baited with lures as described below and collection cups were filled with RV antifreeze (propylene glycol; SPLASH Products, St. Paul, MN) to preserve captured insects. The traps were serviced weekly by straining the antifreeze through disposable paint strainers (190-µm filter tips, TCP Global, San Diego, CA), which were sealed in individual plastic baggies and shipped to the Purdue University Forest Entomology lab for identification. Each week the placement of each trap was shifted one position along the transect to avoid any confounding location effects.

Within each transect, traps baited with the WTB pheromone alone, which served as a positive control, and blank traps (no lure included), which were used as negative controls. All other traps were baited with a combination of WTB pheromone lure [indicated by "+"] and a separate fungal or non-host volatile lure. All lure components were "bubble" lures purchased from Synergy Semiochemical (Burnaby, B.C., Canada), except for WTB lures (ISCA Tech, Riverside, CA) and limonene lures. Limonene lures were made by introducing 1 mL of (R)-(+)-limonene (Sigma-Aldrich, St. Louis, MO) into a semi-permeable polyethylene sachet (press-seal bags, Bagette model 14,770, 5.1 x 7.6 cm, 0.05-mm wall thickness, Cousin Corp., Largo, FL). Bubble lures were replaced monthly and limonene lures were replaced every two weeks. Prior to initiating the trapping experiment each year, monitoring traps baited with WTB pheromone lures were used to confirm WTB activity at each site. Experiments were conducted 9 July–8 October in 2018 (13 collection periods), 12 June–30 September in 2019 (16 collection periods), and 14 July–6 October in 2020 (12 collection periods).

4.2.1 Trapping Experiments with Fungal Volatiles

Four experiments were conducted to determine the extent to which fungal volatiles modified the attraction of WTB to pheromone-baited traps. Fungal volatiles used in the experiments included compounds associated with *G. morbida* (isoamyl alcohol and isobutyl alcohol) (Blood 2016), as well as volatiles identified from the symbiotic fungi of the ambrosia beetles *Xylosandrus crassiusculus* (Motschulsky) and *X. germanus* (Blandford) (Ranger et al. 2021; unpublished data, Ranger et al.). Both of these ambrosia beetles are sympatric with WTB in walnut plantations and volatiles produced by their symbiotic fungi may play a role in WTB host selection (Reed et al. 2015, Klingeman et al. 2017).

Experiment 1 (Exp. 1)

Experiment 1 (2018) was conducted to determine the effect of adding fungal volatiles to pheromone lures on traps. Volatiles identified from *G. morbida* were combined with pheromone lures and placed in one transect at each of the Cottonwood and Yellowhawk sites. Lure treatments included: (1) WTB pheromone (WTB [+]), (2) isoamyl alcohol (IAA+), (3) isobutyl alcohol (IBA+), (4) IAA + IBA (IAA, IBA+), and (5) blank trap. All experiment sites, lure treatments, and release rates are summarized in Table 4.2.

Experiment 2 (Exp. 2)

Experiment 2 (2018) was similar to Exp. 1, but employed an expanded suite of fungal volatiles identified from fungal symbionts of sympatric ambrosia beetles. A single transect was placed at the Russell site, with lure treatments that included: (1) WTB [+], (2) benzyl alcohol (Benz Alc +), (3) hexanol (Hex +), (4) IAA +, (5) methyl benzoate (Meth Benz +), (6) methylphenyl acetate (Methyl Phen +), (7) phenyl alcohol (Phen Alc +), (8) a combination of all fungal volatiles (Blend +), and (9) a blank trap.

Experiment 3 (Exp. 3)

Experiment 3 (2019) was conducted to further test compounds that enhanced attraction to pheromone lures in the previous year. In addition, two putative repellents (limonene and piperitone) were added as treatments in the experiment. A total of three transects of traps were established:

one at the Cottonwood site and two at the Russell site. Lure treatments included: (1) WTB [+], (2) Hex +, (3) IAA +, (4) IBA +, (5) limonene (Lim +), (6) Meth Benz +, (7) piperitone (Piper +), and (8) a blank trap.

Experiment 4 (Exp. 4)

Experiment 4 (2020) was conducted to repeat a test of the ability of fungal volatiles to enhance attraction to pheromone lures. The experiment consisted of five transects: one at the Cottonwood site, two at the Russell site, and two at the Yellowhawk site. Lure treatments included: (1) WTB [+], (2) Hex +, (3) IAA +, (4) IBA +, (5) Meth Benz +, (6) Methyl Phen +, (7) Phen Alc +, and (8) a blank trap.

4.2.2 Fungal and Non-Host Repellents

Three experiments were conducted to determine the extent to which non-host compounds and fungal volatiles repelled WTB from pheromone-baited traps. Non-host compounds often repel bark and ambrosia beetles from colonizing unsuitable hosts, which can then be exploited to repel beetles from valuable trees. Limonene has been previously described as repellent for WTB in the eastern U.S. (Blood et al. 2018), but it was unknown if populations in western states would respond similarly. Benzyl alcohol was repellent to beetles in Exp. 1 (above) and was tested for repellency by itself in one experiment, as described below. Finally, all compounds that were repellent in previous experiments were tested in transects during 2020.

Experiment 5 (Exp. 5)

Experiment 5 (2019) evaluated the repellency of limonene, a compound that has reduced WTB attraction to pheromone-baited traps in other experiments (Audley et al. 2020, Audley et al. 2020a, Blood et al. 2018). Treatments that included limonene with and without a pheromone lure were placed in two transects: one at the Cottonwood site and one at the Yellowhawk site. Lure treatments included: (1) WTB [+], (2) Lim (no pheromone lure), (3) Lim +, and (4) a blank trap.

Experiment 6 (Exp. 6)

Experiment 6 (2019) tested the repellency of benzyl alcohol, a fungal volatile which reduced WTB attraction in Exp. 2. A single transect was placed at the Yellowhawk site that included benzyl alcohol treatments with and without a pheromone lure. The lure treatments included: (1) WTB [+], (2) Benz Alc (no pheromone lure), (3) Benz Alc +, and (4) a blank trap.

Experiment 7 (Exp. 7)

Experiment 7 (2020) compared the repellent activity of compounds that had reduced WTB attraction in previous experiments. The experiment consisted of three transects: one placed at the Cottonwood site and two at the Yellowhawk site. Lure treatments included: (1) WTB [+], (2) Benz Alc +, (3) Lim +, (4) Piper +, and (5) a blank trap.

4.2.3 Push-Pull System

One experiment was conducted to determine the extent to which these compounds that enhanced and reduced attraction to pheromone lures for potential application in a "push-pull" system. Such a system utilizes a known repellent as "push" to repel beetles from a location, and a known attractant as a "pull" to capture the insect in kill-traps or attract it to trap trees (Cook et al. 2007). By utilizing both responses, the desired effect is an increase in the attractive response to the "pull" as a beetle is repelled from another source. Such systems are under development for a variety of bark and ambrosia beetle species (Werle et al. 2019, Byers et al. 2020, Rivera et al. 2020). Compounds used for this experiment were selected based on enhanced attraction and repellent findings in experiments conducted in 2018. One of three compounds that enhanced attraction was paired with benzyl alcohol, which acted as a repellent in 2018, in each of the transects.

Experiment 8 (Exp. 8)

In Experiment 8 (2019), I evaluated the suitability of attractants and repellents from previous experiments to be used in a push-pull management strategy. Specifically, we tested the extent to which the inclusion of a repellent compound (i.e. push) in a linear transect of traps affected the capture of beetles in traps baited with attractive lures (i.e. pull). A total of three

transects were used: one at Russell and two at Yellowhawk. Lure treatments included: (1) WTB [+], (2) Benz Alc +, (3) Meth Benz + or IAA + or Hex +, and (4) a blank trap.

4.2.4 Statistical Analyses

To determine if WTB populations differed among the sites and years, the annual mean and variance of beetles captured by positive-control treatments (WTB [+]) at each site were compared. Because the response variable failed the assumptions of homoscedasticity, a non-parametric Kruskal-Wallis rank sum test with a Dunn's test post-hoc were used to compare means, and a Bartlett's Test of Homogeneity was used to compare variances among the sites for each year.

Because WTB populations were found to be significantly greater at the Yellowhawk location, sites were analyzed separately within each experiment, except for Exp. 3, which only included the Cottonwood and Russell sites. Raw means of the numbers of WTB captured did not meet the assumptions of normality for any of the groups analyzed; therefore, a non-parametric Friedman test with a post-hoc Ryan-Einot-Gabriel-Welsch Q multiple comparison test (with only one transect at a site) or a Kruskal-Wallis rank sum test with a post-hoc Dunn's test (when analyzing more than one transect at a site) were used to analyze results among different lure treatments. All analyses were conducted in R (R Core Team 2020), using the *PMCMRplus* package (Pohlert 2014) for Dunn's test and the *mutoss* package for Ryan-Einot-Gabriel-Welsch Q multiple comparison tests. All tests were conducted at a significance level of $\alpha = 0.05$.

4.3 Results

Over a three-year period 43,481 WTB were captured at all sites, with almost three- and ten-times more WTB captured at the Yellowhawk site (30,664), compared to the Russell (10,476) and Cottonwood (2,341) sites. Significantly more beetles were captured at the Yellowhawk site than the Russell and Cottonwood site over all three years (Kruskal-Wallis $\chi^2 = 151.95$, df = 8, p-value < 0.001; Bartlett's $K^2 = 386.45$, df = 8, p-value < 0.001). Although there were several peak capture periods at each site (Fig. 4.1), corresponding to two or three major emergence periods, there was a consistent background population throughout the active-flight season.

4.3.1 Trapping Experiments with Fungal Volatiles

Exp. 1

A total of 1,674 WTB were captured during Exp. 1, with more than three times the number of beetles at the Yellowhawk site (1,301) than at the Cottonwood (373) site. Differences in the response of WTB to lure treatments varied by site. At the Cottonwood site, the IBA treatment somewhat increased attraction to pheromone-baited traps, whereas the combination of IAA and IBA was similar to the blank trap (Fig. 4.2A; Friedman $\chi^2 = 32.357$, df = 4, p-value < 0.001). At the Yellowhawk site, the combination of IAA and IBA resulted in a marginal increase in the number of beetles captured in baited traps, whereas the individual IAA and IBA treatments were marginally repellent to WTB (Fig. 4.2B; Friedman $\chi^2 = 18$, df = 4, p-value < 0.01).

Exp. 2

A total of 3,437 WTB were captured within a single transect during Exp. 2. The Meth Benz treatment increased attraction of WTB to pheromone-baited traps, while the IAA and Hex treatments showing a marginal increase in beetles captured. Conversely, both the Benz Alc and the Blend treatments repelled beetles from baited traps and were similar to the blank trap (Fig. 4.3; Friedman $\chi^2 = 79.823$, df = 8, p-value < 0.001).

Exp. 3

A total of 2,547 WTB were captured in Exp. 3, with twice as many beetles captured at the Russell (1,761) than at the Cottonwood site (786). The IBA treatment increased the number of beetles captured in pheromone-baited traps, capturing more WTB than all other lure treatments (Fig. 4.4; Kruskal-Wallis $\chi^2 = 43.609$, df = 6, p-value < 0.001). In addition, IAA resulted in a marginal increase in the number of beetles captured, while Hex, Meth Benz, Lim, and Piper all exhibited marginal repellency when placed on pheromone-baited traps.

Exp. 4

A total of 22,217 WTB were captured in Exp. 4, with more than three times the number of beetles captured at the Yellowhawk site (17,743) than at the Russell (4,138) and Cottonwood sites

(336). At the Cottonwood site, the Phen Alc treatment captured more WTB than all other lure treatments, which, in turn, captured more beetles than the blank trap (Fig. 4.5A; Friedman χ^2 = 29.008, df = 7, p-value < 0.001). At the Russell site, the Phen Alc treatment also captured more beetles than all other lure treatments (Fig. 4.5B; Kruskal-Wallis χ^2 = 60.92, df = 7, p-value < 0.001). At the Yellowhawk site, the IBA treatment enhanced attraction to the pheromone lure and captured more beetles than all other lure treatments which were, in turn, similar to the positive control (Fig. 4.5C; Kruskal-Wallis χ^2 = 70.876, df = 7, p-value < 0.001).

4.3.2 Fungal and Non-Host Repellents

Exp. 5

A total of 1,857 WTB were captured in Exp. 5, with more captured at the Yellowhawk site (1,490) than the Cottonwood site (367). The limonene treatment reduced attraction to the pheromone lure at both the Cottonwood (Fig. 4.6A; Friedman χ^2 = 12.315, df = 3, p-value < 0.01), and Yellowhawk sites (Fig. 4.6B; Friedman χ^2 = 17.861, df = 3, p-value < 0.001). At both sites, the response to the combination of limonene and the pheromone lure was similar to that of limonene alone and the blank trap.

Exp. 6

A total of 1,503 WTB were captured at the Yellowhawk site during Exp. 6. The Benz Alc treatment reduced attraction to traps baited with the pheromone lure and the response was similar to both the benzyl alcohol alone and the blank trap (Fig. 4.7; Friedman χ^2 = 21.726, df = 3, p-value < 0.001).

Exp. 7

A total of 2,971 WTB were captured in Exp. 7, with traps at the Yellowhawk site capturing more (2,767) than those at the Russell (95) and Cottonwood (109) sites. At the Cottonwood site, limonene reduced attraction of WTB to the pheromone and the response was similar to that of blank traps. The response to all other lure treatments were similar to that of the positive control (Fig. 4.8A; Friedman χ^2 = 9.6296, df = 4, p-value < 0.05). At the Russell site, all previously

repellent treatments reduced attraction of the pheromone lure below that of the positive control and similar to a blank trap (Fig. 4.8B; Friedman $\chi^2 = 26.877$, df = 4, p-value < 0.001). At the Yellowhawk site, both the benzyl alcohol and limonene treatments acted as repellents, reducing the number of WTB captured in pheromone-baited traps to the level of the blank trap (Fig. 4.8C; Friedman $\chi^2 = 26.996$, df = 4, p-value < 0.001).

4.3.3 Push-Pull System

Exp. 8

A total of 2,267 WTB were captured in Exp. 8, with 2,111 captured in two transects at the Yellowhawk site and 156 captured in one transect at the Russell site. Although the positive control captured more WTB than all other treatments at the Russell site, there were no differences in the response to the lure treatments (Fig. 4.9A; Friedman $\chi^2 = 6.1935$, df = 3, p-value = 0.1026). In one transect at the Yellowhawk site, IAA treatment captured more beetles than all other lure treatments, demonstrating an enhanced attraction to traps baited with the pheromone. The benzyl alcohol treatment, a putative repellent, captured as many beetles as the positive control. (Fig. 4.9B; Kruskal-Wallis $\chi^2 = 12.024$, df = 3, p-value < 0.05). In the other transect at the Yellowhawk site, the pheromone alone attracted more beetles than all other treatments (Fig. 4.9C; Kruskal-Wallis $\chi^2 = 16.246$, df = 3, p-value < 0.05).

4.4 Discussion

The results of this study demonstrate, for the first time, that fungal volatiles modify the attraction of a bark beetle to its pheromone in a field setting. These findings expand upon laboratory studies that found that bark beetles respond to volatiles produced by symbiotic and co-occurring fungi (Davis et al. 2013, Blood et al. 2018, Kandasamy et al. 2019). Although the response of WTB to fungal volatiles varied somewhat between sites and experiments, adult beetles were consistently attracted to traps that contained both *G. morbida* volatiles: isoamyl and isobutyl alcohol. Many bark beetles preferentially colonize stressed trees and the presence of fungi may act as an indicator of reduced host defenses (Raffa et al. 2015, Kandasamy et al. 2019, Rassati et al. 2019). Attraction to symbiotic fungal volatiles may also increase oviposition and larval feeding in areas where fungi concentrate nutrients, thus improving larval survival and development (Ayres

et al. 2000, Six 2012). Thus, fungal volatiles may act as a cue of the host condition that enhances landing behavior in conjunction with conspecific pheromones. Behaviorally, this response to fungal volatiles may be similar to other compounds that are associated with compromised hosts and promote colonization by wood-boring beetles, such as α-pinene in conifers and ethanol in angiosperms (Erbilgin and Raffa 2000, Reddy and Guerrero 2004, Hanks and Millar 2013). In addition to acting as an indicator of host condition, pathogenic fungi can also reduce host defenses and compete with other microorganisms that harm bark beetle progeny (Ayres et al. 2000, Hammerbacher et al. 2013, Hofstetter et al. 2015). Therefore, attraction to fungal-associated volatiles may be an important factor in bark beetle colonization, particularly in cases when a mutualistic relationship exists between WTB and *G. morbida*.

Although some fungal volatiles enhanced beetle attraction to baited traps, benzyl alcohol reduced the attraction of WTB to its pheromone. Not only did benzyl alcohol repel WTB when tested alone, but also when included as part of a blend of fungal volatiles that included compounds that enhanced attraction. Little is currently known about fungal volatiles that may act as repellents to bark beetles that attack hardwood trees. However, in olfactometer bioassays the coniferophagous *Ips typographus* demonstrated the ability to recognize and avoid volatiles from fungi that compete with its own symbiotic fungi (Zhao et al. 2019). Similarly, the repellency of benzyl alcohol may be the consequence of WTB avoiding hosts inhabited by competing fungi, especially as benzyl alcohol was identified from *X. crassiusculus*, an ambrosia beetles that can temporally and spatially overlap with WTB (Reed et al. 2015, Klingeman et al. 2017). Although benzyl alcohol was shown to be produced by fungi, it is also produced by several non-walnut trees and, thus, may be acting as a non-host cue (McNair et al. 2000). Two additional non-host compounds, limonene and piperitone, were also repellents in the present study. Although, limonene is now a known repellent of WTB (Audley et al. 2020a, Audley et al. 2020, Blood et al. 2018), this is the first demonstration of a bark beetle being repelled by piperitone. Piperitone is a fragrant compound isolated from several genera of tropical grasses species of grassy plants and has recently been found to repel *Euwallacea* ambrosia beetles (Byers et al. 2018, 2020). In behavioral terms, piperitone may act as a cue to detect a non-host, as with many volatiles of deciduous trees that deter conifer-infesting bark beetles (Zhang et al. 1999, Jactel et al. 2001, Fettig et al. 2009). Avoiding non-host trees would confer a selective advantage for WTB, as oviposition in unsuitable hosts leads to severely reduced larval survival and development (Raffa et al. 2015).

The results of this study demonstrate that fungal volatiles can modify WTB attraction to its pheromone, yet results often varied among different experiments, sites, and years. Factors that may contribute to this variability include differences in WTB population densities, site characteristics, and lure release rates. Planted monocultures not only concentrate trees in unnatural densities but can also concentrate background host odors and beetle pheromones, such as, in this case, the Yellowhawk site, which had a high incidence of TCD and high WTB density. Moreover, sites with few beetles, such as the Cottonwood site, reduced the statistical power of experimental replicates and diminished the utility of inferences about the biological activity of the compounds tested. Sites in our study also varied in the number of trees present, with the Russell site having far fewer trees than other sites, and adjoining land use and tree age differed somewhat among the sites. In summary, many of the site factors may play a role in the varied response of beetles among the different experiments. In addition to beetle population and site differences, the release rate of lure compounds could explain some of the variation in response of WTB. In previous experiments, repellents were much more effective at high-release rates than low-release rates, although response to fungal volatiles was greater at low concentrations in laboratory experiments (Audley et al., 2020a; Blood, 2016). In our experiments, the methyl benzoate lure was produced as a 20 percent concentration (20:80; methyl benzoate:acetyl tributyl citrate) in 2018, with increased attraction to the pheromone lure, but when changed to a 5 percent concentration it no longer increased attraction to the pheromone. Lastly, in addition to other factors, behavioral responses of bark beetles to their semiochemicals can vary by season and year (Teale and Lanier 1991, Sullivan et al. 2016) and the chemical ecology of WTB behavior remains poorly understood.

Identifying compounds that modify responses to pheromone lures could play an important role in improving future WTB monitoring and TCD management. Increasing the attraction of monitoring lures will aid in detecting incipient WTB populations before densities lead to establishment and further spread. An enhanced semiochemical lure could also aid in tactics such as mass trapping, which has been used to manage populations of *Ips* and *Dendroctonus* bark beetles in large conifer plantings (Ross and Daterman 1997, Schlyter et al. 2001, Wermelinger 2004). Another tactic with application in planted forests is the use of attractive lures to draw beetles to low-value trees at the edges of plots, which can then be removed or treated with insecticides during peak flights (Raty et al. 1995, Prokopy et al. 2003). Repellent compounds have been identified in the current research, as well as other experiments (Audley et al. 2020a, Audley et al. 2020, Blood

et al. 2018), and could aid in repelling beetles from especially valuable trees during peak-flight periods; this could also be especially helpful in black walnut, which requires many years of growth to attain veneer-quality logs. Finally, attractive and repulsive compounds could be used together in a push-pull tactic, which seeks to increase the efficacy of attractive traps, the "pull", by using paired or associated repellents as a "push" (Cook et al. 2007). Although our preliminary results using this type of system failed to improve the number of WTBs captured in traps baited with attractants, additional experiments are needed to test the various repellents and attractants available to evaluate their utility for this strategy.

Although bark beetle-fungi symbioses and associations have been well documented, how beetles utilize volatiles produced by symbiotic organisms as behavioral cues or signals remains relatively unexplored. As our study indicates, fungal volatiles may play a more important role in beetle colonization than previously thought. Additional experiments are needed to determine the spatial scale at which these volatiles operate and additional behaviors they may influence, such as adult feeding and gallery creation. To effectively inform potential management tactics, future work should address quantifying the active space of fungal volatile lures and the influence of release rates on the response of WTB. Knowledge of the behaviorally activity of these semiochemicals will aid in increasing the efficacy of monitoring and management lures, which will be increasingly important as the impact of insect-vectored fungal diseases increases (Hulcr and Dunn 2011, Ploetz et al. 2013).

Table 4.1: Characteristics of three black walnut plantations near Walla Walla, WA .

Site	Area	Trees	Tree age (yrs)	Other Tree Species	Adjoining Areas
Cottonwood	1.25 acres	546	11, 19, 39	black cherry, butternut, conifers	agricultural (wheat), residential
Russell	0.50 acres	250	19	black cherry, river birch, conifers	agricultural (wheat, blueberries), residential
Yellowhawk	1.75 acres	380	11, 19	black cherry	agricultural (corn), residential

Table 4.2: Lure treatments for a series of eight trapping experiments conducted over three years (2018-2020).

			Experiments with Fungal Volatiles				Repellent Volatiles			Push-Pull
Treatment	Abbreviation	Release Rate	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8
WTB pheromone lure	WTB [+]	3-4 mg/day	x	x	x	x	x	x	x	x
Benzyl alcohol	Benz Alc +	4-6 mg/day		x				x	x	x
Benzyl alcohol (no WTB)	Benz Alc	4-6 mg/day						x		
2-Ethyl hexanol	Hex +	2-3 mg/day		x	x	x				x
Isoamyl alcohol	IAA +	4-5 mg/day	x	x	x	x				x
Isobutyl alcohol	IBA +	4-5 mg/day	x		x	x				
Isoamyl, isobutyl alcohol	IAA, IBA +	4-5 mg/day	x							
Limonene	Lim +	20 mg/day			x		x		x	
Limonene (no WTB)	Lim	20 mg/day					x			
Methyl benzoate*	Meth Benz +	10 mg/day		x	x	x				x
Methyl phenylacetate	Methyl Phen +	7-8 mg/day		x		x				
Phenethyl alcohol	Phen Alc +	2.5-3.5 mg/day		x		x				
Piperitone	Piper +	6-8 mg/day			x				x	x
Blend of fungal volatiles	Blend +			x						
Blank (no lures)	Blank		x	x	x	x	x	x	x	x

*The methyl benzoate lure was 20% methyl benzoate in 2018, but due to production difficulties, was changed to 5% methyl benzoate in 2019 and 2020

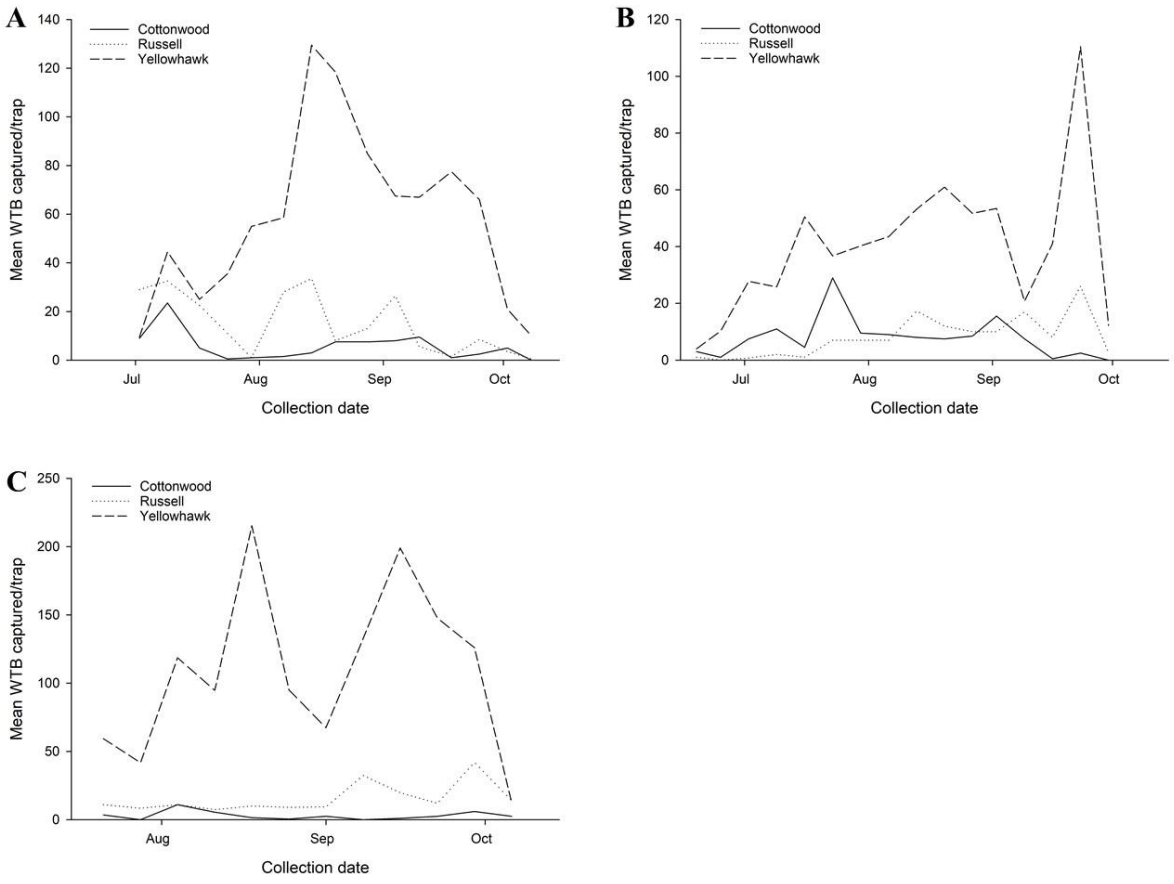

Fig. 4.1: Mean WTB captured in pheromone-baited (WTB [+]) at three sites, Cottonwood, Russell, and Yellowhawk, over three years during July–October of: (A) 2018, (B) 2019, and (C) 2020.

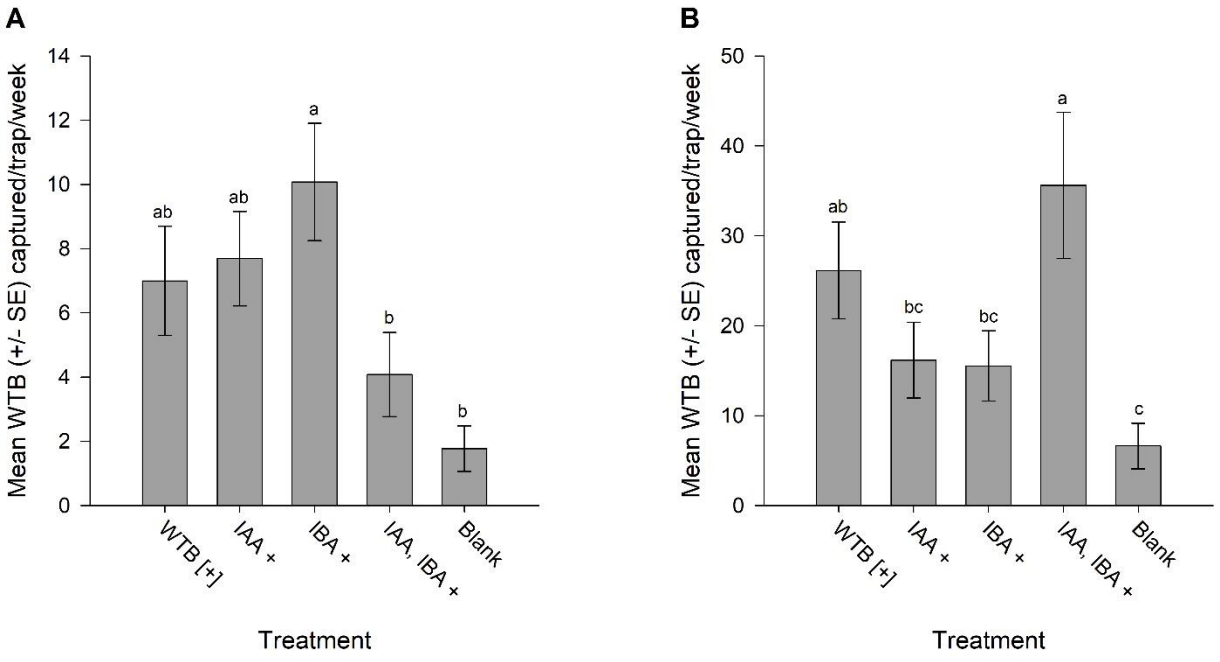

Fig. 4.2: Mean WTB (+/- standard error) captured weekly in multi-funnel traps deployed in two black walnut plantations, (A) Cottonwood and (B) Yellowhawk, during 13 weeks in 2018. Different letters represent significant differences tested at $\alpha = 0.05$ within each site.

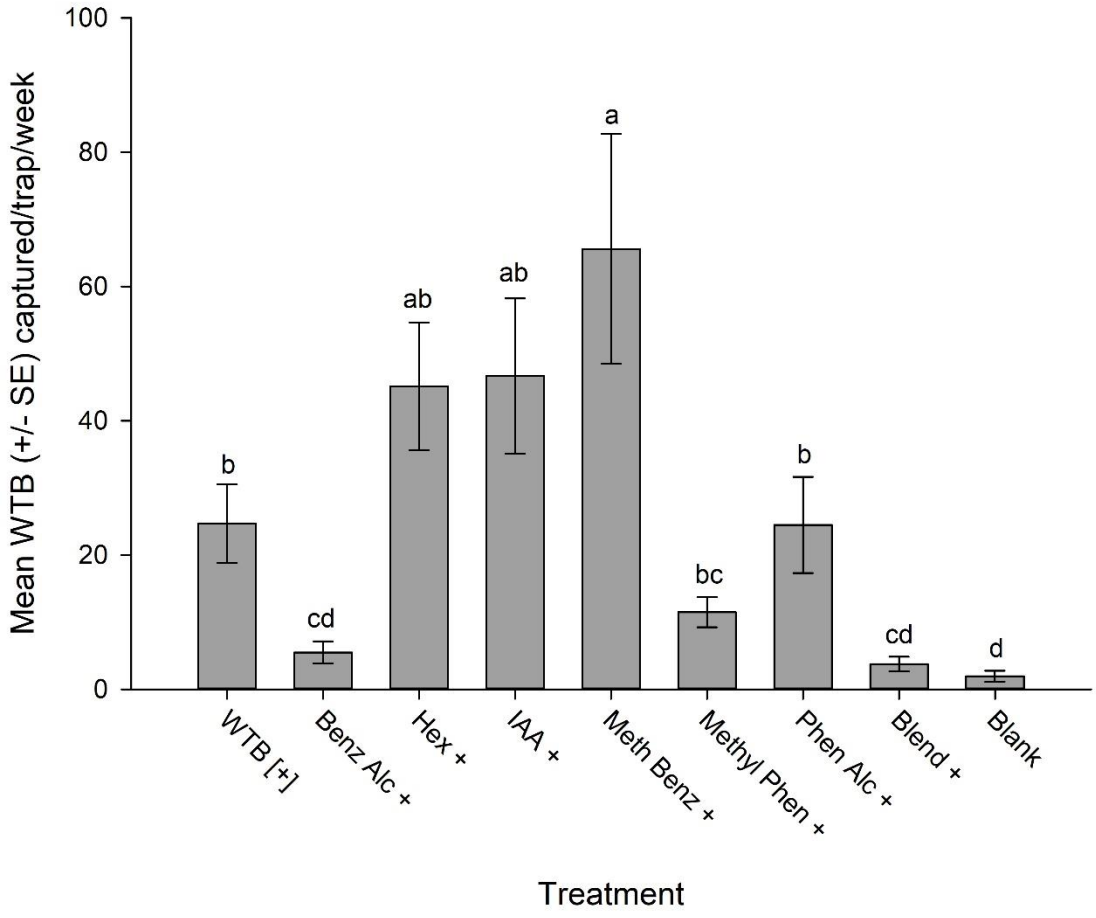

Fig. 4.3: Mean WTB (+/- standard error) captured weekly in multi-funnel traps deployed at the Russell site during 13 weeks in 2018. Different letters represent significant differences tested at $\alpha = 0.05$.

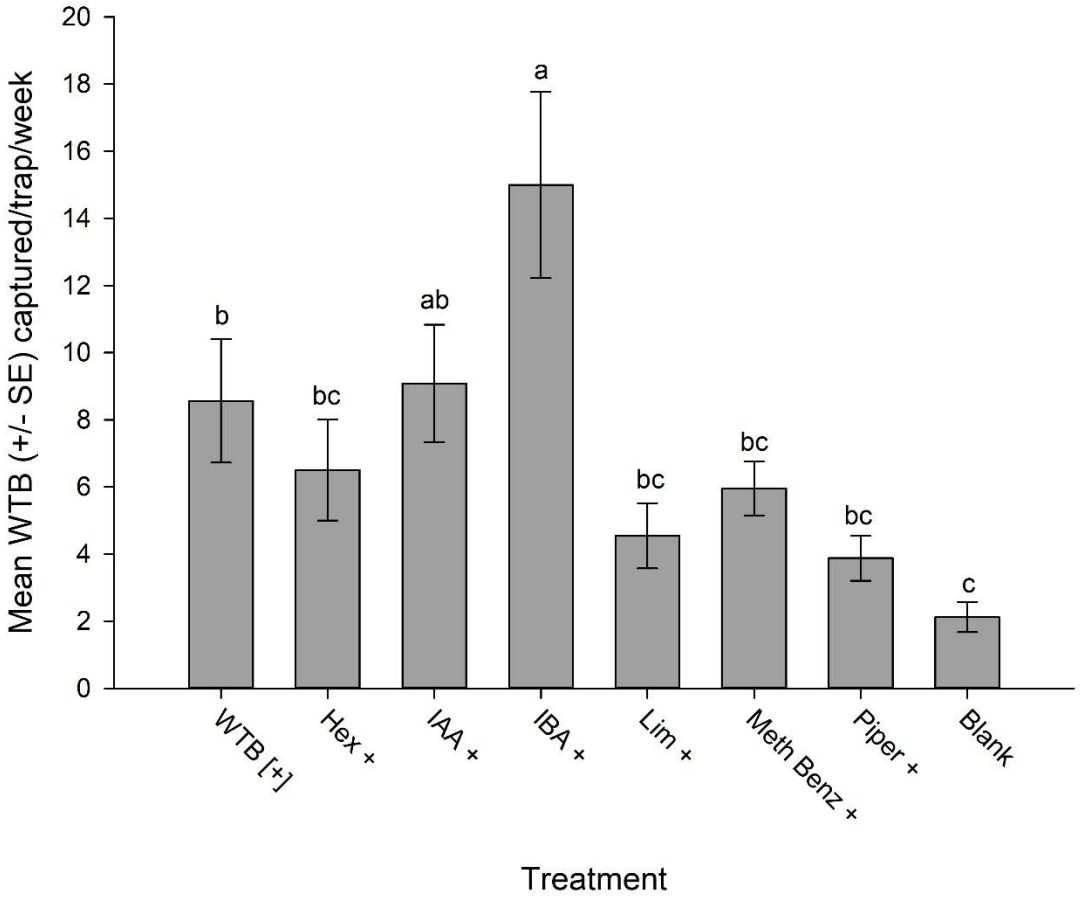

Fig. 4.4: Mean WTB (+/- standard error) captured weekly in multi-funnel traps deployed in black walnut plantations at two sites, Cottonwood and Russell (data combined), during 16 weeks in 2019. Different letters represent significant differences tested at $\alpha = 0.05$.

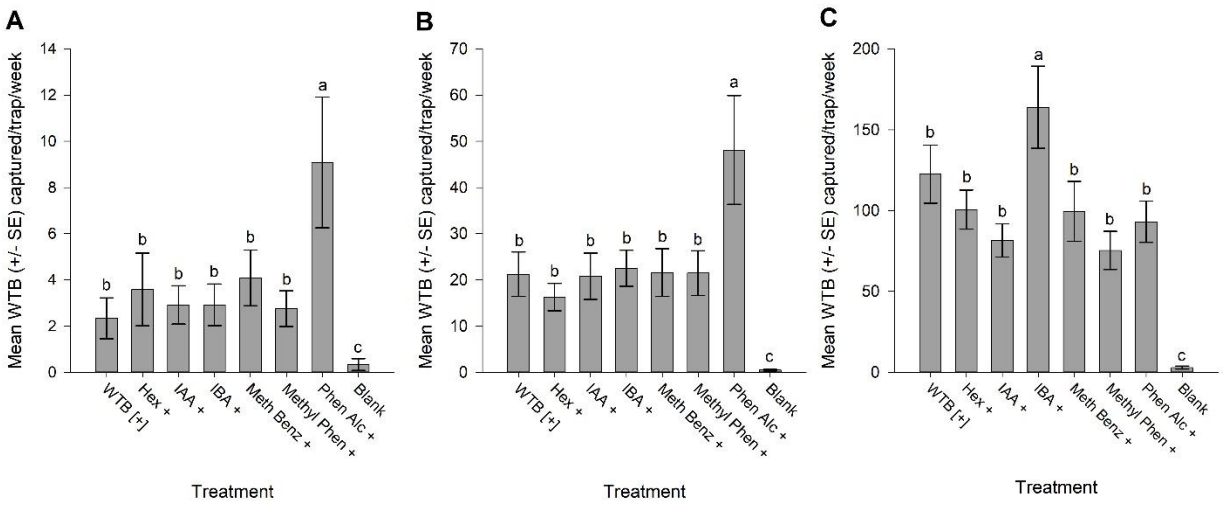

Fig. 4.5: Mean WTB (+/- standard error) captured weekly in multi-funnel traps deployed in three black walnut plantations, (A) Cottonwood, (B) Russell, and (C) Yellowhawk, during 12 weeks in 2020. Different letters represent significant differences tested at $\alpha = 0.05$ within each site.

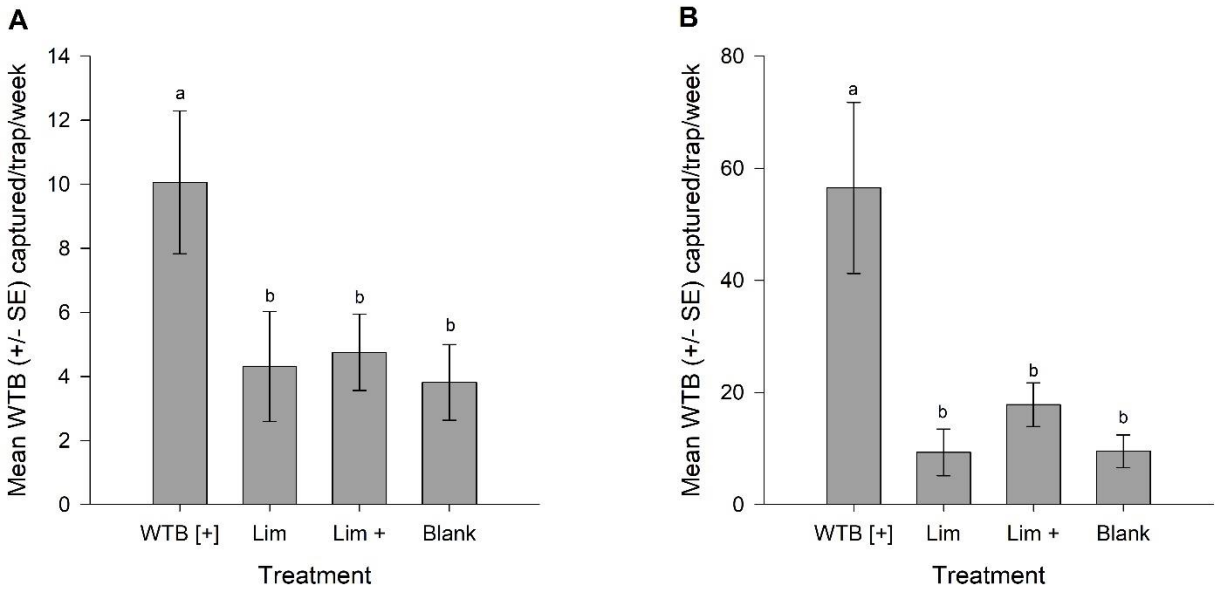

Fig. 4.6: Mean WTB (+/- standard error) captured weekly in multi-funnel traps deployed in two black walnut plantations, (A) Cottonwood and (B) Yellowhawk, during 16 weeks in 2019. Different letters represent significant differences tested at $\alpha = 0.05$ within each site.

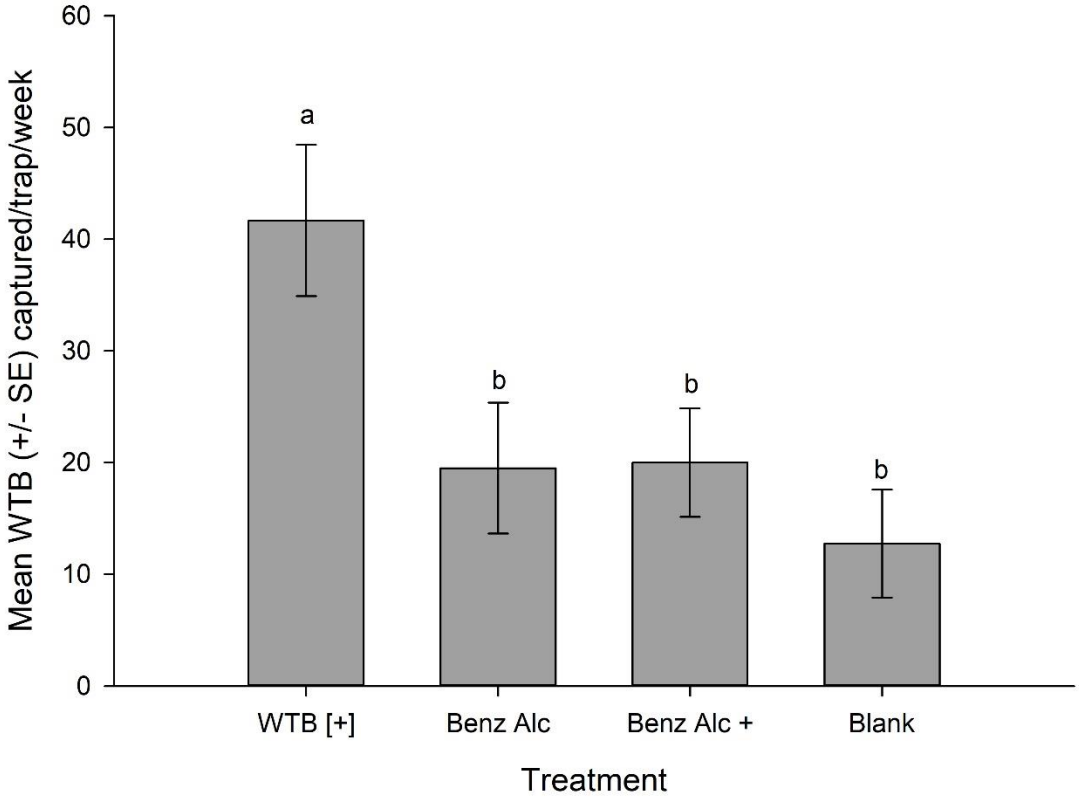

Fig. 4.7: Mean WTB (+/- standard error) captured weekly in multi-funnel traps deployed in one black walnut plantation at the Yellowhawk site during 16 weeks in 2019. Different letters represent significant differences tested at $\alpha = 0.05$.

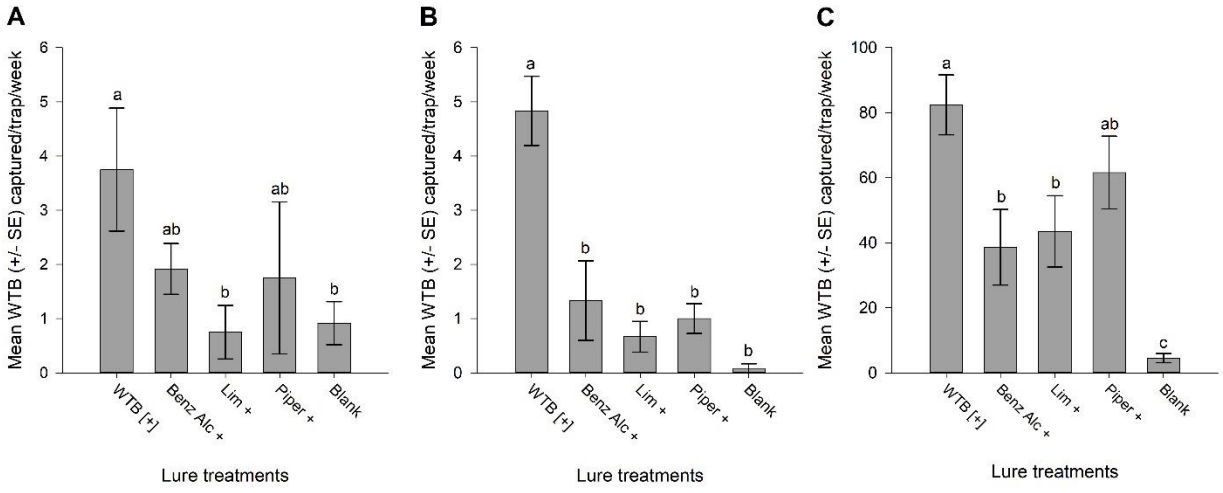

Fig. 4.8: Mean WTB (+/- standard error) captured weekly in multi-funnel traps deployed in three black walnut plantations, (A) Cottonwood, (B) Russell, and (C) Yellowhawk, during 12 weeks in 2020. Different letters represent significant differences tested at $\alpha = 0.05$ within each site.

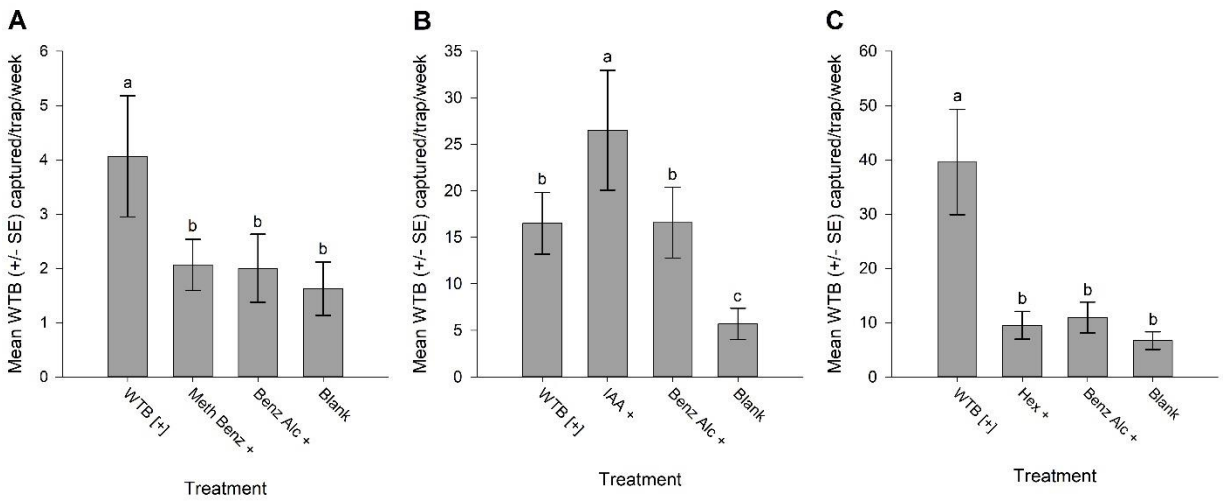

Fig. 4.9: Mean WTB (+/- standard error) captured weekly in multi-funnel traps deployed in three transects within two black walnut plantations, Cottonwood (A) and Yellowhawk (B, C), during 16 weeks in 2019. Different letters represent significant differences within each site tested at $\alpha = 0.05$.

CHAPTER 5. CONCLUSION

The results of laboratory and field experiments presented herein expand on a growing breadth of knowledge regarding the chemical ecology of bark and ambrosia beetles that colonize hardwood trees. Previous to this work, semiochemicals of the peach bark beetle (PBB; *Phloeotribus liminaris*) were unknown, but my findings have demonstrated that high levels of benzaldehyde are produced when female beetles colonize cherry wood and that this volatile compound attracts adult beetles in a field setting. Not only is this the first report of bark beetles utilizing benzaldehyde as a long-range attractant, but it is one of few examples where an insect produces, or enhance production of, host wound volatiles to aid in conspecific aggregation. Also, several volatiles, specifically isoamyl and isobutyl alcohol, produced by symbiotic fungi of the walnut twig beetle (WTB; *Pityophthorus juglandis*) increased attraction of flying beetles to the aggregation pheromone. This result is the first evidence of bark beetles that infest hardwood trees utilizing symbiotic fungal volatiles in host selection. In addition, several volatiles produced by ambrosia beetles that co-occur with WTB may also affect its host selection behavior, perhaps alluding to interspecific eavesdropping by this pest beetle.

In contrast to attractive compounds identified herein, several fungal and non-host volatiles were repellent to both bark and ambrosia beetles. The walnut twig beetle was repelled from pheromone-baited traps in the presence of the fungal volatile benzyl alcohol, while all three invasive ambrosia beetles (*Xylosandrus crassiusculus*, *Xylosandrus germanus*, and *Xyleborinus saxesenii*) were repelled by the same compound to a varying degree. This result suggests that benzyl alcohol may act as a general repellent of bark and ambrosia beetles, although additional research is needed to determine if this is a general phenomenon amongst scolytine beetles. Results of my work also support other recent studies which have observed that limonene, a non-host compound, is broadly repellent to bark and ambrosia beetles. In my research, limonene was repellent to both WTB as well as several ambrosia beetle species. Although piperitone repels *Euwallacea* spp. ambrosia beetles from attractive traps, my findings provide the first evidence that this compound may find application as a management tool for other scolytine species. In results herein, piperitone reduced attraction of both WTB and invasive ambrosia beetles to their respective attractive lures.

Broadly speaking, among my study organisms bark beetle species demonstrated greater response to attractants than ambrosia beetles, which demonstrated only avoidance of fungal volatiles. Many bark beetle species rely on high densities of attacks by conspecifics to overcome host defenses and, thus, there may be a selective advantage to responding to compounds that increase aggregation of adult beetles. Both benzaldehyde and fungal volatiles associated with colonization by PBB and WTB, respectively, contributed to attraction of adult beetles. Only compounds that may convey information about unsuitable hosts, such as benzyl alcohol in the case of the WTB, were avoided by bark beetles. On the other hand, the invasive ambrosia beetles studied here colonize a wide range of hosts and may only rely on general host stress compounds (i.e. ethanol) as attractants. Ambrosia beetles may use fungal volatiles as signals to avoid hosts already colonized by conspecifics, thereby reducing competition and promoting larval survival and development within host xylem. This response of ambrosia beetles to fungal volatiles in my study corroborates the limited number of other field studies conducted with the same or similar ambrosia beetle species.

The findings included here provide management opportunities for bark and ambrosia beetle pests of hardwood trees. The identification of a strong attractant of PBB could lead to development of attract and kill or trap tree tactics to manage this species in the Central Hardwood Forest Region. Using fungal volatiles to increase the attractiveness of pheromone-baited traps may enhance the efficacy of monitoring and management efforts for WTB. The ability to detect these beetles at low population densities is vitally important to safeguard against widespread infestation within the native range of black walnut. Both fungal and non-host volatiles could be used to deter colonization by a variety of bark and ambrosia beetles. Repellent compounds could reduce attacks on particularly valuable trees, or, if the active range of lures permits, be used in area-wide repulsion from the outer edges of tree plantations. The identification of additional attractants and repellents may also aid in the development of push-pull strategies for economically-important bark and ambrosia beetles. For both WTB and invasive *Xylosandrus* spp. beetles, strongly attractive and repellent compounds have been identified and could be optimized to reduce attacks within planted tree stands.

Although my findings represent an important step in understanding the chemical ecology of scolytine beetles that attack hardwood trees, additional areas of research remain poorly understood. New semiochemical-based tools for invasive ambrosia beetles may be developed by

further exploring fungal-associated volatile compounds. Such compounds could also be tested, in combination with the volatiles used herein, in other important pest species, such as the redbay ambrosia beetle which vectors laurel wilt. In addition to testing new compounds, the role of fungal growth media in volatile production has not been well explored and may be important in finding appropriate volatiles for testing. Because fungal volatiles are produced as blends of individual components, it will also be important to test identified compounds in biologically-relevant combinations. These combinations should reflect the ratio of compounds as produced naturally by fungi and be tested in laboratory as well as field settings.

Future use of the semiochemicals identified herein for management will also require additional research. The release rate of volatiles from emitting devices can influence the response of bark and ambrosia beetles and may have influenced the response of those species that were the subject of my research. For example, in laboratory assays WTB responded to low levels of isoamyl and isobutyl alcohol, but exhibited a dose-dependent response to limonene in field trials (Blood et al. 2018, Audley et al. 2020). The active range, or area in which beetles respond to emitted compounds, has not been determined for any of the lures used in my studies. This information will be critical in increasing the efficacy of area-wide monitoring and tree protection tactics that utilize traps and trap trees. For instance, the active range of lures may be used to determine the optimal density of traps to place in a walnut tree plantation to maintain pest beetles below a particular threshold. Finally, the influence of site characteristics, such as tree density and species homogeneity may play a role in effectiveness of semiochemical-based tactics. Testing semiochemicals identified herein in more sites with varying stand characteristics may inform the efficacy of using population manipulation to manage bark and ambrosia beetle populations.

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