DEVELOPMENT AND EVALUATION OF MANAGEMENT STRATEGIES FOR AMBROSIA BEETLES (*SCOLYTINAE*) IN SOUTHEASTERN FRUIT ORCHARDS

by

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(Under the Direction of BRETT R. BLAAUW)

ABSTRACT

Wood-boring ambrosia beetles (Coleoptera: *Scolytinae*) are major economic pests in tree fruit, nut, and woody ornamental orchards. Three projects were conducted from 2022 to 2024 to develop and evaluate different management aspects including monitoring, repellents, a novel strategy that combines lures and repellents, insecticides, and the use of nanocellulose. Traps captured a wide range of species and genera from early March to mid-July. The location, date, and temperature appeared to be factors that contribute to flight and attack activity. Optimal repellents and luring monitoring methods were refined. The viability of both attractants and repellents in a “push-pull” strategy for lowering the frequency of ambrosia beetle attacks on water-stressed apple trees was assessed. The efficacy of a variety of insecticides and nanocellulose was evaluated on ethanol-baited bolt traps. Results identify promising tactics that could potentially be practical and effective for growers in ambrosia beetle monitoring and control in southeastern fruit orchards.

Keywords: specialty tree crop, apples, orchard, ambrosia beetles, integrated pest management, nanocellulose, insecticides, *Scolytinae*

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CHAPTER 1

INTRODUCTION AND NATURAL HISTORY

Wood-boring ambrosia beetles are an economically significant pest in many tree crops such as tree fruits, tree nuts, as well as ornamental nursery trees. The granulate ambrosia beetle, *Xylosandrus crassiusculus*, and black stem borer, *Xylosandrus germanus*, are the two major exotic species that are the most problematic and established in the eastern United States. *Xylosandrus crassiusculus* and *X. germanus* have been found in 32 and 28 states, respectively (Gomez et al. 2018). These beetles cause major problems to the tree crops that include bark staining, perforated stems, oozing sap, branch dieback, and rapid tree death due to burrowing into the trees to grow their fungi food source in galleries that have been made. These beetles are able to grow this special source of nutrition because they introduce symbiotic fungi into the tree as they burrow for both the larval stages and adults to consume (Batra 1963). A variety of stressors such as flood stress leads to the release of ethanol by the trees, which, in turn, attracts the beetles to attack these weakened trees (Ranger et al. 2020).

This research is significant at this time from an economic standpoint because the tree fruit and tree nut market in the US is currently valued at $25 billion by the USDA, and this figure does not even include the valuation of the ornamental nursery tree market. The Georgia Farm Gate Value Report in 2021 valued the tree fruit and tree nut market as well as the ornamental container and field nursery market in Georgia at over $912 million and over $407 million, respectively. One major concern is that ambrosia beetles have been associated with mass dieback of apple trees, now known as “rapid apple decline” or “sudden apple decline,” across major apple-growing regions of the eastern United States (US) (Agnello et al. 2015, Stokstad 2019). The species of ambrosia beetles associated with rapid apple decline in New York and North 2 Carolina, are also common in the southeast. The granulate ambrosia beetle, *X. crassiusculus,* was actually first discovered in the US infesting peach trees in South Carolina but has not been a considerable pest since then (Kovach and Gorsuch 1985). However, with the issues facing apple growers due to rapid apple decline, it is imperative for Georgia growers to have updated information on these potential pests to be prepared to make management decisions if the beetles become a problem. In addition, these ambrosia beetles have a wide host range and already affect a massive number of growers throughout the US, and the number of growers affected could increase if these beetles establish themselves in even more states than they already inhabit.

Key Groups and Distribution

Understanding the distribution of ambrosia beetles can help identify which species have the potential to cause damage to our production industries and determine which species to monitor for. Key pest species of ambrosia beetles, *X. crassiusculus*, *X. germanus*, *X. compactus*, and *Xyleborinus saxesenii* are native to Asia and have become invasive in the US. *Hypothenemus* spp. has had many non-native species introduced to the United States from Africa, Asia, and South America (Haack and Rabaglia 2013). *Xylosandrus crassiusculus* was first discovered in the US in 1974 when specimens were found in a South Carolina peach orchard. (Ranger et al. 2016). This species quickly spread to 32 continental states and Hawaii (Gomez et al. 2018, Ranger et al. 2020), and is now a major pest of woody ornamentals, fruit trees, and nut trees in the southern US (Ranger et al. 2016).

The black stem borer, *X. germanus*, was first discovered in the US in 1932 on grape vines in a New York greenhouse, and this species has become established in 32 states around the US

(Weber and McPherson 1983, Ranger et al. 2016, Galko et al. 2018, Gomez at al. 2018). *Xylosandrus germanus* is now a major pest of woody ornamentals, fruit trees, and nut trees in many Midwestern and Northeastern states (Rabaglia et al. 2006, Ranger et al. 2016).

Xylosandrus compactus is very common in more subtropical to tropical regions, and it was first discovered in the United States in Florida in 1941 (Ngoan et al. 1976, Chong et al. 2009, Greco and Wright 2015). Since it was first found in 1941, *X. compactus* has been found in 13 continental states and Hawaii (Ranger et al. 2020).

Xyleborinus saxesenii was one of the first non-native ambrosia beetles to be discovered in the United States. First found in California in 1911, this species has spread throughout the majority of the United States being found in 45 states (Haack and Rabaglia 2013).

The first species found in the United States belonging to the genus *Hypothenemus*, *H. crudiae*, was discovered in Georgia and Louisiana in 1868 from the native range of Asia; however, many species have been found in the United States with native ranges of Africa and South America as well (Haack and Rabaglia 2013).

General Biology and Fungal Interactions

Most bark beetles feed on the nutrient-rich inner bark of trees; however, ambrosia beetle larvae and adults generally feed on a grown symbiotic fungus that is introduced by the females upon entry into a tree (Harrington 2005). Female beetles are equipped with a sac between the prothorax and mesothorax that is called a mycangium that allows them to transport the symbiotic fungi into the host tree while they are creating the fungus galleries (Batra 1963, 1967; Beaver 1989; Francke-Grosmann 1967; Ranger et al. 2016). *X. crassiusculus, X. germanus, X. compactus, and Xyleborinus saxesenii* all require a symbiotic fungus to farm and feed on to survive. There are currently no species of *Hypothenemus* in North America that have been confirmed to consume fungus; however, a study examining the diet of the coffee berry borer (*Hypothenemus hampei*) has found that this species relies on the fungus *Fusarium solani* to undergo proper development and reproduction (Morales-Ramos et al. 2000).

The fungi *Ambrosiella xylebori* and *A. roeperi* are the major symbionts and food source for *X. crassisusculus* (Harrington et al. 2014, Bateman et al. 2016). *A. hartigii* and *A. gosmanniae* are both associated with the species *X. germanus* (Weber and McPherson 1983, Bateman 2016). Much like *X. crassiusculus*, *X. compactus* relies on *A. xylebori* as well as *Fusaria* spp. (Bateman et al. 2016, Asman et al. 2020). The fungus grown in the galleries are either growing mycelial cells that are dark in appearance when there are no beetles or growing as the food source producing conidia that are white in appearance when beetles are present (Ranger et al. 2016).

The main fungal symbiont of *Xyleborinus saxesenii* is *Raffaelea sulphurea*, but the larvae of this species are actually xylomycetophagus because they can feed on the wood of the tree as well (Deyrup and Atkinson 1987, Biedermann 2012). The fact that the larvae will feed on the wood of the tree is important to note because systemic insecticides have the potential to be effective at managing this species by controlling the larval population in their galleries.

Since most ambrosia beetles feed on these symbiotic fungi and not the tree itself, managing these insects with systemic insecticides provides little benefit. Although these symbiotic fungi comprising the majority of the ambrosia beetles’ food source make management difficult, furthering our understanding of these fungi could lead to different types of management tools for growers. Mycoparasitic fungi of the beetles’ symbiotic fungi as well as entomopathogenic fungi could be explored as possible management tactics.

Reproduction and Oviposition

Ambrosia beetles in the three genera *Xylosandrus*, *Xyleborinus*, and *Hypothenemus* exhibit a variety of similarities in their reproduction. The species in these genera mate with the male siblings and offspring and then choose whether or not to fertilize eggs in the form of arrhenotokous parthenogenesis (Biedermann 2010; Greco and Wright 2015; Vega et al. 2015). Males are haploid and come from unfertilized eggs, while the females are diploid and come from fertilized eggs. Once the females have mated, they will overwinter in the galleries that were constructed until the weather starts to warm up, humidity changes, and light intensity shifts in the spring, and then they vacate the tree to locate a new host to colonize (Chen et al. 2010, Ranger et al. 2016). Once the new host has been found and invaded, *Xylosandrus* spp. and *Xyleborinus saxesenii* will wait to initiate oviposition until after the symbiotic fungus has started to become established in the galleries (Hoffman 1941, Hosking 1973, Ranger et al. 2016, CABI 2022). Timing management strategies to align with spring dispersal may allow for effective, targeted management options.

Life Stages

Understanding the different life stages and timelines of development for ambrosia beetles is crucial for effective management. Determining the most vulnerable stage of development could increase the efficacy of current management tactics by targeting those life stages. Currently, timely spray treatments are fundamental for effective ambrosia beetle management, so understanding the various development times of these beetles would be beneficial because it will allow growers to accurately anticipate when ambrosia beetles will become active during the season to preemptively spray their trees.

Egg

Under Laboratory conditions, the egg of *X. crassiusculus* is approximately 0.41 mm in width and 0.61 mm in length at 29°C and 0.25 mm in width and 0.53 mm in length at 35°C, and it is described as “rod-shaped” with both ends culminating in large curves with soft white, shining color (Qureshi et al. 2021). The females were observed to oviposit a mean of 55.8 to 67.8 eggs per female depending on the temperature (Qureshi et al. 2021). The egg of *X. germanus* is approximately 0.38 mm in width and 0.67 mm in length, and it is described as “ellipsoid-shaped” with soft white, shining color (Hoffman 1941, Ranger 2016). The females exhibited a range of oviposition from 2 to 54 eggs per female (Hoffman 1941). The egg of *X. compactus* is approximately 0.30 mm in width and 0.59 mm in length, and it is described as “oval-shaped” with a smooth white surface (Hara and Beardsley 1979).

The eggs have an observed incubation period of roughly three to four days (Hara and Beardsley 1979). The egg of a widely distributed *Hypothenemus* species, *H. eruditus,* is approximately 0.20-0.25 mm in width and 0.30-0.50 mm in length with an oval shape and white coloration (Huang et al. 2019). The development time from egg to adult was observed to be 28 days on average (Browne 1961). These parameters will not be identical across all species in *Hypothenemus,* but this information provides a general means of comparison between the other species.

The egg of *Xyleborinus saxesenii* is about 0.24-0.26 mm in width and 0.52-0.55 mm in length with an oval shape and translucent white coloration. (Menocal Sandoval et al. 2016, CABI 2022). The eggs are regularly moved and groomed by the adult beetles during the roughly 5-day incubation period (Biedermann 2009).

Larvae

The first instar of *X. crassiusculus* are slightly curved with white coloration and legless (Qureshi et al 2021). Under laboratory conditions, the first instar larvae were roughly 0.39 mm wide and 0.77 mm long at a rearing temperature of 29°C and 0.21 mm wide and 0.63 mm long at a rearing temperature of 35°C (Qureshi et al. 2021). At the fifth and final instar, the mean sizes are 0.71 mm wide by 3.01 mm long and 0.65 mm wide by 2.06 mm long at rearing temperatures of 29°C and 35°C, respectively (Qureshi et al. 2021). *X. germanus* are very similar in shape and form to the larvae of *X. crassiusculus* but undergo three instars before pupation (Weber and McPherson 1983). *X. compactus* larvae have a width of about 0.025 mm and a length of 0.212 mm for the first instar, and the second and final instar of this species has a width of about 0.021 mm and a length of 0.347 mm (Hara and Beardsley 1979).

The larvae of *Xyleborinus saxesenii are* similar in form to the other ambrosia beetle larvae described with three instars, and the larvae of this species have been shown to enlarge the tunnel system as well as groom other individuals and the walls of the gallery (Biedermann et al., 2012).

A known pest species of coffee in the genus *Hypothenemus* is *H. hampei*. The larvae of this species have two instars for the females, only one instar for males, and a prepupal stage (Vega and Johnson 2015). Some researchers believe that the larvae also consume the frass of the adult female beetles in order to obtain the symbiotic fungus needed to disperse and create more colonies (Waterson and Norris 1989).

Pupa

The pupae of *X. crassiusculus* are roughly 0.78 mm to 0.89 mm in width and 2.01 mm to 2.82 mm in length depending on the temperature; the pupae are white until directly prior to the eclosion of the adult where they turn a light brown (Qureshi et al. 2021). The duration of the pupal stage was also observed to be as short as 5.03 days to as high as 9.11 days based on the temperature, with the shortest duration seen at 29°C and longest at 27°C with the beetles evaluated at 27°C, 29°C, 31°C, and 35°C (Qureshi et al. 2021). The larvae *X. germanus* complete a roughly two to three days prepupal stage before actual pupation (Hoffman 1941). Within two days of pupation, the eyes, mandible, and wings start to darken on the white pupa and are easily discernable (Agnello 2020). There are size differences between the pupae of different sexes; the female pupae average about 1.09 mm in width and 2.53 mm in length while the male pupae are smaller with an average width of 0.98 mm and length of 1.78 mm (Hoffman 1941). *X. compactus* also undergoes a prepupal stage, the eyes start to darken around day two of pupation, and everything has darkened prior to eclosion of adult six to 7 days after the start of pupation (Hara and Beardsley 1979).

Xyleborinus saxesenii pupae are motionless during this stage and the pupation and larval development are both located in the same gallery within a colony (Menocal Sandoval et al. 2016). The duration of the pupal stage has been observed to be roughly five days at a rearing temperature of 25°C (Biedermann et al. 2009).

The pupae of the genus *Hypothenemus* have not yet been described; however, it would be safe to assume the general trend of beginning white in color and darkening. The pupae are also most likely smaller in average size when compared to the larger species of *X. crassiusculus* and *X. germanus*. For the species *Hypothenemus hampei*, there is an average of 5.98 pupae inside a singular infested berry on a coffee plant (Hamilton et al. 2019).

Adult

The adult males of ambrosia beetles in the three genera *Xylosandrus*, *Xyleborinus,* and *Hypothenemus* share many similar attributes to each other. The males are smaller and rounder in size with less coloring than the females (CABI 2016, Ranger et al. 2016, CABI 2022). Males are often deformed, less sclerotized, and possess vestigial wings that lack the ability to fly (Hoffman 2941, CABI 2016, Ranger et al. 2016). Males of these genera seldom ever leave the galleries where they were born (Dole et al. 2010, Ranger et al. 2016, CABI 2022).

The genus *Xylosandrus* can be easily characterized from the other members of *Xyleborini* due to the large separation between the procoxae (Rabaglia 2006). The females of the three species *X. crassiusculus, X. germanus,* and *X. compactus* all share similar stout, cylindrical bodies that are rounded at the head and abdomen when looking from a dorsal view (Wood and Bright 1992, Rabaglia et al. 2006, CABI 2008, Ranger et al. 2016). Crassiusculus females are roughly 1.2 mm in width and 2.1-2.9 mm in length, have a pronotum with a brownish-red coloration, and show dark brown coloration on the distal half of their elytra (Ranger et al. 2016). The majority of the head is hidden under a large pronotum which has many blunt serrations on its anterior margin (Kovach and Gorsuch 1985, Rabaglia et al. 2006, Ranger et al. 2016). The steep elytral declivity has a uniform pattern of many small granules throughout which leads to a duller appearance (Ranger et al. 2016). *X. Germanus* are brown to black in coloration with a large pronotum covering their head with a width of roughly 0.87-1.00 mm and a length of 2-2.3 mm (CABI 2019). The elytra possess interstrial setae; however, the stria possess very small, shallow punctures with no setae present (CABI 2019). *X. compactus* females are dark brown in color to the point where they are almost black with a width of approximately 0.7-0.95 mm and a length of approximately 1.4-1.90 mm (Bright 1968). The elytra possess distinct strial and interstrial punctures that are roughly the same size, and the interstria possess a row of setae that are roughly twice as long as the width of the interstria itself. (CABI 2022).

Xyleborinus saxesenii females are approximately 0.71-0.86 mm in width and 2.0-2.4 mm in length with a scutellum that is conical in shape (Bright 1968). Tubercules are present on interstria one, three, and nine; there is an absence of spines at the end of the elytra that distinguishes this species from others in the *Xyleborinus* genus (Menocal Sandoval et al. 2016, CABI 2022).

The adult females of the pest species *Hypothenemus hampei* are roughly 0.61-0.70 mm in width and 1.4-1.6 mm in length (Jaramillo et al. 2006, Baker 2022). However, for the entire genus, this length can range from as small as 0.60mm to as large as 2.2 mm (Johnson et al. 2020). The setae on the elytra for the members of this genus are very distinct because they are neat rows of what looks like raised scales that have been flattened (Huang et al. 2019).

Signs of Infestation

As females bore into a tree in the attempt to establish a colony, the chewed-up wood is pushed out of the attack hole as what is colloquially called a “toothpick” that can be multiple centimeters in length, but these toothpicks cannot be relied on because they can break apart from wind or rain (Ranger 2016). Oozing sap, sap staining on bark, dieback of branches, perforated stems, wilting, and tree collapse are all signs of ambrosia beetle infestation (Hara & Beardsley 1979, Greco and Wright 2015, Ranger et al. 2016)

Rapid apple decline or sudden apple decline (RAD or SAD) is a condition in apple trees that presents the symptoms of lesions that turn necrotic, yellowing or reddening of leaves, and dying tissue at the grafting connection point of rootstock and trunk; tree collapse/death can happen within weeks of these symptoms first becoming noticeable (Singh et al. 2019, Stokstad

2019). Currently, the major contributing factors of RAD are not completely known, but ambrosia beetles are correlated with the decline with nearly all trees displaying symptoms of RAD showing infestations of ambrosia beetles (Villani & Walgenbach 2017).

Hosts

Ambrosia beetles have a wide variety of host trees, with hundreds of different species that they have been known to attack, and they frequently infest avocado trees, coffee plants, fruit trees, nut trees, and trees in woody ornamental nurseries (Greco & Wright 2015, Hulcr & Stelinski 2017, Ranger et al. 2020). Common fruit and nut trees found in many orchards such as apples (*Malus* spp.), figs (*Ficus* spp.), cherries, plums, and peaches (*Prunus* spp.), pears (*Pyrus* spp.), avocados (*Persea* spp.), grapes (*Villis* spp.) pecans (*Carya illinoinensis*), and chestnut (*Castanea* spp.) have all experienced attacks and infestations by ambrosia beetles (Weber and McPherson 1983, Ranger et al. 2016, Ranger et al. 2020). Species of tree may not be as big of a factor for ambrosia beetle attack selection as the increased ethanol production of stressed and weakened trees (Ranger 2010, 2013).

Management

Essentially the only current effective management strategy for ambrosia beetles is the use of pyrethroids (such as permethrin and bifenthrin) in a preventative manner, but recent research has shown that verbenone and methyl salicylate are effective repellents for ambrosia beetles (Reding et al. 2010, Atkinson et al. 2011, Hughes et al. 2017). No systemic insecticides have been shown to be effective at causing adult beetle mortality largely due to the fact that the beetles bore into the trees without ingesting any of the wood (Addesso et al. 2019). The preventative use of bifenthrin and permethrin involves the spraying of these insecticides every 10-14 days when the beetles are in their spring flight (Hudson and Mizell 1999, Ranger et al.

2016). The emergence of beetles can be difficult to forecast, so it is important to employ other strategies to maintain tree health and reduce the stress that trees undergo which can make them more attractive to attacks (Ranger et al. 2016). In order to effectively reproduce, these beetles require the successful establishment of their symbiotic fungi, so studies have looked into the use of other fungi to halt the symbiotic fungi from developing. The entomopathogenic fungi *Beauveria spp.* and *Metarhizium spp.* are being evaluated in combination with the mycoparasitic fungi *Trichoderma* to manage the beetle itself as well as the fungal symbionts, and this combination has shown promising results in impeding the development of the fungal symbiont and increased mortality of the adult beetles (Castrillo et al .2016, Reverchon et al. 2021). Future studies looking into the use of *Beauveria spp., Metarhizium spp*., and *Trichoderma* should explore the most effective delivery method of this combination of entomopathogenic and mycoparaitic fungi as well as attempt to discover possibly more effective microorganisms to utilize (Reverchon et al. 2021). Despite these recent advances in different monitoring and management techniques for these pests, more research is necessary to better understand the effectiveness of different insecticides and discover more ideal methods of species-specific monitoring and management strategies (Gugliuzzo et al. 2021). The current knowledge pertaining to behavior and ecology must be expanded upon to develop the most successful and sustainable methods of management of ambrosia beetles.

Monitoring

The practice of monitoring ambrosia beetles for first flights and peak activity throughout the season is crucial for the timing of spraying insecticides or employing different management tactics. The first flight of ambrosia beetles after overwintering depends very heavily on the temperature of the environment, so predicting the start of their activity can be difficult. Luckily there are known attractants for these beetles that can make monitoring effective and inexpensive for growers.

Attractants

Ethanol has been shown to readily attract the females of *X. crassiusculus, X. germanus, X. compactus, Xlyeborinus saxesenii,* and *Hypothenemus spp.* (Ranger et al. 2010, Messing 2012, Chen et al. 2021). Microbial degradation and anaerobic respiration occur in stressed or dying trees, and ethanol is released in large quantities from these processes as a by-product (Kimmerer and Kozlowski 1982, Kimmerer and MacDonald 1987). Many abiotic and biotic factors can elicit the release of ethanol from trees such as anoxic conditions, hyperoxia, freezing, disease, etc. (Ranger et al. 2010, Bui et al. 2019). These stressors cause the production of acetaldehyde to sharply increase in the trees and this compound is soon transformed into ethanol to be transported through the xylem of the tree to the leaf tissue (Ranger 2010). Once in the leaf tissue, some of the ethanol is changed into acetone and acetaldehyde from oxidative metabolism, but the majority is released as volatile ethanol (Cossins 1978, Kreuzwieser et al. 1999). Methanol is produced by healthy trees and production is greatly increased in response to mechanical damage; however, methanol is not a strong attractant for ambrosia beetles (Von Dahl et al. 2006, Ranger et al. 2010). A study by Ranger et al. in 2010 that involved injecting trees with volatile compounds such as ethanol, acetaldehyde, acetone, and methanol found that ethanol was the most effective attractant. Ethanol traps can be home-made using 70-95% ethanol, but there is much less labor involved in purchasing commercial lures because it eliminates the frequent refilling of the home-made lures (Ranger et al. 2016). Ambrosia beetle captures are generally higher with higher concentrations of ethanol. The addition of α-pinene and conophthorin showed no consistent increase in attraction over ethanol alone (Steininger et al. 2015, Addesso 2019).

Traps

A variety of traps such as the Lindgren funnel trap, tree bolts, and baited bottle traps have been utilized to successfully monitor ambrosia beetles. Each of these three traps proves useful in different aspects of the beetles' behavior. The Lindgren funnel trap is a viable method to catch beetles while in flight, the baited bottle trap lures flying beetles, and the tree bolts are useful to see the number of actual ambrosia beetle attacks on wood. The Lindgren funnel trap was created in 1983 when Dr. BS Lindgren layered multiple funnels on top of each other with a collection tube at the bottom to collect the beetles caught. These funnel traps are easy to deploy and show similar efficacy to sticky traps (Lindgren 1983). A newer style of ambrosia beetle trap are baited bottle traps, which consist of 2-liter bottles with windows that are made by cutting away the plastic on the side, and a collection tube is modified to screw into the top of the bottle. These traps are hung upside down, filled with soap water, and then baited with an ethanol lure to attract ambrosia beetles (Steininger et al. 2015, Ranger et al. 2016). Due to the fact that these bottle traps can be made very easily with very little cost, this type of monitoring trap has become commonly utilized. The height at which these bottle traps are deployed exhibits differences in catch rates for certain species. When traps were deployed at 0.5 m, 1.7 m, and 3.0 m, more *X. germanus* were captured at 0.5 m than at the other heights; more *X. crassiusculus* were captured at 0.5 m and 1.7 m when compared to traps at 3 m (Reding et al. 2010). Since different species of ambrosia beetles are more prevalent in certain areas of the United States, this difference in capture rate at different trap heights is important based on the location where the monitoring is taking place and which species are being targeted. The tree bolt monitoring traps are very useful for assessing the prevalence of beetle attacks. These traps are made by cutting off roughly 20-30 cm length sections of tree trunks or branches that are a width of about 10cm. There are two generally used ways to bait these bolts to attract ambrosia beetles. One method is to soak the tree bolt in ethanol for 24 hours prior to deployment, and the other widely used method is to core the bolt with a drill to make an approximately 1 cm wide hole that can go 10 cm deep into the bolt. Once the tree bolt is cored, ethanol is poured into the hole and the hole is closed with a cork (Ranger et al. 2016). The number of ambrosia beetle attacks over time can be quantified by regularly checking the tree bolts for entry holes and marking the holes found (Redding and Ranger 2020). Any studies involving the use of spray treatments will most likely elect to use the cored tree bolts filled with ethanol as opposed to those soaked for 24 hours because the act of soaking bolts in the ethanol could potentially alter the efficacy of a spray compared to sprays made to trees under normal field conditions.

Research Objectives

The overall goal of this study is to assess the different methods of ambrosia beetle trapping, monitoring, and management. The information gained from this study will aid in the creation of a more comprehensive method to manage ambrosia beetle attacks. Objective 1 is to obtain more information to determine the most effective trapping techniques between different types of traps, concentrations of ethanol lures, and repellents. Objective 2 is to test a possible novel management tactic by employing a push-pull technique consisting of repellents and lures on water-stressed apple trees. Objective 3 is to compare different insecticides to determine if any others besides bifenthrin and permethrin could be effective in management and to evaluate possible tree protection techniques through the use of nano-cellulose gel in tandem with insecticides and repellents. More specifically:

1. Objective 1a of our study is to evaluate and improve upon the current species-specific trapping methods and the dispersal of ambrosia beetles. Different trap types as well as

different lures and ethanol concentrations could possibly affect the capture rate of ambrosia beetles.

2. Objective 1b of our study is to determine the best combination of repellents that successfully repel more beetles when the most effective lure found from objective 1(a) is present. Better ambrosia beetle repellents would be extremely useful for growers to help reduce the number of ambrosia beetle attacks.
3. Objective 2 of our study is to combine the use of the most effective repellents and lures found in Objective 1 to develop and evaluate the efficacy of a novel "push-pull" strategy to reduce ambrosia beetle attacks. The establishment of a new management practice that could reduce the amount of insecticide needed would benefit the environment as well as the potential slow insecticide resistance that is developing.
4. Objective 3a of our study is to determine effective comprehensive management tactics by comparing the effectiveness of the new and current active ingredients of insecticides. It would be very useful for farmers to have more insecticides available for them to use rather than the spraying of solely permethrin and/or bifenthrin.
5. Objective 3b of our study is to evaluate the efficacy and duration of protection of treatment with nanocellulose gel. Nanocellulose could potentially create a physical barrier of protection without pesticides, or it could increase the duration of effectiveness of bifenthrin, permethrin, or repellents when used in a mixture so the frequency of spraying by growers could be lessened.

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CHAPTER 2

EVALUATING THE EFFICACY OF DIFFERENT TRAPS FOR MONITORING AND REPELLENT COMPOUNDS ON AMBROSIA BEETLES (*SCOLYTINAE*)

Introduction

Ambrosia beetles (subfamily *Scolytinae*) are bark beetles that bore into the wood of living tree crops and cause considerable damage to the health of the tree and the entire cropping system economically (Ree & Knutson 1997, Ranger et al. 2016, Gugliuzzo et al. 2021). There has been an increase in ambrosia beetle-associated tree death, particularly with apples in states like New York and North Carolina and in young pecan plantings in Georgia. These beetles cause major problems to tree crops through bark staining, perforated stems, oozing sap, branch dieback, and rapid tree death due to burrowing into the trees to grow their fungi food source in galleries that have been made (Greco and Wright 2015, Ranger et al. 2016). This research is extremely important from an economic standpoint because the tree fruit and tree nut market in the US is currently valued at $25 billion by the USDA. The Georgia Farm Gate Value Report in 2021 valued the tree fruit & tree nut market in Georgia at over $912 million.

While ambrosia beetles may not be the sole cause of tree death, a variety of stressors, such as flood stress or drought, can increase the susceptibility of trees to attack by the beetles. Tree stress leads to the release of ethanol by the trees, attracting the beetles to attack these weakened trees, which can subsequently lead to tree decline (Ranger et al. 2020). The granulate ambrosia beetle, *Xylosandrus crassiusculus*, and black stem borer, *Xylosandrus germanus*, are the two major exotic species that are the most problematic and established in the eastern United

States. *X. crassiusculus* and *X. germanus* have been found in 32 and 28 states, respectively (Gomez et al. 2018). The majority of all ambrosia beetles are attracted to ethanol emitted from young, stressed, or dying trees (Ranger et al. 2010, Messing 2012, Chen et al. 2021). Different abiotic and biotic factors that lead to the release of ethanol by trees include (but are not limited to): hyperoxia, anoxia, disease, and freezing (Ranger et al. 2010, Bui et al. 2019). These stressors cause ethanol to be transported through the xylem of the tree into the leaf tissue and released as volatile ethanol (Cossins 1978, Kreuzwieser et al. 1999, Ranger et al. 2010).

Management of ambrosia beetles can be very difficult because the beetles do not feed on the wood of the trees that they bore into, instead, they introduce a symbiotic mycangial fungus to cultivate inside the tree as the colony's sole source of nutrition (Batra 1963, Addesso 2019). As few as one gallery in a tree can lead to the major decline of seedlings (Hara & Beardsley 1979). Because they do not ingest the wood, systemic pesticides would not be useful in controlling ambrosia beetle populations. The preventative use of the repellent insecticides bifenthrin and permethrin has been the only viable insecticide observed and entails the spraying of these insecticides every 10-14 days when the beetles are in their spring flight (Hudson and Mizell 1999, Ranger et al. 2016). The monitoring methods of these beetles need to be as effective as possible to determine the optimal time to start spraying insecticides.

Ethanol-baited traps are widely used to successfully monitor ambrosia beetle activity because ethanol has been shown to be the most effective attractant tested (Ranger et al. 2010, Galko et al. 2019). The additives α-pinene and conophthorin were tested with ethanol, but the efficacy of these mixtures was not consistently increased when compared to ethanol alone (Addesso 2019). The three trap types most thoroughly tested in their ability to capture ambrosia beetles are the Lindgren funnel trap, tree bolt traps, and bottle traps; however, it has been discovered that the bottle traps were more effective at catching *X. crassiusculus* in flight than the Lindgren funnel trap (Coyle et al. 2005, Reding et al. 2011). Baited bottle traps consist of 2-liter bottles with windows that are made by cutting away the plastic on the side. These traps are hung upside down, filled with soap water, and then baited with an ethanol lure to attract ambrosia beetles (Steininger et al. 2015, Ranger et al. 2016). The height at which these bottle traps are deployed also shows a difference in the catch rates for certain species; more *X. crassiusculus* were captured at 0.5 m and 1.7 m when compared to the traps deployed at 3 m (Reding et al. 2010). The tree bolt monitoring traps are very useful for assessing the prevalence of beetle attacks in the area, and growers can make these traps by cutting off roughly 20-30 cm length portions of tree trunks or branches that are at least 3.81 cm in diameter. The diameter must be at least 3.81 cm to delay the desiccation of the ethanol from the bolt trap (Reding & Ranger 2020). There are two generally used ways to bait these bolts to attract ambrosia beetles. One method is to soak the tree bolt in ethanol for 24 hours prior to deployment, and the other widely used method is to core the bolt with a drill to make an approximately 1 cm wide hole that can go 10 cm deep into the bolt. Once the tree bolt is cored, ethanol is poured into the hole and the hole is closed with a cork (Ranger et al. 2016).

Pyrethroids such as permethrin and bifenthrin may have been the only repellent insecticide known for ambrosia beetles, but research has started to show that verbenone and methyl salicylate are effective repellents for ambrosia beetles (Borden et al. 2001, Reding et al. 2010, Atkinson et al. 2011, Hughes et al. 2017). While verbenone has been used in managing pine beetles since 1988, methyl salicylate was primarily used as a repellent for aphids until more recently (Hardie et al. 1994, Progar 2003). The use of these repellents could potentially open a door for the development of novel strategies for ambrosia beetle management that do not require the heavy spraying of pyrethroids.

Despite the advances in different monitoring and managing techniques for these pests, more research is necessary to better understand the effectiveness of luring methods and repelling compounds to develop more ideal methods of species-specific monitoring and management strategies (Gugliuzzo et al. 2021). The main objectives of this study were to determine the efficacy of different commercially purchased lures for bottle traps, the most effective tree bolt luring methods, and the compound(s) that could aid in repelling ambrosia beetles before they attack a stressed tree.

Materials and Methods

Study site. In 2022, the University of Georgia Horticulture Research Farm in Watkinsville, GA was selected for this study. There was a large variety of trees and other crops being grown on this site for various research projects that are not managed with the commercial standard insecticide management guideline (Table 1). The crops nearest to where the experiments took place were pecan trees, peach trees, blueberries, and grapevines.

Traps, lures, and repellents. Tree bolts and bottle traps were utilized. The tree bolts were made from crape myrtle (*Lagerstroemia* spp.) with a diameter of 3.81-5.0 cm and a length of ~20 cm. A diameter of at least 3.81 cm was required for proper coring of the bolts and to prevent rapid desiccation of the bolts soaked in ethanol (Reding and Ranger 2020). The same ethanol was used to treat both the cored and soaked bolts. The soaked bolts were soaked in ethanol for 24 hours prior to deployment (Fig. 1A). For the filled bolt traps, a 15 cm deep hole was drilled through the center of the bolt leengthwise, the hole was filled with ethanol, and the top of the hole was closed with a cork (Fig. 1B). Both types of tree bolts had a small eye hook drilled into the top of the bolt to feed a zip-tie through to hang the bolts from a metal shepherd's hook (Fig. 1). Every week, all bolts were assessed for attacks, the cored bolts were refilled with ethanol, and the soaked bolts were replaced every 2 weeks due to a loss in attractiveness to ambrosia beetles (Reding and Ranger 2020).

The bottle traps utilized were made from 2-liter clear juice bottles (Berlin Packaging, Chicago, IL) with two windows cut out across from each other both with widths of 5 cm and lengths of 9 cm. A small hole was made in the bottom of the bottle to hang the low-release lures (ChemTica USA LLC, Durant, OK) and any repellents utilized from a paper clip (Fig. 2A). The repellents consisted of BeetleBlock Verbenone pouches (ChemTica) and Predalure Methyl Salicylate (90-day) pouches (ChemTica USA LLC, Durant, OK). The Ultra-High-Release ethanol lures (ChemTica) were situated inside the bottle trap (Fig. 2B). Two larger holes were spaced out on either side of the bottom of the bottle to feed a zip tie through to hang the bottle trap upside down on a metal shepherd's hook. The cap of the juice bottle was affixed to a cap of a 50 ml centrifuge tube, so the captured beetles were collected into the tube (Fig. 2A, 2B). All the cutting and affixing of the caps was performed with a wood-burning wand and hot glue. Soap water was made using Dawn dish soap (Procter and Gamble, Cincinnati, OH) and added to the bottle traps to break the surface tension of the water to drown visiting beetles. The lures were replaced every 4 weeks.

Trap/lure-type experiment. This experiment was conducted at the University of Georgia Horticulture Research Farm in 2022 (Table 1). The crape myrtle tree bolts and bottle traps were employed for this experiment. The treatments were 1) a tree bolt soaked in 10% for 24 hours, 2) a tree bolt soaked in 90% ethanol for 24 hours (Fig. 1A), 3) a cored tree bolt filled with 75 ml of 10% ethanol, 4) a cored tree bolt filled with 75 ml of 90% ethanol (Fig. 1B), 5) a bottle trap baited with a low-release pouch style ethanol lure (ChemTica) (Fig. 2B), 6) a bottle trap baited with an ultra-high release (UHR) ethanol lure (ChemTica), 7) a tree bolt with no ethanol, and 8) a bottle trap with no ethanol lure. These treatments were replicated four times in a randomized complete block design (RCBD) along the wood line of the farm with traps spaced 10 m apart. This experiment was conducted when there was ambrosia beetle activity at the study site over 42 days. While waiting to dissect the bolts to identify the ambrosia beetles inside, the bolts were kept in storage at the lab.

Repellent type experiment. This experiment was conducted at the University of Georgia Horticulture Research Farm in 2022 (Table 1). The bottle traps were utilized in this experiment. The treatments were bottle traps with 1) no ethanol lure or repellents, 2) UHR ethanol lure, 3), UHR ethanol lure + a verbenone pouch (Fig. 2A), 4) UHR ethanol lure + a methyl salicylate pouch, and 5) UHR lure + a verbenone pouch + a methyl salicylate pouch. These treatments were replicated four times in an RCBD along the wood line of the farm. Each shepherd hook with a treatment hanging from it was placed 10m apart. This experiment was conducted when there was ambrosia beetle activity at the study site over 28 days.

Evaluation. All of the traps for both experiments were assessed every 7 days. The soap water in the bottle traps was drained, the collection tubes were replaced, the full collection tubes were taken to the UGA Peach Entomology Lab, and the bottle traps were refilled with soap water. The tree bolts were assessed for attack holes and new attacks were circled with a sharpie. The tree bolts were dissected at the end of the trap-type experiment to try to recover beetles inside the bolts. Ambrosia beetles caught in all traps were identified to genus or species level depending on the specimen primarily using an ambrosia beetle guide from UF IFAS Extension (Bateman and Hulcr 2017).

Data analysis. The statistical analysis of data from both experiments was conducted using the statistical software JMP Pro 17. For the Trap/lure-type experiment the number of beetles collected in the bottle traps both overall and at each sample date were subjected to generalized linear mixed model analysis (GLMM) with a Poisson distribution and a least squares mean (LSmeans) Tukey's honestly significant difference post hoc test (Tukey's HSD) (α = 0.05). The lure type on the bottle traps acted as the treatment, and each of the bottle traps was the replication. The number of new attack holes on the bolt traps throughout this experiment were subjected to generalized linear mixed model analysis (GLMM) with a Poisson distribution and a least squares mean (LSmeans) Tukey's honestly significant difference post hoc test (Tukey's HSD) (α = 0.05). The lure type of the bolt acted as the treatment, and each of the bolts was the replication. The count data was square-transformed due to low count data collected during the trial period. Date and rep were considered random variables.

For the repellent type experiment, the number of beetles collected in the bottle traps both overall and at each sample date were subjected to generalized linear mixed model analysis (GLMM) with a Poisson distribution and a least squares mean (LSmeans) Tukey's honestly significant difference post hoc test (Tukey's HSD) (α = 0.05). The repellent type acted as the treatment, and each of the bottle traps was a replication. Date and rep were considered random variables.

Results

A total of 405 ambrosia beetles were collected from bottle traps in 2022. The most abundant beetle species collected from the bottle traps at the UGA Horticulture Research Farm in 2022 were *Hypothenemus* spp. accounting for 50% of the overall captures. The second and third most abundant species found were *X. crassiusculus* and *Xyleborinus Saxesenii* at 21% and 14%, respectively. The fourth most abundant species found was *Cnestus mutilatus* at 8%. The remaining 7% consisted of *X. compactus*, *Xyleborinus ferrugineus*, and other miscellaneous species (Fig. 3).

The total number of beetles collected in the bottle traps showed that the type of lure significantly impacted the number of beetles that were captured (F = 38.42; df = 2, 69; *P* < 0.0001). The ultra-high-release lures caught significantly more beetles than the low-release lures, and both of these lures caught significantly more beetles than the non-baited bottle trap (Fig. 4A). It was also seen that the date of sample collection had a significant impact on the number of beetles captured (F = 49.54; df = 5, 66; *P* < 0.0001) (Fig. 4B). There were significant differences in the species of beetle captured in the bottle traps with distinct lure types. The number of *Hypothenemus* spp. caught in the bottle traps containing high-release ethanol lures caught significantly more beetles than the low-release and non-baited treatments (F=23.83; df = 2, 69; *P* < 0.0001) (Fig. 5A). *Xylosandrus crassiusculus* showed a significantly higher number of beetles caught for the bottle traps baited with low-release ethanol (F = 15.25; df = 2, 69; *P* < 0.0001) (Fig. 5B). *Xyleborinus saxesenii* showed significantly more beetles in the high-release ethanol traps when compared to the low-release ethanol traps and non-baited traps (F = 8.41; df = 2, 69; *P* = 0.0005) (Fig. 5C).

There was a significant difference between the number of attack holes observed on bolt traps with different lure treatments (F = 7.05; df = 4, 115; *P* < 0.0001). The bolt filled with 10% ethanol was not significantly different from the non-baited bolt or the bolt soaked in 10% ethanol. The bolt soaked in 10% was not significantly different from the bolt filled with 90% or the bolt soaked with 90% as well. The bolt soaked in 90% and the bolt filled with 90% showed significantly more attacks than the blank bolt and the bolt filled with 10% ethanol (Fig. 6).

Repellent type experiment. In 2022, the type of repellent had a significant effect on the number of beetles captured at the UGA Horticulture Research Farm (F = 15.83; df = 4, 75; $P < 0.0001$). The combination of verbenone and methyl salicylate had significantly fewer beetles captured than the bottle with no repellent and the bottle with methyl salicylate; the combination was not significantly different from the number of beetles captured in the blank bottle trap with no ethanol or repellents. The verbenone alone did not have any significant differences in the number of beetles captured from the combination of verbenone and methyl salicylate (Fig. 7A). The sample date also showed a significant difference in the number of beetles captured with the collection for the 4th week on 1 June 2022 showing significantly more beetle captures than the preceding three weeks (F = 9.62; df = 3, 76; $P < 0.0001$) (Fig. 7B).

Discussion

The results show that the genus *Hypothenemus* accounted for the majority of the ambrosia beetles collected in bottle traps during the time of sampling. It is possible that these dates for the experiment were well after the peak flight of *X. crassiusculus* because this species and other members of *Xylosandrus* were shown to be the most abundant types of ambrosia beetles captured in Georgia (Monterrosa et al. 2022). The ultra-high-release lure (ChemTica) was shown to be the most effective at luring ambrosia beetles to bottle traps when compared to the low-release ethanol lure. Interestingly, *X. crassiusculus* showed a preference for the low-release ethanol lures; however, we believe that this could be partly due to the depth of the wood line and where the low-release treated bottle trap was located. Bottle traps baited with ultra-high-release ethanol are an effective method of monitoring ambrosia beetle flight activity; however, the labor involved in creating a bottle trap could lead to growers preferring to use baited tree bolts as their monitoring technique.

Although not significantly different from the bolt soaked in 10% ethanol, the bolt soaked in 90% ethanol and the bolt filled with 90% ethanol were determined to be the most effective luring techniques when using bolts. This determination was made due to the fact that the bolt soaked in 10% ethanol showed significantly similar attacks to the bolt filled with 10% ethanol, and this bolt filled with 10% ethanol showed significantly fewer attacks than the bolts that were filled and soaked with 90% ethanol. Using a bolt soaked in 90% ethanol for 24 hours could potentially alter the efficacy of a spray compared to sprays made to trees under normal field conditions, so any spray trials should employ the cored and ethanol-filled bolts instead of the bolts soaked in ethanol for 24 hours. Either bolts soaked in 90% ethanol or filled with 90% should be an effective tool for growers to monitor the attacking activity of ambrosia beetles in their area. Growers can make these soaked bolts to monitor ambrosia beetle attack activity by cutting ~20 cm long pieces of wood with 3.81-5.0 cm in diameter and soaking these bolts in readily available 90% ethanol. Growers can also opt for the cored bolts by getting bolts of the same size as the soaked bolts, drilling a ~15 cm hole through the top of the bolts, filling the hole with 90% ethanol, and sealing the hole with a cork.

The repellent-type experiment showed a general trend of the combination of verbenone and methyl salicylate being the most effective combination of repellents out of all the ones tested because it was the only repellent treatment that did not significantly differ from the blank bolt with no ethanol lure or repellents in terms of ambrosia beetle captures. Growers who are interested in the use of repellents as an additional method of ambrosia beetle control should consider the use of verbenone in tandem with methyl salicylate as the most effective repellent of this study.

Date of sample collection exhibited a statistically significant effect on the number of ambrosia beetles captured, and these findings highlight the variability of ambrosia beetle flight prevalence that can be due to a variety of environmental factors influencing the activity of these beetles. It is imperative that growers who wish to control as much of the damage caused by ambrosia beetles as possible closely monitor the activity of these beetles in order to deploy management tactics such as spray, repellents, etc. in a manner timely enough to have the most protection for when ambrosia beetles are the most prevalent during the season.

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Table 1. Study site location, experiment type, and sampling periods for 2022.

Cropping system	**Location**	**Experiment type**	**GPS coordinates**	**Sampling period** 2022
Various	Watkinsville, GA	Lures	33.887977, -83.416765	13 April – 1 June
Various	Watkinsville, GA	Repellents	33.885308, -83.416502	4 May – 1 June

Fig. 1. A) Bolt trap soaked in ethanol for 24 hours and B) drilled bolt trap filled with ethanol and capped with a cork.


A
B

Fig. 2. Bottle trap with A) ultra-high-release ethanol lure (orange arrow) and verbenone repellent pouch (red arrow) and B) low-release ethanol lure (blue arrow).

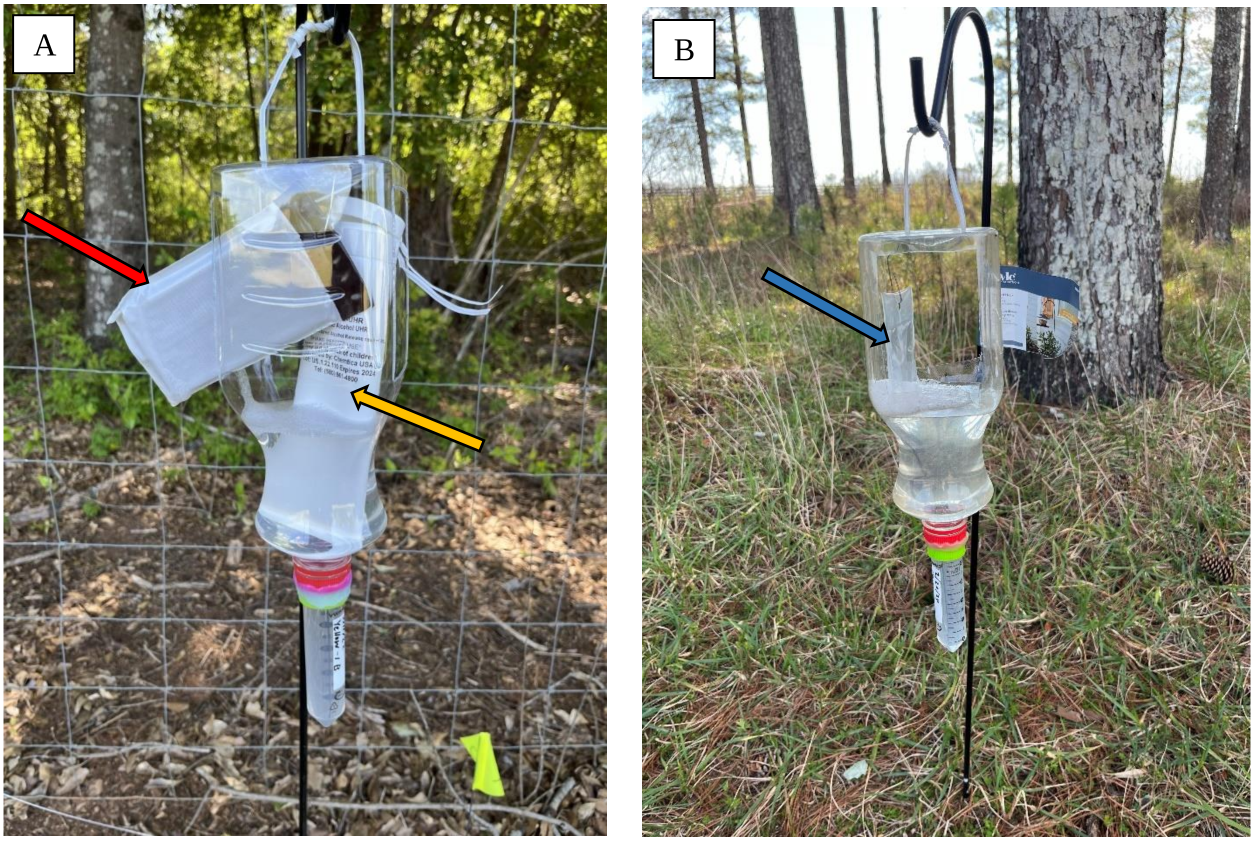
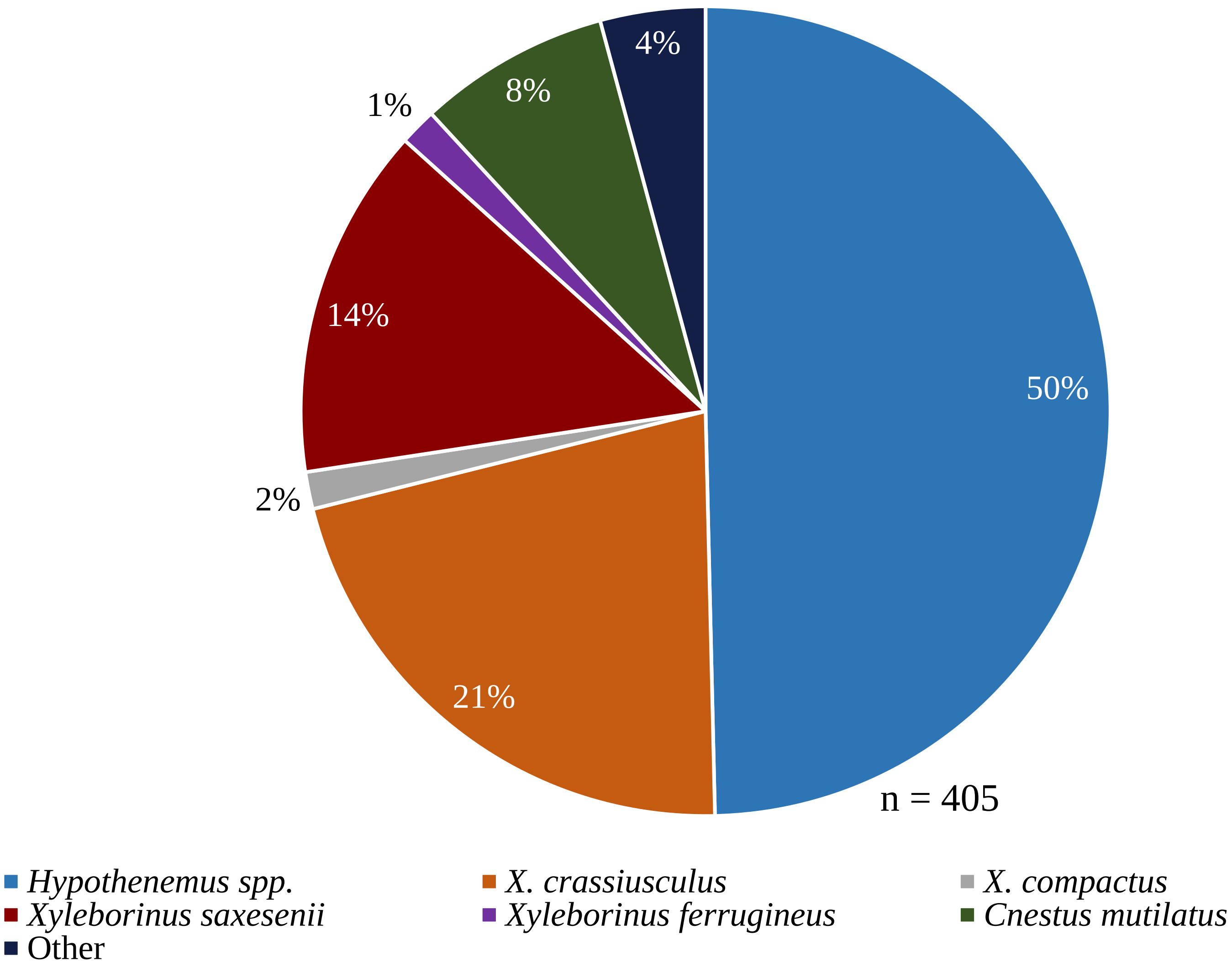

A
B

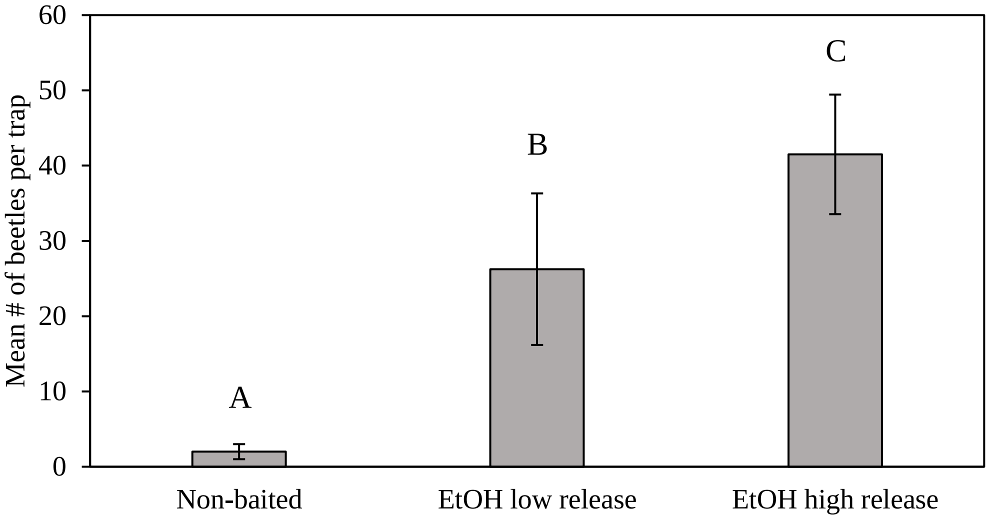
Fig 3. Percentage of genera and species captured in all bottle traps in 2022 at the UGA Horticulture Research Farm in Watkinsville, GA.



50%
21%
2%
14%
1%
8%
4%
n = 405
Hypothenemus spp.
X. crassiusculus
X. compactus
Xyleborinus saxesenii
Xyleborinus ferrugineus
Cnestus mutilatus
Other

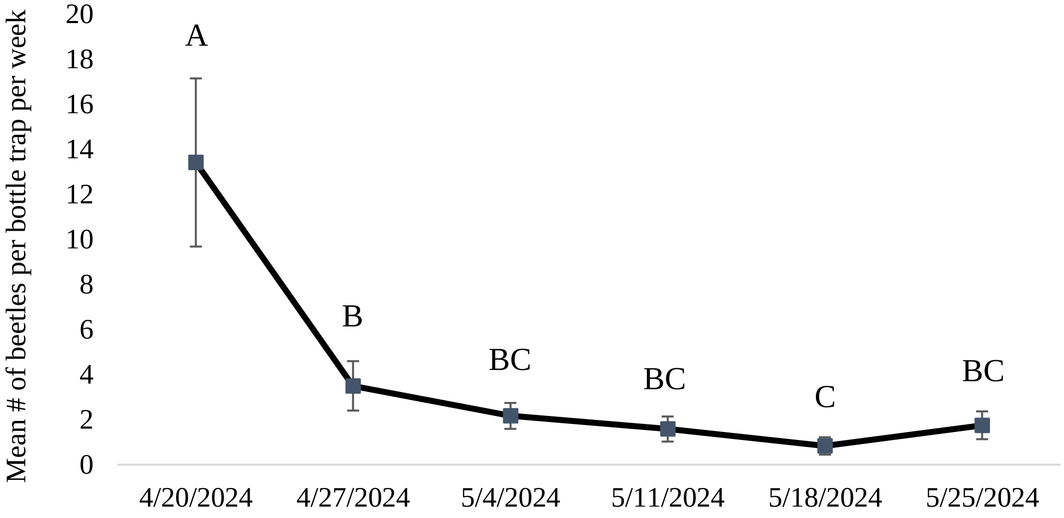
Fig. 4. Mean ± SEM of ambrosia beetles collected per bottle trap for A) the type of lure in the bottle trap and B) the sample date. Letters not connected by the same letter are significantly different (Tukey's HSD, α = 0.05).

A



Mean # of beetles per trap
0
10
20
30
40
50
60
A
B
C
Non-baited
EtOH low release
EtOH high release

B



Mean # of beetles per bottle trap per week
0
2
4
6
8
10
12
14
16
18
20
A
B
BC
BC
C
BC
4/20/2024
4/27/2024
5/4/2024
5/11/2024
5/18/2024
5/25/2024

Fig. 5. Mean ± SEM of ambrosia beetles collected per bottle trap for the type of lure in the bottle trap for A) *Hypothenemus* spp., B) *X. crassiusculus*, and C) *Xyleborinus saxesenii*. Letters not connected by the same letter are significantly different (Tukey's HSD, α = 0.05).

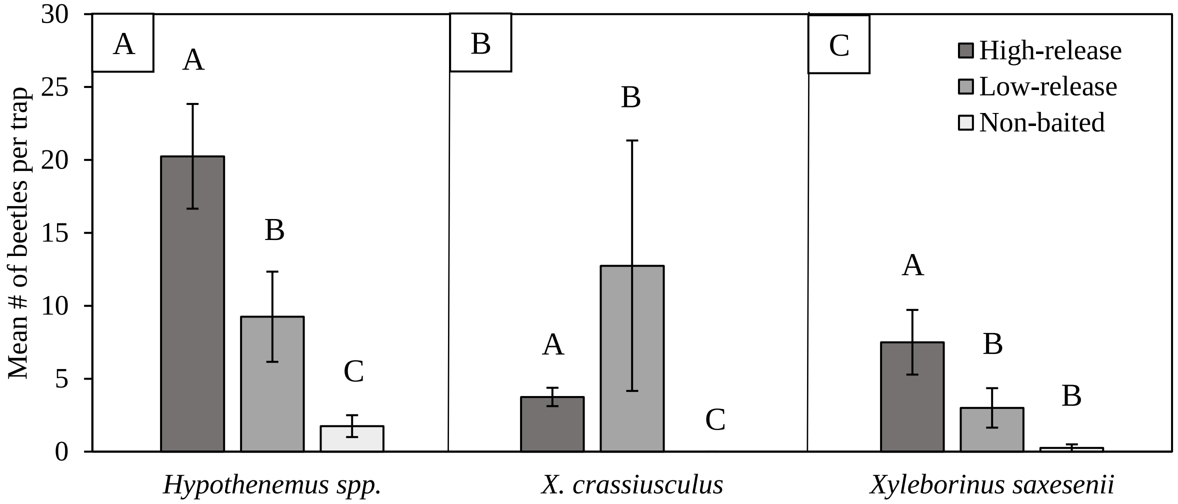
Mean # of beetles per trap
30
25
20
15
10
5
0
A
B
C
High-release
Low-release
Non-baited
A
B
C
A
B
C
A
B
B
Hypothenemus spp.
X. crassiusculus
Xyleborinus saxesenii

Fig. 6. Mean ± SEM of ambrosia beetle attacks per bolt trap for the type of luring method applied to the bolt. Letters not connected by the same letter are significantly different (Tukey's HSD, $\alpha = 0.05$).

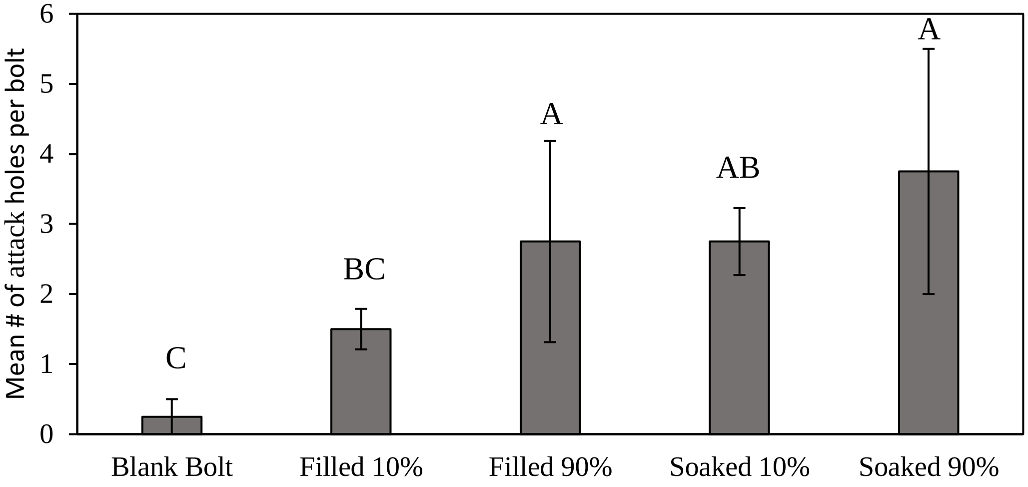
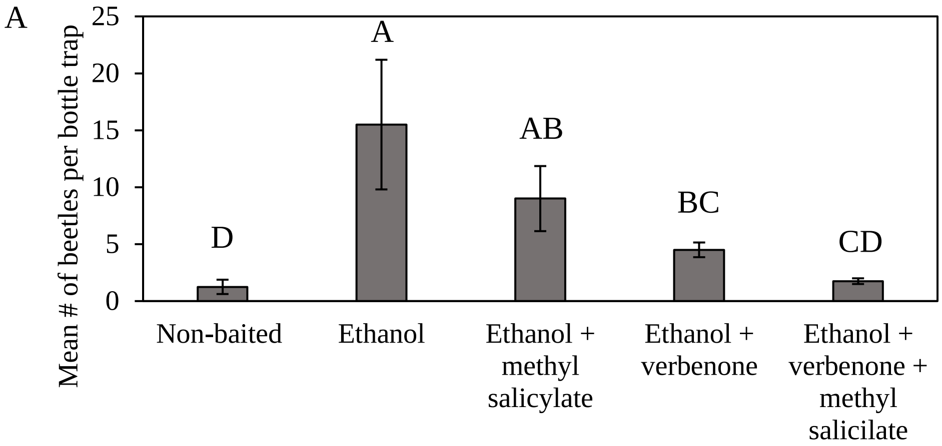
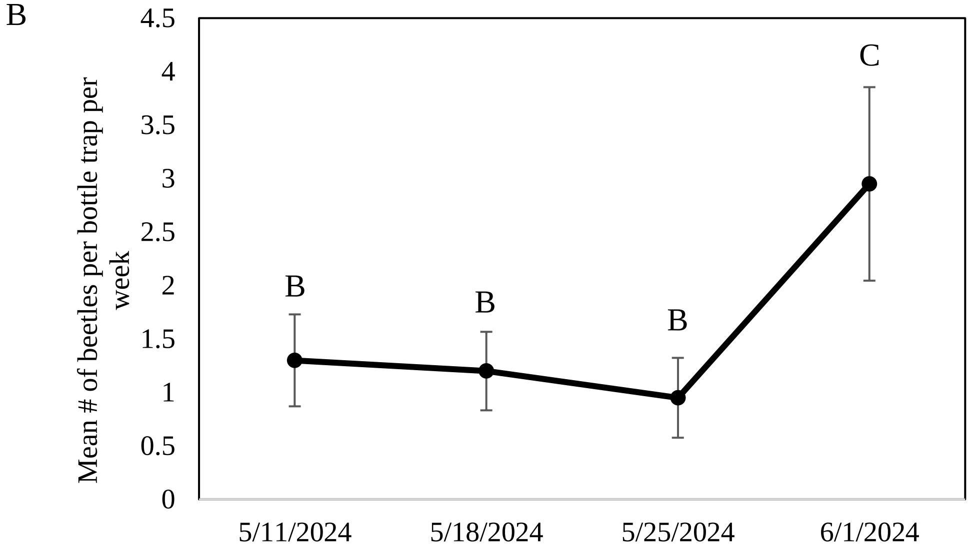

6
5
4
3
2
1
0
Mean # of attack holes per bolt
C
BC
A
AB
A
Blank Bolt
Filled 10%
Filled 90%
Soaked 10%
Soaked 90%

Fig. 7. Mean ± SEM of ambrosia beetles captured in bottle traps for A) the type of repellents applied to the bottle traps and B) the dates of collection. Letters not connected by the same letter are significantly different (Tukey's HSD, $\alpha = 0.05$).


A
Mean # of beetles per bottle trap
25
20
15
10
5
0
D
A
AB
BC
CD
Non-baited
Ethanol
Ethanol + methyl salicylate
Ethanol + verbenone
Ethanol + verbenone + methyl salicilate


B
Mean # of beetles per bottle trap per week
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
B
B
B
C
5/11/2024
5/18/2024
5/25/2024
6/1/2024

CHAPTER 3

DOES THE USE OF ATTRACTANTS AND REPELLENTS IN A PUSH-PULL MANAGEMENT STRATEGY INFLUENCE AMBROSIA BEETLE (*SCOLYTINAE*) ATTACKS ON APPLE TREES?

Introduction

Wood-boring ambrosia beetles (subfamily *Scolytinae*) are bark beetles that pose a considerable threat to the health of trees in an orchard and the economic status of the cropping system (Ree & Knutson 1997, Ranger et al. 2016, Gugliuzzo et al. 2021). Apple orchards in the northeastern United States such as New York and North Carolina have recently seen the emergence of ambrosia beetles as a major pest species (Agnello et al. 2015, Villani & Walgenbach 2017). The 2020 Farm Gate Valuation of Georgia apple production was nearly $10 million, so, even though ambrosia beetles are not a major problem for Georgia apple growers at the moment, it is important to preemptively refine management tactics in case these beetles do become a major economic pest in Georgia apple orchards in the future. The USDA National Agricultural Statistics Service valued the apple industry in the United States at over $3 billion in 2022/2023. The apple industry in the United States is a massive market, so it is imperative that management practices for ambrosia beetle control continue to be improved. Many different situations can cause stress to apple trees, but a major factor has been determined to be flood stress, which increases the susceptibility of trees to attack by ambrosia beetles (Ranger et al. 2020). Tree stress leads to the release of ethanol by the trees, attracting the beetles to attack these weakened trees, which can subsequently lead to tree decline (Ranger et al. 2020).

Rapid apple decline or sudden apple decline (RAD or SAD) is a mysterious condition in apple trees that presents the symptoms of lesions that turn necrotic, yellowing or reddening of leaves, and dying tissue at the grafting connection point of rootstock and trunk; tree collapse/death can happen within weeks of these symptoms first becoming noticeable (Singh et al. 2019, Stokstad 2019). It was hypothesized by researchers that a wide variety of factors such as ambrosia beetles and their fungal associations, cold damage, fungal pathogens, viruses, weather, microbial communities, etc. all contribute to causing RAD/SAD (Agnello et al. 2017, Peter 2018, Singh et al 2019, Wright et al. 2020). As of now, there seems to be no definitive answer on which factors cause RAD, and the magnitude of the effect that any one specific factor has is not known. However, it has been seen that in many apple orchards across North Carolina, nearly every tree that has symptoms of RAD has had some level of ambrosia beetle infestation (Villani & Walgenbach 2017).

Apple trees in much of the northeast are generally planted very close together (as close as 1.2 m apart for dwarf apple trees), staked, and trellised, but the majority of dwarf apple trees in Georgia are usually spaced at least 2.1 m apart. With these trees in the Northeast generally much closer together than in Georgia, the Northeastern apple trees could be facing stress through competition with the other trees, flooding of orchards, freezing, and diseases spreading more rapidly. Microbial degradation and anaerobic respiration occur in stressed or dying trees, and ethanol is a by-product of these processes and is released in large quantities (Kimmerer and Kozlowski 1982, Kimmerer and MacDonald 1987). Many abiotic and biotic factors can elicit the release of ethanol from trees such as anoxic conditions, freezing, and disease that is more readily spread between trees that are closer together (Ranger et al. 2010, Bui et al. 2019, Wallis et al. 2021). Acetaldehyde production sharply increases in the trees due to these stressors, and this compound is soon transformed into ethanol and transported through the xylem of the tree to accumulate in the tissues of the leaves (Ranger et al. 2010). Once the ethanol arrives to the leaf tissue, a small amount of it is changed via oxidative metabolism processes into acetone and acetaldehyde, but the majority of the compounds in the leaf tissue still consist of volatile ethanol that is released into the air (Cossins 1978, Kreuzwieser et al. 1999). Methanol is a compound that is normally created and released by healthy trees and production is greatly increased in response to any mechanical damage that the trees may endure; however, methanol has been shown to not be a strong attractant for ambrosia beetles (Von Dahl et al. 2006, Ranger et al. 2010).

Currently, the only known effective insecticides are the pyrethroids permethrin and bifenthrin, and these insecticides may actually be more repellent than insecticidal for the ambrosia beetles (Van Der Laan & Ginzel 2013, Ranger et al. 2016). These sprays have been shown to be ineffective on beetles already established inside of the trees because the bark of the tree acts as a protective barrier from the insecticides (Reding et al. 2010, Van Der Laan & Ginzel 2013). Systemic insecticides cannot be utilized with much efficacy because ambrosia beetles do not eat the actual wood of the tree; the females introduce a mycangial fungus inside the tree to grow and feed on (Batra 1983, Addesso et al. 2019). Because there are no viable pesticides available to control ambrosia beetles once established inside a tree, it is imperative that management tactics that lower the number of initial attacks be developed to reduce the number of beetles entering those trees. The major objective of this study was to determine the efficacy of the repellents verbenone and MeSA in tandem with UHR bottle traps as a push-pull management tactic on the number of ambrosia beetle attacks on water-stressed apple trees.

Materials and Methods

Study site. In 2023 and 2024, the University of Georgia Horticulture Research Farm in Watkinsville, GA was selected for this study. There was a large variety of trees and other crops being grown on this site for various research projects that are not managed with the commercial standard insecticide management guideline (Table 2). The crops nearest to where the experiments took place were pecan trees, peach trees, blueberries, and grapevines.

Traps and lures. Baited bottle traps were utilized in the experiment. The bottle traps were made from 2-liter clear juice bottles with two windows cut out across from each other both with widths of 5 cm and lengths of 9 cm. Two holes were spaced out on either side of the bottom of the bottle to feed a zip tie through to hang the bottle trap upside down on a metal shepherd's hook. The cap of the juice bottle was affixed to a cap of a 50 ml centrifuge tube, so the captured beetles were collected into the tube (Fig. 8C). All the cutting and affixing of the caps was performed with a wood-burning wand and hot glue. Soap water was made using Dawn dish soap (Procter and Gamble, Cincinnati, OH) and added to the bottle traps to break the surface tension of the water to drown visiting beetles. All bottles had an ultra-high release (UHR) lure (ChemTica USA LLC, Durant, OK) to attract beetles, and tubes were collected and replaced every 7 days.

Repellents and trees. The repellents utilized were a MeSA pouch emitter and a verbenone pouch emitter used in tandem with each other. A zip tie was run through the top of both repellent pouches, and they were hung approximately halfway up the trees (Fig. 8A).

Water-stressed golden delicious apple trees (*Malus domestica*) that were 1.22-1.52 m in height were utilized in this experiment. The trees were water-stressed by placing the pots of the trees into a garbage bag, placing that into a second larger empty pot, completely saturating the soil with water, and then tying the garbage bag around the bottom of the tree to stop any water escape through evaporation (Fig. 8B). Saturation of the soil was checked periodically, and more water was added to the pots if needed. All trees were assessed for ambrosia beetle attacks approximately every 2-3 days.

Push-Pull experiment. There were three variations of this experiment to determine the efficacy of a novel ambrosia beetle strategy using attractants and repellents: the first was conducted in May 2023, the second in July 2023, and the third in March 2024 (Table 2).

Experiment 1. Baited bottle traps and repellents were utilized in tandem in this experiment. The treatments were 1) a water-stressed apple tree as the control tree (Fig. 8B) and 2) a water-stressed apple tree with verbenone + MeSA emitter pouches and a perimeter of 8 evenly spaced UHR ethanol-baited bottle traps ~6 meters away from the water-stressed tree (Fig. 8C). These treatments were replicated five times. The control tree was placed 12.2 meters away from the treatment tree (~6 meters away from the nearest baited bottle trap). Control trees were placed on the wood line of the farm, and the treatment grids were placed as close to the wood line as space allowed on the farm. This experiment was conducted when there was ambrosia beetle activity at the study site over 14 days.

Experiment 2. Baited bottle traps and repellents were utilized in tandem in this experiment. The treatments were 1) a water-stressed apple tree as the control tree and 2) a water-stressed apple tree with verbenone + MeSA emitter pouches and a perimeter of 8 evenly spaced UHR ethanol-baited bottle traps ~6 meters away from the water-stressed tree. These treatments were replicated five times. The control tree was now placed 36.5 instead of 12.2 meters away from the treatment tree (30.5 instead of 6 meters away from the nearest baited bottle trap). Control trees were placed on the wood line of the farm, and the treatment grids were placed as close to the wood line as space allowed on the farm. This experiment was conducted when there was ambrosia beetle activity at the study site over 14 days.

Experiment 3. Baited bottle traps and repellents were utilized in tandem in this experiment. The treatments were 1) a water-stressed apple tree as the control tree and 2) a water-stressed apple tree with verbenone + MeSA emitter pouches and a perimeter of 8 evenly spaced UHR ethanol-baited bottle traps ~6 meters away from the water-stressed tree. These treatments were now replicated seven times instead of five times. The control tree was placed 36.5 meters away from the treatment tree (30.5 meters away from the nearest baited-bottle trap). Control trees were placed on the wood line of the farm, and the treatment grids were placed as close to the wood line as space allowed on the farm. This experiment was conducted when there was ambrosia beetle activity at the study site over 14 days.

Evaluation. All of the bottle traps during all three experiments were collected every 7 days. The soap water in the bottle traps was drained, the collection tubes were replaced, the full collection tubes were taken to the UGA Peach Entomology Lab, and the bottle traps were refilled with soap water. All apple trees were assessed for attack holes approximately every 2-3 days, and new attacks were circled with a sharpie. The number of ambrosia beetles caught in the bottle traps for each replication was recorded, and beetles were stored in 70% ethanol.

Data analysis. The statistical analysis of data for all three experiments was conducted using the statistical software JMP Pro 17. Only the tree attack data from experiment 3 was analyzed because experiments 1 and 2 had nearly no attacks observed. For experiment 3 the number of attack holes observed both overall and at each sample date were subjected to generalized linear mixed model analysis (GLMM) with a Poisson distribution and a least squares mean (LSmeans) Tukey's honestly significant difference post hoc test (Tukey's HSD) ($\alpha = 0.05$). The presence or absence of the push-pull tactic and sampling date acted as the treatments, and each of the trees was a replication. The number of beetles captured in the bottle traps will compared across all three experiment dates for the first week, second week, and combined two weeks of beetle collection. For experiments 1, 2, and 3 the number of beetles collected in the bottle traps both overall and at each sample date were subjected to generalized linear mixed model analysis (GLMM) with a Poisson distribution and a least squares mean (LSmeans) Tukey's honestly significant difference post hoc test (Tukey's HSD) ($\alpha = 0.05$). Date and rep were considered random variables.

Results

Experiments 1 and 2. In 2023 the combined total of ambrosia beetle attacks for experiments 1 and 2 (totaling 28 days of assessment) was 5. There were 2 attack holes in the control trees and 1 attack hole in the treatment trees for experiment 1. There was 1 attack hole in both the control and treatment trees for experiment 2. Not enough attack data was collected from these two experiments to run any meaningful statistical analysis.

Experiment 3. In 2024 the number of ambrosia beetle attacks was significantly lower in the water-stressed trees treated with the push-pull tactic than in the water-stressed control trees after 14 days (F = 27.16; df = 1, 82; $P < 0.0001$) (Fig. 9A). The average number of attacks for each sample date was not significant until ~10 days after deployment (F = 115.19; df = 5, 78; $P < 0.0001$) and this 3/16/2024 sample date had significantly more new attacks than any other sample date (Fig. 9B). The last sample date on 20 March 2024 had significantly more attacks than the sample dates before 16 March 2024, but had significantly fewer attacks than that penultimate sample collection date (Fig. 9B).

For the first collection week of experiments 1, 2, and 3 there were significantly more beetles captured in the May bottle traps in 2023 compared to the March 2024 bottle traps, and March 2024 bottle traps caught significantly more beetles than July 2023 (F = 56.60; df = 2, 14; *P* < 0.0001) (Fig. 10A). For the second collection week, March 2024 bottle traps had significantly more attacks than the May and July 2023 bottle traps (F= 522.64; df = 2, 14; *P* < 0.0001) (Fig. 10B). Overall, the March 2024 bottle traps caught significantly more beetles than May and July of 2022 bottle traps, and May 2023 bottle traps caught more beetles than July 2023 (F = 524.79; df = 2, 14; *P* < 0.0001) (Fig. 10C) (Table 2).

Discussion

From the ambrosia beetle attack data collected from experiment 3 in 2024 that showed significant differences between treatments, the push-pull tactic appears to be a viable method of ambrosia beetle management. Although the management tactic significantly lowered the amount of ambrosia beetle attacks in water-stressed apple trees, the economic significance of this finding has yet to be determined. The economic threshold level of ambrosia beetles in apple trees is not currently known, so more research would have to be conducted to determine the economic threshold in order to determine whether or not this management tactic would be economically advantageous to apple growers. The degree to which ambrosia beetles contribute to rapid apple death (RAD) is also currently unknown, so more research needs to be conducted in that area to prove whether or not this management tactic can lessen RAD in apple orchards. Location appears to be a major factor in the severity of ambrosia beetle attacks, but the proximity to different crops, degree of shading the trees are in, etc. could all also play a role in ambrosia beetle attack severity.

Although there were 352 beetles caught in bottle traps in May and July, only 5 attack holes were observed throughout 28 days of sampling. Even though there is ambrosia beetle flight activity, that does not always mean that there is beetle attack activity. The bottle traps deployed in March 2024 caught significantly more beetles in 14 days compared to the first two experiments combined, with 2029 beetles in 14 days vs. 352 in 28 days (Fig.10C). It is safe to assume from the number of beetles captured and attack holes assessed in March 2024, that this timeframe was the beginning of peak flight and attack activity of ambrosia beetles in this area. May and July of 2023 were most likely substantially past the timeframe of peak flight and attack activity. Peak flight and attack activity of ambrosia beetles appears to be somewhat of a short window of time.

Research conducted in Mississippi, Virginia, and Ohio in 2016 and 2017 found that a push-pull strategy using ethanol-baited bottle traps and verbenone as the repellent did not lower the number of ambrosia beetle attacks on water-stressed trees satisfactorily; however, the data collected from experiment 3 in this study shows promising results of this strategy with ultra-high-release ethanol baited bottle traps and the combination of verbenone and methyl salicylate as the repellent (Werle et al. 2018). Verbenone as the push component with ethanol-baited bottle traps significantly reduced the number of ambrosia beetle attacks in a 2020 study on avocado trees, but the effective radius of the repellent was found to be less than 1 m (Rivera et al. 2020). This small effective radius of the repellents could cause the labor involved with setting up this management tactic on a large orchard scale to be much higher than what was originally thought. This increase in labor due to the possible need to add more repellents closer together could lower the likelihood of growers electing to use this management tactic.

In summary, our results show that the push-pull tactic of ultra-high-release bottle traps and the combination of verbenone and methyl salicylate is effective at lowering the amount of ambrosia beetle attacks on water-stressed apple trees. The efficacy of this management tactic in an economic sense when materials and labor costs involved are taken into account is not yet known. At this current time, this management strategy should not be recommended to large-scale growers until the economic threshold of these beetles is determined and a proper cost-benefit analysis of this tactic can be performed.

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Table 2. Study site location, experiment type, and sampling periods for 2023 and 2024.

Cropping system	**Location**	**Experiment**	**GPS coordinates**	**Sampling period**	
				2023	2024
Various	Watkinsville, GA	Push-Pull 1	33.887204, -83.416731	4 May – 18 May	
Various	Watkinsville, GA	Push-Pull 2	33.887204, -83.416731	3 July – 17 July	
Various	Watkinsville, GA	Push-Pull 3	33.887204, -83.416731		6 Mar – 20 Mar

Fig. 8. Water-stressed A) treatment tree with verbenone emitter pouch (yellow arrow) and methyl salicylate emitter pouch (red arrow) and B) control tree with no repellent pouches. C) Water-stressed treatment tree (green arrow) with a perimeter of 8 evenly spaced UHR bottle traps (blue arrow) ~6 m away from the treatment tree.

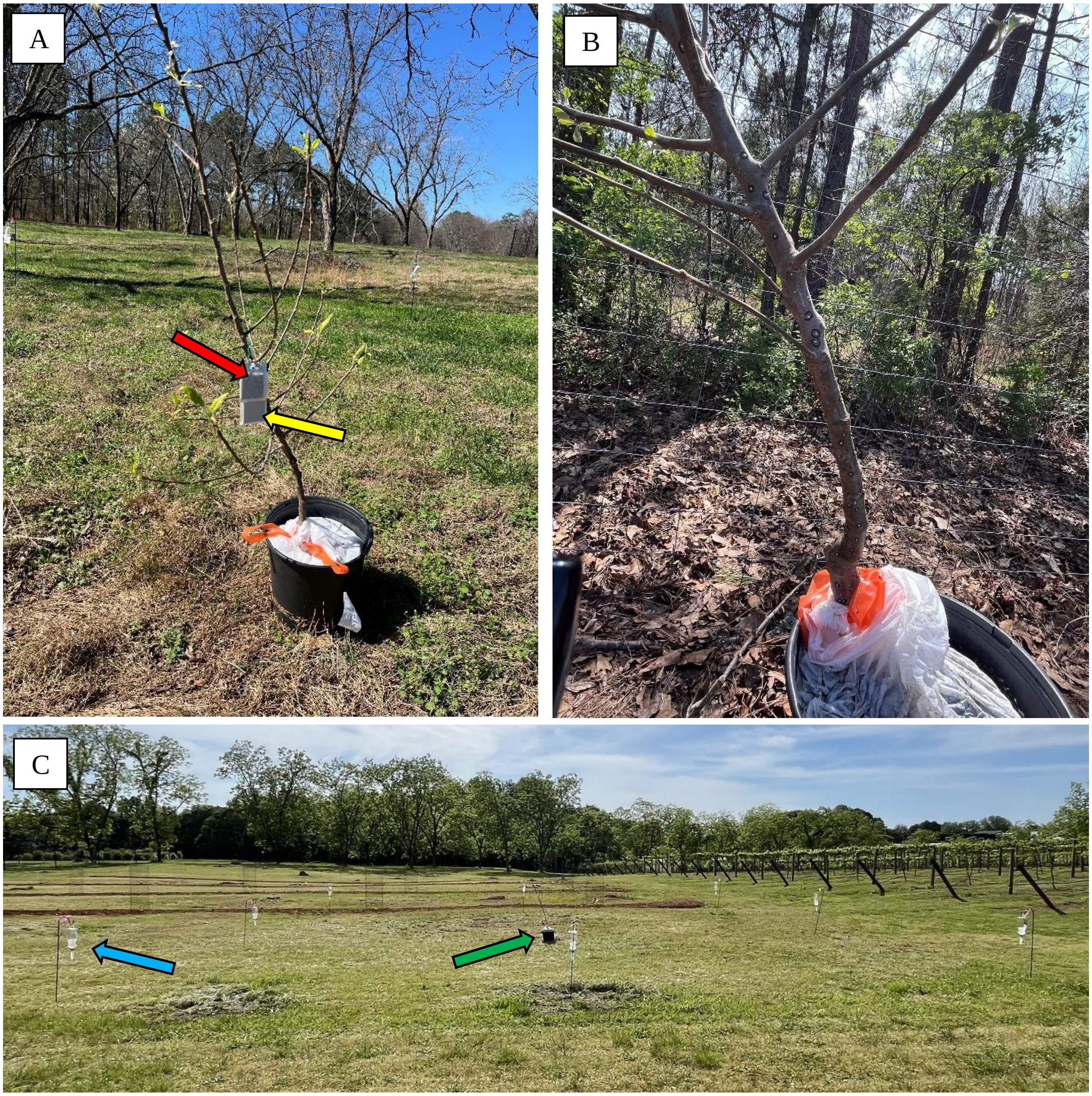
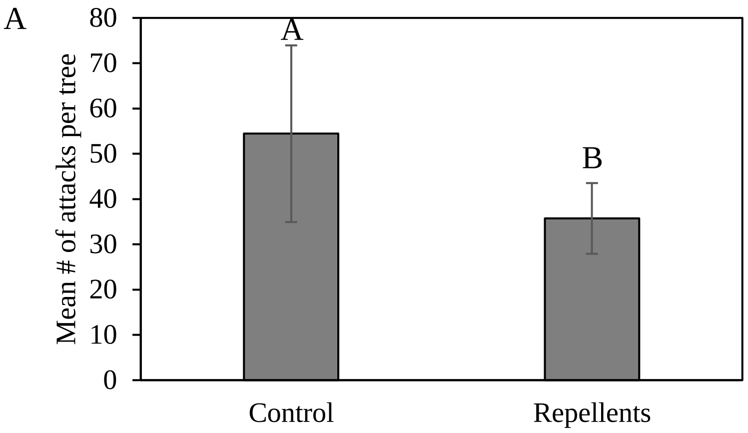

A
B
C

Fig. 9. Mean ± SEM of ambrosia beetle attacks per tree during experiment 3 for A) the treatment of the water-stressed tree and B) the dates of tree attack assessment. Letters not connected by the same letter are significantly different (Tukey's HSD, $\alpha = 0.05$).


A
80
70
60
50
40
30
20
10
0
Mean # of attacks per tree
A
B
Control
Repellents

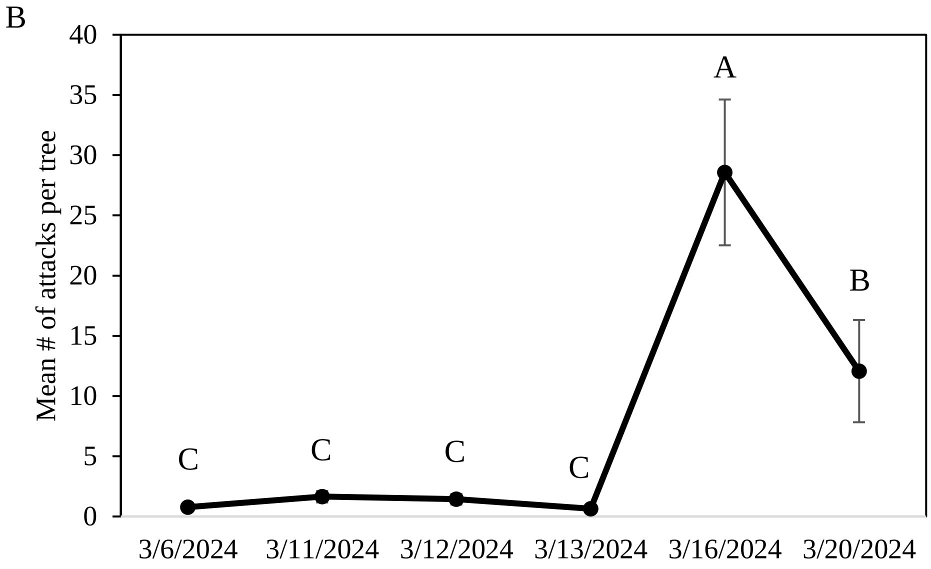
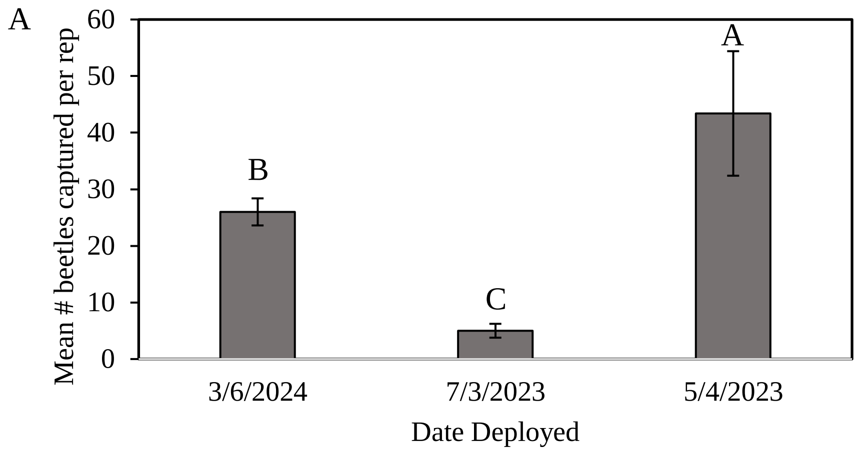
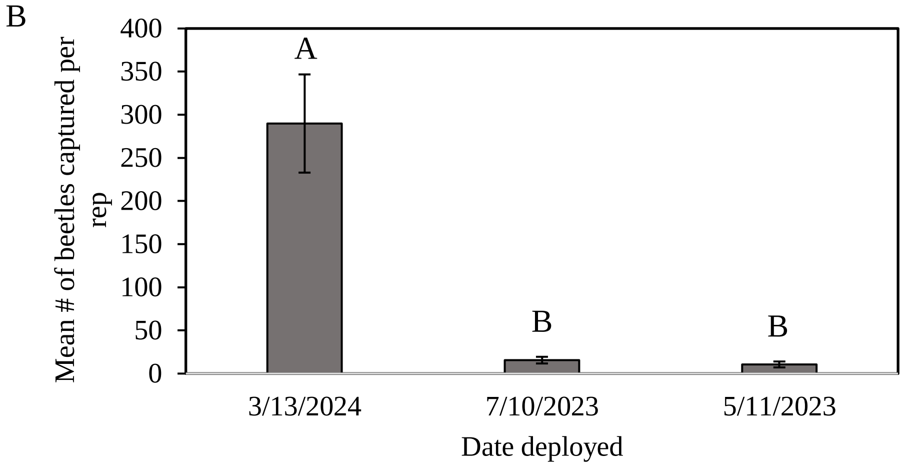
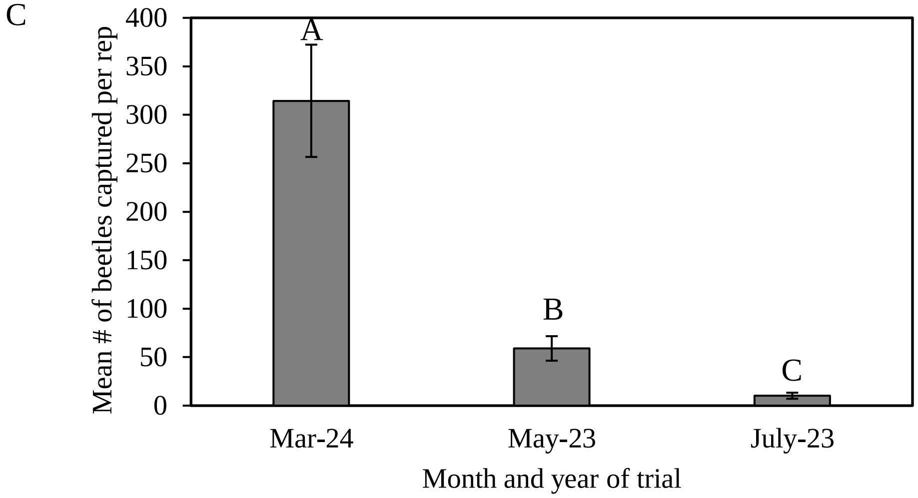

B
40
35
30
25
20
15
10
5
0
Mean # of attacks per tree
A
B
C
C
C
C
3/6/2024
3/11/2024
3/12/2024
3/13/2024
3/16/2024
3/20/2024

Fig. 10. Mean ± SEM of ambrosia beetles captured in experiments 1, 2, and 3 per rep for A) the first week of captures, B) the second week of captures, and C) the overall captures. Letters not connected by the same letter are significantly different (Tukey's HSD, $\alpha = 0.05$).


A
Mean # beetles captured per rep
60
50
40
30
20
10
0
B
C
A
3/6/2024
7/3/2023
5/4/2023
Date Deployed


B
Mean # of beetles captured per rep
400
350
300
250
200
150
100
50
0
A
B
B
3/13/2024
7/10/2023
5/11/2023
Date deployed


C
Mean # of beetles captured per rep
400
350
300
250
200
150
100
50
0
A
B
C
Mar-24
May-23
July-23
Month and year of trial

CHAPTER 4

COMPARISON OF DIFFERENT INSECTICIDES AND INFLUENCE OF NANOCELLULOSE GEL ON AMBROSIA BEETLE (*SCOLYTINAE*) ATTACKS

Introduction

Ambrosia beetles are a major economic pest of tree fruit, tree nut, and woody ornamental nurseries (Reding et al. 2010, Ranger et al. 2016, Gugliuzzo et al. 2021). The granulate ambrosia beetle, *Xylosandrus crassiusculus*, and black stem borer, *Xylosandrus germanus*, have been found in 32 and 28 states, respectively, and these are the two major exotic species that are the most problematic and established in the eastern United States (Schedl 1963, Weber & McPherson 1983, Gomez et al. 2018). These beetles are more likely to attack young, stressed trees, and these trees are located by the beetles from the release of ethanol from the trees (Ranger et al. 2010). Trees stressed by conditions such as anoxic conditions, freezing, and disease will have their production and release of ethanol greatly increased (Ranger et al. 2010, Bui et al. 2019, Wallis et al. 2021). Acetaldehyde production sharply increases in trees when they are enduring stressful conditions, this compound is then converted into ethanol, and this ethanol is transported through the xylem of the tree to leaf tissue where it is released as a volatile compound (Ranger et al. 2010). Before the ethanol is released from the tree, oxidative metabolism processes convert a small amount of it into the compounds acetone and acetaldehyde; however, the large majority of these compounds accumulated in the leaf tissue consists of the volatile ethanol that the trees release (Cossins 1978, Kreuzwieser et al. 1999).

Essentially the only current effective management strategy for ambrosia beetles is the use of pyrethroids (such as permethrin and bifenthrin) in a preventative manner (Reding et al. 2010, Atkinson et al. 2011, Williamson et al. 2023). The beetles bore into trees without ingesting any of the tree's wood, so systemic insecticides have proven to be ineffective at lowering the number of beetle attacks thus far (Addesso et al. 2019). The females will carry in a mycangial symbiotic fungus to introduce inside of the tree to grow as the food source for all life stages of these beetles (Batra 1963, Addesso et al. 2019). The preventative use of the repellent insecticides bifenthrin and permethrin involves spraying the trees with these insecticides every 10-14 days during the spring flight of the beetles (Hudson and Mizell 1999, Ranger et al. 2016).

Pyrethroids influence the nervous system of insects by acting as sodium channel modulators (IRAC 2024). When exposed to this type of insecticide, insects show very irritated/excited behavior, and this could be the repellent aspect of these insecticides by causing ambrosia beetles to become irritated and leave a tree they would otherwise attack (Meyer & Shafer 2006, Yan et al. 2011).

Nanocellulose is a nanomaterial that has a high surface area, strength, and binding activity, it is also non-toxic to animals and the environment (Ilyas et al. 2023). It can be created with a variety of materials such as algae and wood pulp (Sam et al. 2017). This nanocellulose has the potential to act as a physical barrier and/or retain the efficacy of different insecticides and repellents for a longer period of time when mixed together before spraying. The main objective of this study was to evaluate the efficacy of insecticides of differing modes of action, nanocellulose as a physical barrier to attacks, and the combination of nanocellulose with repellents and effective insecticides on lowering the number of ambrosia beetle attacks observed on tree bolt traps baited with 50% ethanol.

Study sites. Two of the three experiments were conducted at the University of Georgia Horticulture Research Farm in Watkinsville, GA in 2023. There was a large variety of trees and other crops being grown on this site for various research projects that are not managed with the commercial standard insecticide management guideline (Table 3). The crops nearest to where the experiments took place were pecan trees, peach trees, blueberries, and grapevines. In 2024, a pecan orchard in Athens, Georgia was selected as the study site for the third experiment (Table 3). This orchard has pecan trees of varying ages as well as a small orchard of peach trees, and the trees in this orchard are maintained under very minimal insecticide spray applications for pest management.

Traps and sprays. Cored and filled tree bolts were utilized in all three experiments. The tree bolts were made from young peach tree wood (*Prunus spp.*) with a width of 3.81-5.0 cm and a length of ~20 cm. A width of at least 3.81 cm was required for proper coring of the bolts (Reding and Ranger 2020). A 15 cm hole was drilled through the center of the bolt, the hole was filled with 50% ethanol, and the top of the hole was closed with a cork. A small eye hook was drilled into the top of the bolt to feed a zip-tie through to hang the bolts from a metal shepherd's hook (Fig. 11A, B, C). Approximately every 2-3 days, all bolts were assessed for attacks, and were refilled with 50% ethanol every week.

The sprays and active ingredients for the insecticide experiment were Brigade 2EC (bifenthrin; FMC Corporation, Philadelphia, PA), Perm-up 3.2EC (permethrin; UPL Limited, Mumbai, India), Exirel (cyantraniliprole; FMC Corporation, Philadelphia, PA), Apta (tolfenpyrad; Nichino America Inc, Wilmington, DE), Carbaryl 4L (carbaryl; Drexel Chemical, Memphis, TN), Altacor (chloratraniliprole; FMC Corporation, Philadelphia, PA), and Celite 610 (diatomaceous earth; Brandt, Inc, Tampa, FL) at their appropriate rates with about 15 ml used for each insecticide mixture per treated bolt. The sprays for the nanocellulose experiment included nanocellulose alone, nanocellulose + bifenthrin, nanocellulose + permethrin, and nanocellulose + verbenone at their appropriate rates with about 15 ml used for each nanocellulose mixture. The sprays for the nanocellulose and insecticide experiment included all of the sprays that were utilized in both previous experiments.

Insecticide experiment. The peach tree bolts were utilized in this experiment on the UGA Horticulture Research Farm in 2023 (Table 3). The treatments were cored peach tree bolts filled with 50% ethanol and sprayed with approximately 15 ml of 1)Brigade 2EC (bifenthrin) at 100 ul per 100 ml, 2) Perm-up 3.2EC (permethrin) at 78 ul per 100 ml, 3) Exirel (cyantraniliprole) at 160 ul per 100 ml, 4) Apta (tolfenpyrad) at 211 ul per 100 ml, 5) Carbaryl 4L (carbaryl) at 0.75 ml per 100 ml, 6) Altacor (chloratraniliprole) at 33.7 mg per 100 ml, 7) Celite 610 (diatomaceous earth) at 2.4 g per 100 ml, 8) a bolt filled with ethanol and no spray, and 9) a blank bolt with no ethanol or spray (Fig. 11A). These treatments were replicated six times in an RCBD along the wood line of the farm. Each shepherd hook with a treatment hanging from it was placed 10 m apart. This experiment was conducted when there was ambrosia beetle activity at the study site over 21 days.

Nanocellulose experiment. The peach tree bolts were utilized in this experiment on the UGA Horticulture Research Farm in 2023 (Table 3). The treatments were cored peach tree bolts filled with 50% ethanol and sprayed with approximately 15 ml of 1) CelluForce NCC (nanocellulose crystals gel; CelluForce, Montreal, Quebec, Canada) at 25 ml per 100ml, 2) nanocellulose gel + bifenthrin at 100 ul per 100 ml, 3) nanocellulose gel + permethrin at 78 ul per 100 ml, 4) nanocellulose gel + verbenone at 6.75 g per 100 ml, and a blank bolt with no ethanol or spray (Fig. 11B). These treatments were replicated five times in an RCBD along the wood line of the farm. Each shepherd hook with a treatment hanging from it was placed 10 m apart. This experiment was conducted when there was ambrosia beetle activity at the study site over 21 days.

Nanocellulose/insecticide experiment. The peach tree bolts were utilized in this experiment on the pecan orchard in Athens, Georgia in 2024 (Table 3). This experiment is essentially a combination of experiments 1 and 2 into one larger experiment. The treatments were cored peach tree bolts filled with 50% ethanol and sprayed with approximately 15 ml of 1)Brigade 2EC (bifenthrin) at 100 ul per 100 ml, 2) Perm-up 3.2EC (permethrin) at 78 ul per 100 ml, 3) Exirel (cyantraniliprole) at 160 ul per 100 ml, 4) Apta (tolfenpyrad) at 211 ul per 100 ml, 5) Carbaryl 4L (carbaryl) at 0.75 ml per 100 ml, 6) Altacor (chloratraniliprole) at 33.7 mg per 100 ml, 7) Celite 610 (diatomaceous earth) at 2.4 g per 100 ml, 8) CelluForce NCC (nanocellulose gel) at 25 ml per 100 ml, 9) nanocellulose gel + bifenthrin at 100 ul per 100 ml, 10) nanocellulose gel + permethrin at 78 ul per 100 ml, and 11) nanocellulose gel + verbenone at 6.75 g per 100 ml, 12) a bolt filled with ethanol and no spray, and 13) a blank bolt with no ethanol or spray (Fig. 11C). These treatments were replicated five times in an RCBD along the wood line of the pecan orchard. Each shepherd hook with a treatment hanging from it was placed 10 m apart. This experiment was conducted when there was ambrosia beetle activity at the study site over 21 days.

Evaluation. The number of attacks observed on the tree bolts across all three experiments was recorded and marked with a Sharpie approximately every 2-3 days, and the bolts were refilled with 50% ethanol every 7 days. The tree bolts were dissected at the end of experiment 1 to try to recover beetles inside the bolts. Ambrosia beetles recovered were identified to genus or species level depending on the specimen primarily using an ambrosia beetle guide from UF IFAS Extension (Bateman and Hulcr 2017).

Data analysis. For the insecticide and nanocellulose experiments, the number of new attack holes observed on the tree bolt traps during the duration of the experiment was subjected to GLMM analysis with a Poisson distribution and a least squares mean (LSmeans) student's t all pairwise comparison ($\alpha = 0.05$). Date and rep were considered random variables.

For the nanocellulose/insecticide experiment, the number of new attack holes observed on the tree bolt traps overall and at each sample date was subjected to generalized linear mixed model analysis (GLMM) with a Poisson distribution and a least squares mean (LSmeans) student's t all pairwise comparison ($\alpha = 0.05$). Date and rep were considered random variables.

Results

Nanocellulose experiment. In 2023, a total of 24 new attacks were discovered across all treatments and repetitions during the 21-day trial. There was a significant difference in the number of attacks on bolts between the different treatments ($F = 2.83$; df = 4, 220; $P < 0.0254$). The two mixtures of nanocellulose and bifenthrin/permethrin showed significantly fewer attack holes than the blank bolt filled with 50% ethanol. The nanocellulose by itself and the mixture of nanocellulose and verbenone were not significantly different from any of the treatments (Fig. 12A).

Insecticide experiment. In 2023, a total of 31 new attack holes were discovered across all treatments and repetitions during the 21-day trial. There were no significant differences in the amount of ambrosia beetle attacks on tree bolts sprayed with different insecticides ($F = 1.15$; df = 8, 315; $P = 0.3276$) with the cyantraniliprole treatment experiencing zero attacks throughout the

21-day trial (Fig. 12B). A total of 7 beetles were recovered from the bolts: 4 *X. crassiusculus*, 2 *Xyleborinus saxesenii*, and 1 unidentified specimen.

Nanocellulose/insecticide. In 2024, a total of 1328 new attack holes were discovered across all treatments and repetitions during the 21-day trial. There were many significant differences in the amount of ambrosia beetle attacks on tree bolts sprayed with different treatments (F = 16.05; df = 12, 507; P < 0.0001). None of the treatments showed a significantly similar number of attacks to the blank bolt with no ethanol or spray (Fig. 13). The 50% ethanol-baited bolt with no spray showed a significantly similar number of attacks to chlorantraniliprole, bifenthrin + nanocellulose, carbaryl, and nanocellulose + verbenone. The baited bolts sprayed with cyantraniliprole, diatomaceous earth, and tolfenpyrad had a significantly higher number of attacks compared to the 50% ethanol-baited bolt with no spray (Fig.13). The bolt treated with nanocellulose, permethrin + nanocellulose, bifenthrin, and permethrin had significantly lower attacks than the 50% ethanol-baited bolt with no spray. Permethrin was the only treatment that had significantly fewer attacks than the nanocellulose-treated bolt (Fig. 13).

The sample dates had significant differences in the number of attacks (F = 129.89; df = 7, 512; P < 0.0001). The sample collection 11 days after deployment showed a significantly higher number of beetle attacks than any other collection date (Fig. 14). The sample collection 4 days after deployment had the lowest number of attacks, and this number of attacks assessed was not significantly different from the sample collection 18 days after deployment. The first 11 days of sampling showed a general increase in the number of attacks assessed as the number of days deployed increased (Fig. 14).

Discussion

It was unfortunate that there were no significant differences seen in the 2023 insecticide trial at the UGA Horticulture Research Farm, and this was most likely due to the fact that the peak flight had already passed when the traps were deployed in late March of 2023. The initial flight was seen to start around roughly 15°C average daily temperature with the peak activity following soon after (Monterrosa et al. 2022). The daily average temperature at the research site from February 20th to March 9th ranged from 20.66-12.84°C with an average daily temperature of 16.81°C. The first flight most likely started near February 20th and the peak flight most likely occurred sometime in March before the bolts were deployed. The cyantraniliprole experiencing no attacks appears to be a very odd coincidence because it shows a general trend of being the least effective insecticide in the 2024 insecticide/nanocellulose experiment.

The 2023 nanocellulose trial at the UGA Horticulture Research Farm was also well after the estimated initial and peak flight of ambrosia beetles because the bolts for this trial were deployed at the beginning of June. The two mixtures of nanocellulose and bifenthrin and permethrin appear to be effective at lowering the amount of ambrosia beetle attacks when compared to 50% ethanol-baited bolts with no spray, and, although not significantly different, these two mixtures show a general trend of being more effective at lowering the number of ambrosia beetle attacks than the nanocellulose alone and the mixture of nanocellulose and verbenone. We were expecting to be able to compare the bifenthrin + nanocellulose and permethrin + nanocellulose to permethrin alone and bifenthrin alone from these two 2023 trials; however, the number of attacks was so low that no meaningful comparisons could be made. The insecticide/nanocellulose trial in 2024 was performed so a meaningful comparison could be ascertained.

The 2024 insecticide/nanocellulose trial at the Georgia pecan orchard appears to catch the initial flight and the start of a peak flight of ambrosia beetles. The temperature from 20 February to 3 March never reached about 15°C in average daily temperature. The daily average temperature from 3 March to 9 March ranged from 14.65-15.82°C with an average daily temperature of 15.12°C (Watkinsville-Hort weather station data). Both the temperature at the start and the gradual increase in the number of attacks on tree bolts lead us to believe that the initial flight of ambrosia beetles was captured during this trial in 2024. The spray treatments cyantraniliprole, diatomaceous earth, and tolfenpyrad are not recommended for ambrosia beetle management because all three of these sprays had a higher number of attacks than the 50% ethanol-baited bolt that had no spray treatment. The spray treatments chlorantraniliprole, bifenthrin + nanocellulose, carbaryl, and nanocellulose + verbenone are also not recommended for ambrosia beetle management because all 4 of these treatments did not show fewer attacks than the 50% ethanol-baited bolt with no spray. The mixture of nanocellulose and bifenthrin may not have shown viable efficacy in ambrosia beetle control because the two substances do not mix properly and the Bifenthrin readily leaches out of the mixture, or possibly because the nanocellulose interacts with the insecticide and lowers its efficacy. More research would need to be conducted to determine the interactions between the two compounds.

Spray treatments of nanocellulose, nanocellulose + permethrin, bifenthrin, and permethrin all show promise of effectively lowering the number of ambrosia beetle attacks. Although it significantly lowered the amount of ambrosia beetle attacks compared to the 50% ethanol-baited bolt with no spray, nanocellulose shows a trend of not being as effective at reducing the number of attacks as the other three treatments because it did not show a significantly lower number of attacks than chlorantraniliprole. Permethrin alone shows a trend of increased efficacy in reducing ambrosia beetle attacks because the nanocellulose + permethrin and bifenthrin did not show significantly different numbers of attacks than the nanocellulose alone. Much like the case with nanocellulose + bifenthrin, the mixing of nanocellulose with permethrin seems to lower the efficacy of permethrin in ways that are not yet understood. The nanocellulose could possibly reduce the volatility of the pyrethroids, thus reducing their repellency. More research would have to be conducted to learn the interactions that these two compounds have when mixed together. The nanocellulose alone may have reduced the number of ambrosia beetle attacks by creating a physical barrier that made it harder for the beetles to bore inside the bolt, but the results from this treatment were not satisfying enough to recommend this spray treatment to growers.

These findings of the treatments involving permethrin and bifenthrin showing trends of being the most effective insecticide for ambrosia beetle control is consistent with other studies involving insecticides (Švihra et al. 2004, Fettig et al. 2006, Williamson et al. 2023). Permethrin shows the potential of being more effective at ambrosia beetle control than bifenthrin, but more research would have to be done to solidify any differences between the two insecticides.

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Table 3. Study site location, experiment type, and sampling periods for 2023 and 2024.

Cropping system	Location	Experiment	GPS coordinates	Sampling period	
				2023	2024
Various	Watkinsville, GA	Insecticide	33.887204, -83.416731	25 Mar – 14 April	
Various	Watkinsville, GA	Nanocellulose	33.887204, -83.416731	1 June – 17 July	
Pecan	Athens, GA	Insecticide/ Nanocellulose	33.879249, -83.289697		7 Mar – 28 Mar

Fig. 11. Tree bolts drilled and filled with 50% ethanol and capped with a cork for the A) 2023 insecticide trial at the UGA Horticulture Research Farm in Watkinsville, GA, B) 2023 nanocellulose trial at the UGA Horticulture Research Farm in Watkinsville, GA, and C) 2024 insecticide/nanocellulose trial at the pecan orchard in Athens, GA.

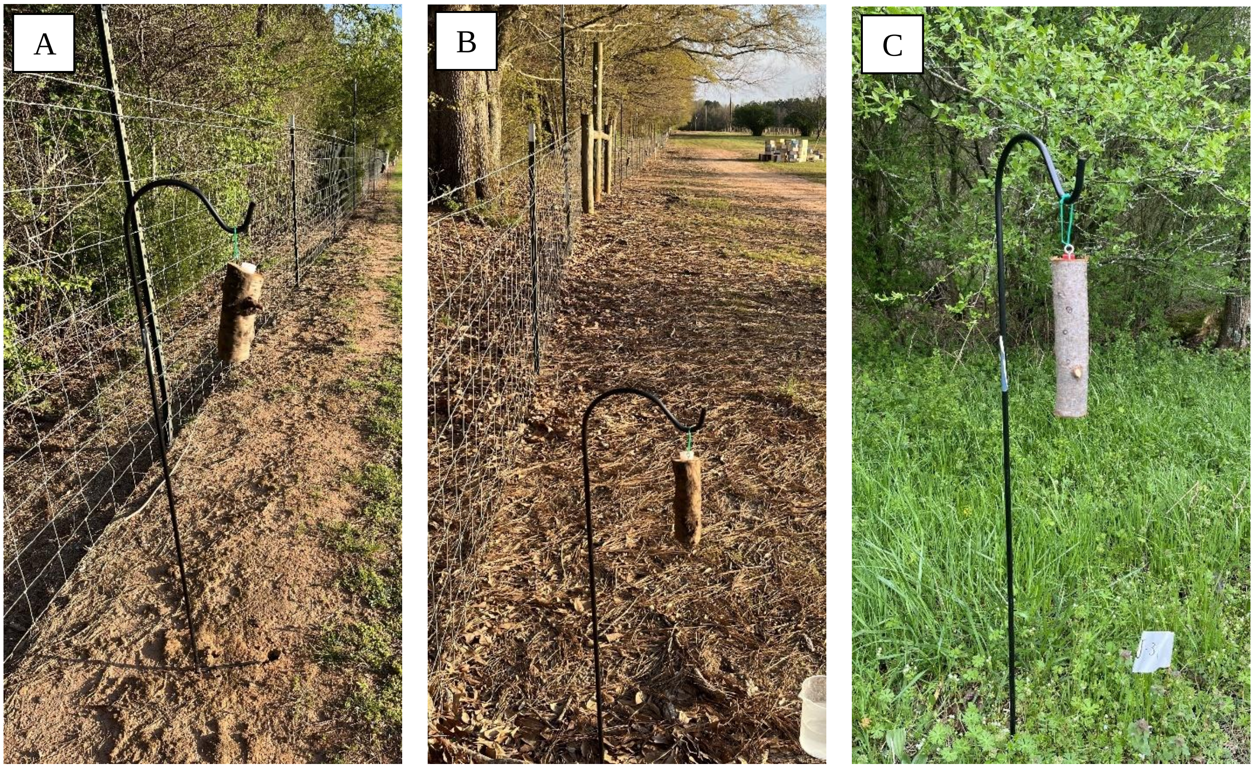

A
B
C

Fig. 12. Mean ± SEM of ambrosia beetle attacks per bolt for A) treatments in the 2023 nanocellulose trial and B) treatments in the 2023 insecticide trial. Letters not connected by the same letter are significantly different (Student's t-test, α = 0.05).

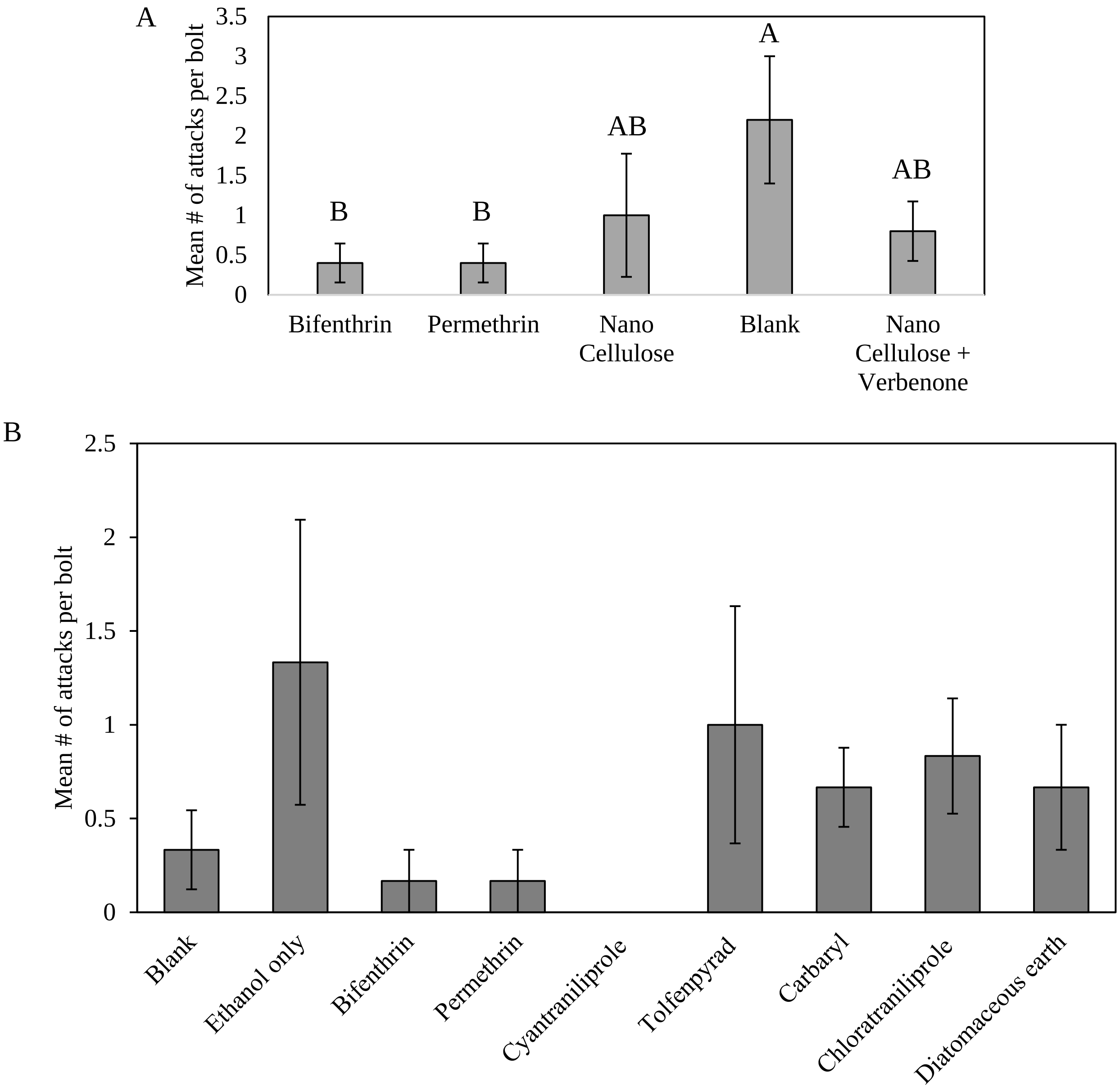
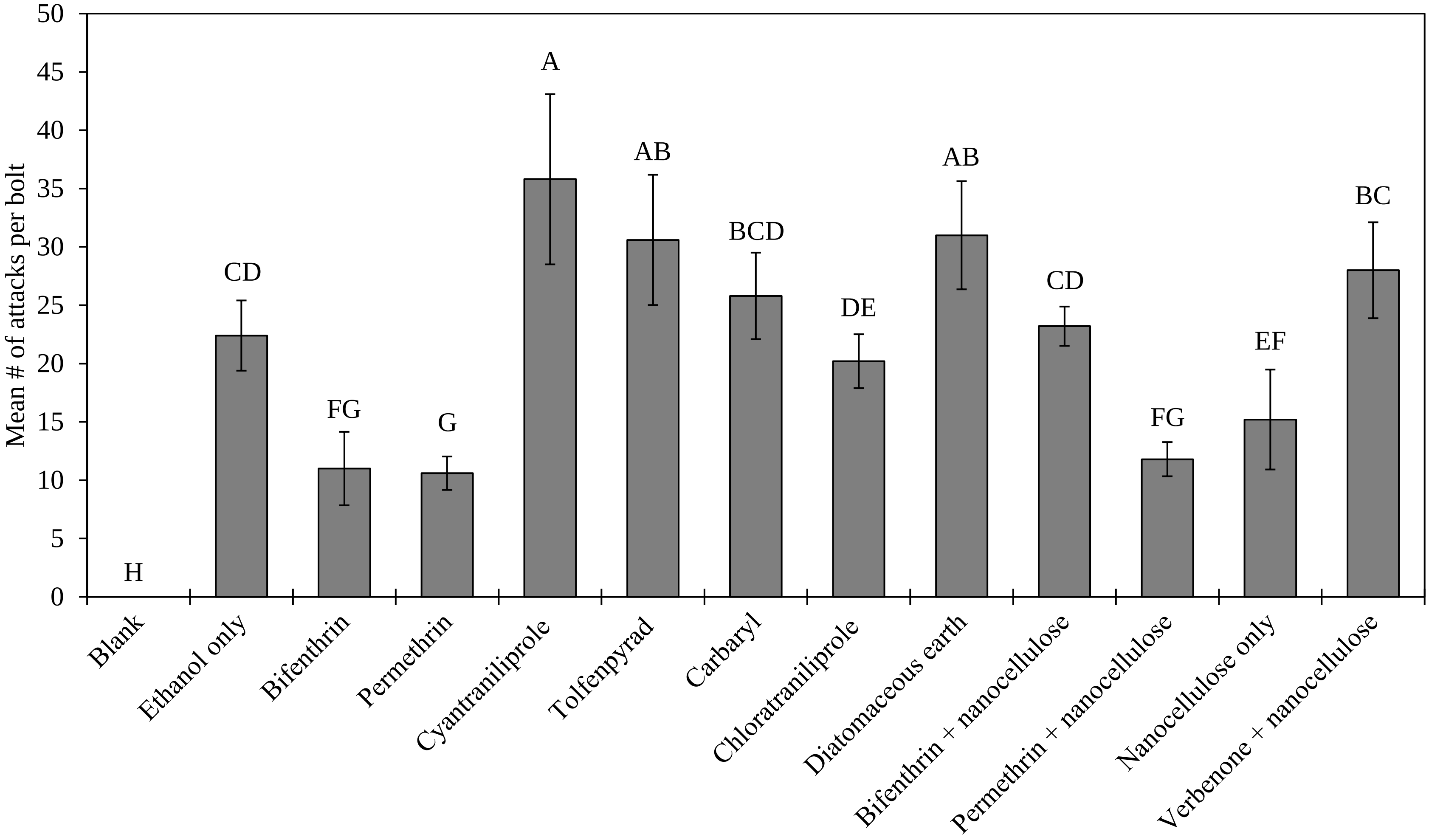
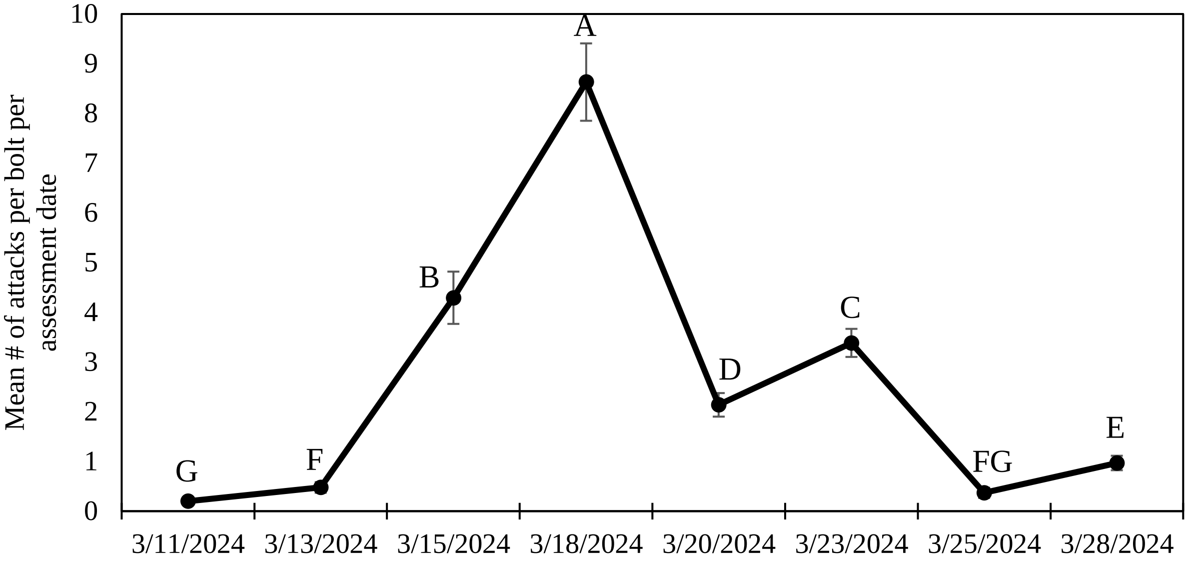

A
Mean # of attacks per bolt
3.5
3
2.5
2
1.5
1
0.5
0
B
B
AB
A
AB
Bifenthrin
Permethrin
Nano Cellulose
Blank
Nano Cellulose + Verbenone
B
Mean # of attacks per bolt
2.5
2
1.5
1
0.5
0
Blank
Ethanol only
Bifenthrin
Permethrin
Cyantraniliprole
Tolfenpyrad
Carbaryl
Chloratraniliprole
Diatomaceous earth

Fig. 13. Mean ± SEM of ambrosia beetle attacks per bolt for treatments in the 2024 insecticide/nanocellulose trial. Letters not connected by the same letter are significantly different (Student's t-test, $\alpha = 0.05$).



Mean # of attacks per bolt
0
5
10
15
20
25
30
35
40
45
50
H
CD
FG
G
A
AB
BCD
DE
AB
CD
FG
EF
BC
Blank
Ethanol only
Bifenthrin
Permethrin
Cyantraniliprole
Tolfenpyrad
Carbaryl
Chloratraniliprole
Diatomaceous earth
Bifenthrin + nanocellulose
Permethrin + nanocellulose
Nanocellulose only
Verbenone + nanocellulose

Fig. 14. Mean ± SEM of ambrosia beetle attacks per bolt for the date of bolt assessment. Letters not connected by the same letter are significantly different (Student's t-test, $\alpha = 0.05$).


Mean # of attacks per bolt per assessment date
10
9
8
7
6
5
4
3
2
1
0
3/11/2024
3/13/2024
3/15/2024
3/18/2024
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CHAPTER 5

CONCLUSIONS

Ambrosia beetles continue to be an economically significant pest in tree fruit and nut orchards and woody ornamental nurseries around much of the United States. They have the potential to become major economic pests in many agricultural industries in Georgia; however, there have only been a few studies performed in Georgia. Trials evaluating the efficacy of repellents and luring methods were conducted at the University of Georgia Horticulture Research Farm in 2022. The treatments from the luring experiment and repellent experiment consisted of the treatments: bolts soaked in 10% and 90% ethanol, filled with 10% and 90% ethanol, a control (bolt with no ethanol), a bottle trap baited with an ultra-high-release ethanol lure (UHR), a bottle trap baited with a low-release ethanol lure, a bottle trap with no ethanol, a UHR bottle trap with a verbenone pouch, a UHR bottle trap with a methyl salicylate (MeSA) pouch, a UHR bottle trap with both verbenone and MeSA pouch, a bottle trap with just UHR, and a bottle trap with no ethanol or repellents. Both the soaked and the cored bolts treated with 90% ethanol attracted more beetles than the blank bolts. Growers may prefer to utilize soaked bolts to reduce the labor involved in bolt trap creation. The UHR attracted more beetles than the low-release ethanol lures in the bottle traps. The combination of verbenone and MeSA repelled more beetles than bottle traps with just MeSA. The combination of MeSA exhibits a trend of being possibly more repellent than verbenone alone. There were differences in the number of attacks and beetles collected between the date of sample collection and trap location. These results provide practical information for growers in the area of monitoring tactics and viable repellents and highlight the importance of consistent monitoring for proper ambrosia beetle management.

In 2023 and 2024 at the University of Georgia Horticulture Research Farm, the efficacy of the combined use of repellents and ethanol-baited bottle traps in a push-pull style to lower the amount of ambrosia beetle attacks on water-stressed trees was conducted. Three similar experiments were conducted, and the treatments for the experiments included a water-stressed apple tree with verbenone and MeSA pouches with a perimeter of 8 ultra-high-release baited (UHR) bottle traps ~6 m from the treatment tree and a control water-stressed tree (no repellents or bottle traps). In the May 2023 experiment, the control trees were placed ~12.2 m from the treatment tree. In the July 2023 experiment, the control trees were placed ~36.5 m from the treatment tree. The experiment in 2024 maintained this ~36.5 m distance between treatment and control trees, but two more repetitions were added to this experiment making for a total of 7 repetitions compared to the 5 repetitions of experiments 1 and 2. The two experiments from 2023 could not have the tree attack data analyzed due to extremely small count data (< 10 attacks total). The experiment from 2024 showed a reduced number of ambrosia beetle attacks on the water-stressed trees with this push-pull strategy implemented. The number of beetles captured in bottle traps around the treatment trees during the first week of collection, the second week of collection, and overall, for the three different experiments were different from one another. This showed that the attack and flight activity of the beetles were different between the dates that these experiments were conducted. These results provide practical information for growers in the area of a potential new management strategy that is shown to be effective at lowering the amount of ambrosia beetle attacks on stressed apple trees.

In 2023 at the University of Georgia Horticulture Research Farm, the efficacy of insecticides and nanocellulose in ambrosia beetle management were evaluated separately. The efficacy of both insecticides and nanocellulose in ambrosia beetle control were evaluated in a larger, combined experiment at a Georgia pecan orchard in 2024. The treatments for all three experiments consisted of cored peach tree bolts filled with 50% ethanol and sprayed with bifenthrin, permethrin, cyantraniliprole, tolfenpyrad, carbaryl, chloratraniliprole, diatomaceous earth, nanocellulose gel, nanocellulose gel + bifenthrin, nanocellulose gel + permethrin, nanocellulose gel + verbenone, a bolt filled with 50% ethanol and no spray, and a blank bolt with no ethanol or spray. The insecticide trial in 2023 could not be analyzed due to a low number of attacks. Evaluating nanocellulose in 2023 showed that mixtures of nanocellulose + bifenthrin and nanocellulose + permethrin reduced the number of ambrosia beetle attacks compared to the control bolt baited with 50% ethanol. The insecticide/nanocellulose trial in 2024 showed reduced numbers of attacks on bolts sprayed with nanocellulose, nanocellulose + permethrin, bifenthrin, and permethrin. Permethrin on its own shows a trend of currently being the most effective insecticide for ambrosia beetle control available to growers based on the data collected from this trial. Until more research is conducted with nanocellulose, permethrin, and bifenthrin still appear to be the only viable insecticide options for growers that seek to manage ambrosia beetles.

Future studies that evaluate different aspects of the push-pull tactic including trap layout, trap number, the effective radius of repellents and lures, different forms of repellents, and the efficacy of the “push” and “pull” components separately are needed to refine this management tactic. Nanocellulose and how it interacts with pyrethroids when mixed needs to be explored to determine how nanocellulose influences the efficacy of the pyrethroids and if nanocellulose by itself could actually create a physical barrier on trees that lowers the number of ambrosia beetle attacks. Ultimately, an economic threshold for ambrosia beetles in fruit and nut trees must be established in order to determine if we would be justified in recommending any of these management tactics that were evaluated to growers.