PHENOLOGY AND MONITORING OF AMBROSIA BEETLES IN ORNAMENTAL NURSERIES, TREE FRUIT, AND PECAN ORCHARDS IN GEORGIA

by

ALEJANDRA MONTERROSA

(Under the Direction of Shimat V. Joseph and Angelita L. Acebes-Doria)

ABSTRACT

Ambrosia beetles (Coleoptera: Curculionidae) are wood-boring pests in ornamental nurseries, fruit, and pecan orchards. Seasonal flight activity and species abundance were investigated in nurseries, fruit, and pecan orchards using ethanol-baited bottle traps. *Xylosandrus crassiusculus* (Motschulsky) and *X. germanus* (Blandford) were abundant pest species in all systems. Three experiments were done in 2019 and 2020 to improve monitoring to help refine management strategies. Phenology of ambrosia beetles was studied and results showed that flight activity was dependent on warmer temperatures in spring and continued intermittently throughout the growing season. When testing ethanol type and placement on wooden bolt traps, results showed greater attraction to bolts with ethanol added in the core than with ethanol pouch placed on the outside. The impact of age of ethanol lures on attractancy was evaluated, resulting in trap captures with eight-week-old ethanol pouches that were not significantly different from four-week-old pouches.

INDEX WORDS: *Xylosandrus* spp., phenology, bolt trap, ethanol lures

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ALEJANDRA MONTERROSA

B.S., GORDON STATE COLLEGE, 2018

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

MASTER OF SCIENCE

ATHENS, GEORGIA

2021

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ALEJANDRA MONTERROSA

Major Professor:	Shimat V. Joseph Angelita L. Acebes-Doria
Committee:	Brett R. Blaauw William G. Hudson

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
May 2021

ACKNOWLEDGEMENTS

I am grateful to both of my major advisors, Dr. Shimat V. Joseph and Dr. Angelita L. Acebes-Doria, who were both very encouraging and extremely helpful throughout this degree program. They showed me great patience and understanding. I am also grateful for Dr. Brett R. Blaauw and Dr. William G. Hudson for their wonderful advice throughout this program as well as for reviewing this thesis.

I highly appreciate Caleb Julian, Mary Hannah Hardin, Brooklyne Wassel, Pamela Halliday, and the many others for their help and effort put into assisting me with trap setups and data collection. I would like to thank Dr. Uttam Bhattarai for helping me analyze the data. I would also like to thank my family and friends that encouraged and supported me throughout these years to further my education and finish this degree.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Ornamental nurseries, tree fruit, and nut orchards and are highly valued commodities in Georgia. In 2018, the farm gate value for ornamentals was at $888 million USD, which grew by ~$42 million USD since 2017 (Wolfe and Stubbs 2019). About 11 million hectares of land in Georgia was used for ornamental horticulture in 2018. Ornamental horticulture's production systems—field nurseries, container nurseries, greenhouses, turfgrass, and landscape horticulture all contributed $8 billion USD to Georgia's economy and provided 83,000 jobs in 2018. The 2018 farm gate value for fruits and nuts were $614 million USD, which decreased since 2017 (Wolfe and Stubbs 2019). In 2018, nuts and fruits contributed $1.9 billion USD to Georgia's economy and provided 13,000 jobs (Wolfe and Stubbs 2019). When it comes to apples and peaches, those crops made up about 10% of all of the fruit commodities (Wolfe and Stubbs 2019). The combined farm gate value is about $56 million USD using about 4,046 hectares of Georgia land (Wolfe and Stubbs 2019). Pecans made up 35.5% of the fruit and nuts commodity with a farm gate value of $218 million USD (Wolfe and Stubbs 2019). About 76,080 hectares in Georgia were used for pecan orchards in 2018 (Wolfe and Stubbs 2019). Clearly, these three commodities are important to Georgia's economy.

Ambrosia beetles are serious pests of ornamental field nursery, tree fruit, and nut orchards (Ree and Knutson 1997, Agnello et al. 2014, Reed et al. 2015, Addesso et al. 2019). Ambrosia beetles belong to order Coleoptera, family Curculionidae and within subfamily Scolytinae. Scolytinae contains many tribes including Xyleborini which is one of the most diverse tribes within Scolytinae (Gomez et al. 2018). The tribe Xyleborini has some of the most widely distributed beetles as they share traits such as fungal farming and haplodiploidy (Gomez et al. 2018). The genus *Xylosandrus* within tribe Xyleborini is comprised of approximately 54 species worldwide (Dole et al. 2010). Several *Xylosandrus* species such as *Xylosandrus crassiusculus* (Motschulsky), *Xylosandrus germanus* (Blandford) and *Xylosandrus compactus* (Eichhoff) have been introduced to North America and are causing economic losses (Dole et al. 2010).

The granulate ambrosia beetle (formerly referred to as Asian ambrosia beetle), *X. crassiusculus,* and black stem borer (also referred to as the black timber bark beetle), *X. germanus* are serious pests in ornamental nurseries (Oliver and Mannion 2001, Ranger et al. 2016). The black twig borer, *X. compactus,* has recently begun to have a pest status in ornamental nurseries and tree nuts (Ranger et al. 2016, Greco and Wright 2015) but not confirmed in Georgia. These beetles bore into the heartwood of young, stressed trees. The tunnels created by these adult beetles form galleries and later the tunnels widen to form brood chambers where the adult beetles introduce a symbiotic fungus that they farm and use to feed larvae and adults (Oliver and Mannion 2001, Ranger et al. 2016, Addesso et al 2019). The attacks on trees do not always cause tree death but often cause branch dieback which can negatively impact the aesthetics (Oliver and Mannion 2001, Ranger et al. 2016).

Distribution

X. crassisculus, X. germanus, and *X. compactus* are all ambrosia beetle species native to Asia. *X. crassiusculus* is established throughout Central America, the Caribbean, Oceania, and East and West Africa, North America and Europe (Ranger et al. 2016). *X. crassiusculus* was first found in North America in 1974 in South Carolina from a peach orchard (Ranger et al. 2016). The beetle is now established in 31 states in the eastern US, and in Hawaii (Ranger et al. 2016, Ranger et al. 2020). In recent years, *X. crassiusculus* emerged as a major pest in the mid-Atlantic and southern U.S. states (Ranger et al. 2016).

X. germanus is native to Asia but now found throughout Europe, and North America. In 1932, *X. germanus* was the first recorded in North America from grape vines grown in a greenhouse in New York (Weber and McPherson 1983b, Ranger et al. 2016, Galko et al. 2018). Currently, it is found in 32 US states (Ranger et al. 2016). Within the U.S., it is established in the northern, mid-Atlantic, southern, southeastern, midwestern, and northwestern U.S. (Rabaglia et al. 2006, Ranger et al. 2016). *X. germanus* is a major pest in the Midwest and Northeast regions (Ranger et al. 2016) and is also reported in Canadian provinces of British Columbia, Quebec, Nova Scotia, and Ontario (Rabaglia et al. 2006, Ranger et al. 2016). It was first discovered in Germany in 1951 and now is present in most of Europe (Galko et al. 2018).

X. compactus is common in subtropical and tropical environments (Greco and Wright 2015). It is reported from Africa, Asia, the Pacific Islands, New Zealand, South America, and North America (Chong et al. 2009, Greco and Wright 2015). In the U.S., *X. compactus* was first found in Florida in 1941 (Ngoan et al. 1976, Chong et al. 2009, Greco and Wright 2015). It is now established in 13 US states in the south, southeast, midwest, and Hawaii (Ranger et al. 2020).

General Biology

Within the genus *Xylosandrus*, the adult beetles are sexually dimorphic. The females have a stouter and more cylindrical body, and they readily fly. The males are much smaller with a more "spheroid" body and are mostly flightless and rarely leave the galleries (Dole et al. 2010, Ranger et al. 2016). The females disperse from the host tree seeking a new tree host. When they land on new host, they bore into the tree trunks creating tunnels and galleries. The tunnels widen to become the brood chamber where they rear larvae (Addesso et al. 2019). The complete life cycle varies among *Xylosandrus* species. Although the life cycle of *X. germanus* is not completely understood, the developmental time from egg to adult is about 4 to 5 weeks (Hoffmann 1941, Weber and McPherson 1983b). A generation for *X. crassiusculus* can span between 20 days to 4 months, depending on the precise location and region the beetle is in (Mizzel et al. 1994). *X. compactus* completes its life cycle in about 58 days at temperatures ranging between 23 and 27 °C.

Life Stages

Egg

The egg of *X. germanus* is about 0.67 mm in length and 0.38 mm in width. It is "ellipsoid" in shape, shiny, soft, translucent, and white in color (Hoffman 1941, Ranger et al. 20016, CABI 2019). The females oviposit approximately 2-54 eggs (Hoffman 1941). The egg of *X. crassiusculus* has not been formally described but is thought to be similar to the egg of *X. germanus* (Ranger et al. 2016). The egg of *X. compactus* is approximately 0.59 mm long and 0.30 mm wide (Hara and Beardsley 1979). It is oval shaped, smooth, and white in color (Hara and Beardsley 1979). The incubation period for the eggs is about 4 days (Hara and Beardsley 1979).

Larvae

The newly hatched larva of *X. germanus* is white, elongate and apodous but it soon becomes curved (Hoffman, 1941, Ranger et al. 2016, CABI 2019). The color of larval head capsule is light brown but it darkens with time (Weber and McPherson 1983, Ranger et al. 2016). The larva of *X. crassiusculus* has not been formally described but is thought to be similar to *X. germanus*

(Ranger et al. 2016). The larvae of *X. germanus* and *X. crassiusculus* undergo three instars (Weber and McPherson 1983). *X. compactus* larvae are apodous, creamy white in color, and have a pale brown head capsule (Ngoan et al. 1976). There are two larval stages with the first instar measuring 0.212 mm in length and 0.025 mm in width and the second instar measuring 0.347 mm in length and 0.021 mm in width (Hara and Beardsley 1979).

Pupa

X. germanus larvae undergo a prepupal stage that lasts for 2 or 3 days (Hoffman 1941). The pupae are white in color. The female pupae are larger in size averaging 2.53 mm in length and 1.09 mm in width whereas, the male pupae average 1.78 mm in length and 0.98 mm in width (Hoffman 1941). The *X. germanus* pupae have legs, wings and a moveable abdomen (Ranger et al. 2016). A couple of days after pupation, the eyes darken, and about a day before molting, the mandibles and hind wings change colors to a red brown and blue, respectively (Hoffman 1941). The pupa of *X. crassiusculus* has yet to be formally described but is similar to the pupa of *X. germanus*. Similar to *X. germanus*, *X. compactus* has a prepupal stage. The pupae are a white color during the first two days (Hara and Beardsley 1979) then their eyes turn brown in color. By the fifth day, the mandibles of the pupae darken and followed by darkening of the wings. The body of the female pupae darken at this stage (Hara and Beardsley 1979). The pupal stage lasts from 6 to 7 days (Hara and Beardsley 1979).

Adult

The *X. germanus* adult female has a stout, cylindrical body and is uniformly dark brown to black in color with a shiny declivity (Ranger et al 2016). Adults are about 2.0 to 2.4 mm in length and about 1.0 mm in width (Ranger et al. 2016). The head of the beetle is hidden under the pronotum (Ranger et al. 2016). There are striations running along the declivity with alternating rows of setae. The adult female *X. crassiusculus* has a stout, cylindrical body and a reddish brown pronotum with the elytra being a darker brown color (Ranger et al. 2016). The female of *X. crassiusculus* is slightly larger than *X. germanus* female, ranging from 2.1 to 2.9 mm in length and 1.2 mm in width (Ranger et al. 2016). The head of *X. crassiusculus* is also prominently covered by the pronotum. The elytral declivity lacks striae and has a granulate texture which gives it a dull appearance (Rabaglia 2006). Female *X. compactus* have a stout, cylindrical body and a uniform shiny black color (Hara and Beardsley 1979, Greco and Wright 2015). The adult female is 1.6-1.8 mm in length and 0.72-0.74 mm in width (Hara and Beardsley 1979). The body size of *X. compactus* is smaller than *X. germanus* with strial setae on the shiny declivity (Rabaglia 2006).

The adult male beetles of *X. crassiusculus*, *X. germanus*, and *X. compactus* are all very similar in their characteristics. The males of all three species are smaller than the females, lighter in color and more sphere-like in shape (Ranger et al. 2016). The males are pale, less sclerotized with rudimentary hind wings and therefore, they do not fly (Hoffman 1941, Ranger et al. 2016). They rarely come outside their galleries (Dole et al. 2010, Ranger et al. 2016).

Fungal associations

While carving out the galleries, the females introduce a symbiotic fungus that they carry in specialized organs called the mycangia (Francardi et al. 2017). The mycangium is located between the prothorax and mesothorax (Ranger et al. 2016). As the beetles bore into the heartwood of the tree, the fungal spores are transferred and deposited into the tunnels. The fungal colony develops around the walls of tunnels and becomes the food source for both the adults and the larvae (Oliver and Mannion 2001). The *X. crassiusculus* and *X. germanus* females do not initiate oviposition until the fungi are established in the gallery (Weber and McPherson 1983, Ranger et al. 2016). There are several species of fungi associated with *X. germanus*, including *Ambrosiella hartigii* and *A. grosmanniae* (Weber and McPherson 1983, Bateman et al. 2016). *A. xylebori and A. roeperi* are associated with *X. crassiusculus* (Bateman et al. 2016). *A. xylebori* and *Fusaria* spp. fungi are associated with *X. compactus* (Bateman et al. 2016). There are two forms of the fungus—a white and dark form that develops in the presence and absence of the adults, respectively (Ranger et al. 2016). The dark form consists of mycelial cells that grow in the absence of beetles, whereas the white form produces conidia and serves as food source (Ranger et al. 2016).

Reproduction and Oviposition

Ambrosia beetles reproduce by arrhenotokous parthenogenesis (Greco and Wright 2015) where the females mate with their male siblings and selectively fertilize their eggs. The eggs that are not fertilized become haploid males and fertilized eggs become diploid females. The mated female beetles overwinter in the galleries (Ranger et al. 2016) and emerge from the overwintering hosts seeking a new host in the spring when the temperature warms up. Males remain in the galleries of overwintering hosts and rarely leave the host. Once the females colonize the new hosts, they wait until the symbiotic fungus develops in the brooding chambers before they begin oviposition (Hoffman 1941, Weber and McPherson 1983, Ranger et al. 2016). *X. germanus* oviposits one egg per day whereas, *X. crassiusculus* oviposits one to six eggs per day (Weber and McPherson 1983, Ranger et al. 2016). Female *X. compactus* oviposits 2-16 eggs during their lifetime (Hara and Beardsley 1979). Once the eggs hatch, the larvae spread and settle uniformly within the gallery and begin feeding on the fungus (Weber and McPherson 1983).

Signs of infestation

When the females bore through the stem, it generates wood dust and frass in the entry holes which appear as “toothpicks” on the tree trunk. However, the toothpicks may not be intact due to rain and high winds but entry holes will be present (Mizell et al. 1994, Greco and Wright et al. 2015, Ranger et al. 2016). In some tree species, the boring damage can trigger sap production and staining from the entrance holes on the trunk (Ranger et al. 2016). On young ornamental trees, branch die back, and wilting have been reported (Hara and Beardsley 1979, Greco and Wright 2015, Ranger et al. 2016). Internal injury to the trees that may show indications of previous ambrosia beetle activity is tissue discoloration within the tissue surrounding the tunnels; however, several reasons could be the cause of discoloration such as host defense, introduced fungus, or other pathogens that may have been introduced into the tunnels (Ranger et al. 2016). Although not all affected trees die, the damage symptoms affect the aesthetic appeal which negatively impact marketability—particularly in ornamental nurseries (Ranger et al. 2016).

Hosts

Ambrosia beetles are attracted to young, stressed trees (Ranger et al. 2010) When trees are in stress they emit several stress signals or volatile chemicals, particularly ethanol, which are attractive to the ambrosia beetles (Ranger et al. 2010). The ambrosia beetles attack healthy looking trees as well as recently dead trees (Weber and McPherson 1983b). They attack a wide range of tree species including trees in ornamental nurseries, tree fruits and nuts, coffee plantations, and avocado groves (Greco and Wright 2015, Ranger et al. 2016, Hulcr and Stelinski 2017, Ranger et al. 2020). *X. germanus* and *X. compactus* attack more than 200 species of trees in 51 families (Chong et al. 2009, Ranger et al. 2016). *X. crassiusculus* attack more than 120 tree species (Ranger et al. 2016). Although they attack coniferous and deciduous trees, attacks on deciduous thin barked trees are more often reported(Ranger et al. 2020). *X. germanus, X.*

crassiusculus, and *X. compactus* attack trees regardless of age (Ranger et al. 2020). *X. germanus* and *X. crassiusculus* prefer tree trunks over the branches whereas, *X. compactus* attacks mostly tree branches and twigs rather than trunks (Ranger et al. 2020). Examples of ornamental trees attacked by ambrosia beetles include dogwood (*Cornus* spp.), redbud (*Cercis* spp.), maple (*Acer* spp.), oaks (*Quercus* spp.), fir (*Abies* spp.), and pine (*Pinus* spp.) (Ranger et al. 2016, Ranger et al. 2020). In addition, ornamental shrubs such as hydrangea and gardenia are commonlybe attacked by ambrosia beeltes (Chong et al. 2009). Tree fruit and nut orchards, such as apples (*Malus* spp.), cherry and peach (*Prunus* spp.), avocados (*Persea* spp.), grape (*Villis* spp.) pecans (*Caraya* spp.), walnut (*Jugans* spp.), and chestnut (*Castanea* spp.) are also potential targets (Weber and McPherson 1983a, Ranger et al. 2016, Ranger et al. 2020).

Why *Xylosandrus* species are challenging to manage in production systems?

There are several reasons why management of these *Xylosandrus* ambrosia beetles are challenging. First, the key pest species are polyphagous beetles, in terms of the host plants they attack (Atkinson and Equihua-Martinez 1986, Oliver and Mannion 2001, Ranger et al. 2020). Because of wide host range, it is challenging to target single or a few tree species with management tools. Secondly, ambrosia beetles directly bore into tree trunks, without ingesting the wood, and bypass the vascular system of the trees reducing exposure to systemic insecticides (Ranger et al. 2016, Ranger et al. 2020). Also, their symbiotic relationship with a fungus and habit of feeding only on fungal colony further prevents insecticide exposure through ingestion (Ranger et al. 2016, Addesso et al. 2019, Ranger et al. 2020). Thirdly, ambrosia beetles reproduce by arrhenotokous parthenogenesis where fertilized eggs develop into females and unfertilized eggs to males (Greco and Wright 2015). This reproduction mechanism favors production of more females than males (Greco and Wright 2015). For example, in *X. germanus* and *X. compactus* female to male ratio is 9:1 and 10:1 respectively (Hara and Beardsley 1979, Ranger et al. 2016).

Monitoring

Monitoring for ambrosia beetles is important when it comes to ambrosia beetle control. The first emergence of the beetles after overwintering is temperature dependent and therefore can be unpredictable. With monitoring the first flight can be detected along with peak activity throughout the season and pesticide sprays can be timed accordingly. There are different types of monitoring attractants and traps that are effective and relatively inexpensive for users.

Attractants

Females of *X. germanus*, *X. crassiusculus.* and *X. compactus* are attracted to ethanol (Ranger et al. 2010). During bud break and stressful situations, ethanol is naturally produced by trees (Ranger et al. 2010). The trees could be under stress because of drought, flooding, freezing, disease, poor irrigation practices, and low fertility, which causes trees to increase their production of acetaldehyde (Ranger et al. 2010). Acetaldehyde is subsequently converted into ethanol and transported through xylem vessels (Ranger et al. 2010). In contrast, healthy trees produce methanol, which is not attractive to ambrosia beetles (Ranger et al. 2010). Ranger et al. (2010) showed that ethanol was the most attractive volatile compound among other volatiles such as acetaldehyde, acetone, and a non-stress related volatile (methanol) to ambrosia beetles (specifically, *X. germanus*) when injected into trees (Ranger et al. 2010). Ethanol lures are commercially available and can be purchased containing 70-95% ethanol with varied release rate (Ranger et al. 2016). Previously, Steininger et al. (2015) showed that non-denatured ethanol captured more ambrosia beetles compared to two brands of hand sanitizers (Purell and Germ-X). Between two hand sanitizers, ambrosia beetle captures were greater in the product with higher ethanol concentration (Steininger et al. 2015). Studies using semiochemical additives with ethanol such as α-pinene and conophthorin have not proven effective either as attractants or repellants (Addesso et al. 2019).

Traps

Several trap types including Lindgren funnel trap, bottle trap, and wooden bolts have been investigated for ambrosia beetle monitoring. Lindgren funnel traps have been used to intercept beetles during flight, plastic bottle traps have been used to attract and capture beetles during flight, and bolt traps have been used as bait to assess beetle presence and attack severity. In 1983, B. Staffan Lindgren proposed using a trap consisting of multiple funnels with a collection cup attached to the bottom of the funnels to trap ambrosia beetles and pine beetles. The funnel traps were easy to setup and user-friendly and trap captures were comparable to sticky traps (Lindgren 1983). Bottle traps are constructed using 2-liter soda bottles with window(s) (one or multiple) cut out from the side. They are baited with ethanol lures and hung upside down with soapy water in the bottle to capture the ambrosia beetles (Steininger et al. 2015, Ranger et al. 2016). The bottle traps are now widely used for monitoring ambrosia beetles because they are inexpensive and easy to construct. Studies placing ethanol-baited bottle traps at various heights showed that *X. germanus* captures were higher at 0.5 m above the ground, whereas *X. crassiusculus* captures were similar at 0.5 or 1.7 m above the ground (Reding et al. 2010). Another monitoring tool is the wooden bolt trap that is increasing its popularity among growers. These traps are constructed using tree trunks cut into bolts (about 10 cm diameter and 30-60 cm long). The bolts are baited with ethanol by submerging the bolt in ethanol and soaking for 24 h or drilling a vertical 1-cm hole at the core of the bolt (up to 10 cm deep). Ethanol is added into the hole and covered with a cork (Ranger et al. 2016). The attacks from *X. germanus* and *X. crassiusculus* can be monitored by systematically counting and marking the entry holes on the bolts (Reding and Ranger 2020).

Management

Because of the beetles' wood-boring behavior and not ingesting the wood, there are no systemic insecticides that can directly cause adult mortality (Addesso et al. 2019). Instead, insecticides (especially pyrethroids) are used as repellents and are applied preventatively (Reding et al. 2010). The insecticide applications must be precise and should coincide or be early enough with emergence of the ambrosia beetles from the overwintering hosts in late winter or spring (Addesso et al. 2019). Currently, preventive applications of pyrethroids, such as permethrin or bifenthrin on the trunks of trees is recommended at every 10 to 14 days during the spring flight period (Hudson and Mizell 1999, Ranger et al. 2016). Because exact beetle emergence time is hard to predict, other cultural management strategies that maintain healthy trees and reduce stress are recommended (Ranger et al. 2016). Fungal colonization is essential to initial oviposition, thus, strategies that affect fungal development have been studied using *Beauveria bassiana* strains and *Trichoderma* sp. strains. These entomopathogenic and mycoparasitic fungi interrupt the development of symbiotic fungi and reduce the survival of adult beetles (Castrillo et al. 2011, Castrillo et al. 2013, 2016). Future studies using *Beauveria bassiana* strains and *Trichoderma* sp. strains could refine management strategies against ambrosia beetles.

Stress factors should be monitored throughout nurseries and orchards to be able to take approriate measures for management. Signs of stress, although not always visible, may include branch dieback, wilting folioage, and stunted growth (Ranger et al. 2016). When stress factors are present, it should be known that the trees affected are predisposed to ambrosia beetle attack.

Future Studies

There are several substances that could be potentially useful when it comes to decreasing tree stress and ethanol release from the tree. One of the substances is known as biochar and is incorporated into the soil as an additive that could potentially be useful in studies dealing with certain types of stress. Biochar is produced using pyrolysis and is an organic material, and although not many studies have been done it is thought to help with water retention (Verheijen et al. 2010). Another additive to stress relief could potentially be kaolin clay. The application of kaolin clay spray on trees leaves behind a white reflective surface that could distort the beetle's view of the tree (Werle et al. 2017), as well as add a layer of protection from being scorched by the sun, which can reduce tree stress. Also, it is not clear if kaolin clay contributes to reduced release of ethanol and be less attractive to invading ambrosia beetles. Although kaolin clay does not cause beetle mortality, it could alter beetle movement, feeding pattern, and oviposition (Werle et al. 2017).

Research Objectives

For the first objective, studies on ambrosia beetles have been done across the country, however, phenology data in Georgia has not been updated since the early 1990s. The past study looked into one pest species, the granulate ambrosia beetle, and only in nurseries. A current study would be beneficial to see which pest species are present now across several commodities to update beetle activity across the state. For the second objective (a), surveys showed that growers will use store bought ethanol as the attractant for their ambrosia beeltle traps for monitoring. We wanted to test if there is a difference on attractancy of store bought and commercially available ethanol and if the placement of the ethanol in or on the trap was different. For objective 2b, this study was chosen to examine the effects of lure age to see if the attractancy of the lure extends beyond the recommended 4 weeks.

1. Objective 1 of our study was to determine the seasonal incidence and abundance of ambrosia beetles to predict peak flight activity in different regions and commodities throughout Georgia. Since ambrosia beetle activity can differ from region to region this objective is important for refining management practices throughout different regions in Georgia by allowing growers to time their pesticide sprays more accurately.
2. Objective 2(a) of our next study was to improve ambrosia beetle monitoring traps by investigating attractiveness of different types of ethanol lures (commercially available ethanol lure and store bought ethanol) with bolt traps. This objective can impact monitoring of ambrosia beetles by giving more grower friendly and less costly options for monitoring.
3. Objective 2 (b) was also to improve monitoring of ambrosia beetles by observing the effects of commercially available aged ethanol lures on beetle captures. This can help refine monitoring strategies and reduce the cost of monitoring.

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CHAPTER 2

PHENOLOGY OF AMBROSIA BEETLES (COLEOPTERA: CURCULIONIDAE) IN ORNAMENTAL NURSERIES, TREE FRUIT, AND PECAN ORCHARDS THROUGHOUT GEORGIA

A. Monterrosa, S. V. Joseph, B. R. Blaauw, and A. L. Acebes,

To be submitted to the Journal of Environmental Entomology

Abstract

Ambrosia beetles (*Xylosandrus* spp.) are problematic in ornamental nurseries and are emerging as serious pests in orchard crops. An updated survey of ambrosia beetle species and their corresponding phenology was needed in Georgia to aid in refining management practices for these beetles. In 2019 and 2020, ambrosia beetles were monitored across nine sites at ornamental nurseries, tree fruit, and pecan orchards in Georgia. At each site, six ethanol-baited bottle traps were deployed with three traps along the edge of a wood-line and three traps placed 30-m from the edge of the nurseries and orchards. Traps were deployed from mid-January through September and checked weekly. Captured beetles were counted and identified. The most abundant pest species, *X. crassiusculus, X. germanus* and emerging pest species, *X. compactus*, were analyzed further to investigate seasonal flight activity. At most sites, flight activity began in February when average temperatures reached >7.2 °C and continued until the termination of the study in late August and early September. Across most sites, increased flight activities were observed in March, April, and May, corresponding to temperatures reaching >15.5 °C. This survey was the first to study the relative abundance and phenology of ambrosia beetles in three different agricultural systems in the southern US. The study found that flight activity can occur in temperatures lower than ~20 °C, even in temperatures as low as ~7.2 °C.

Key Words: *Xylosandrus crassiusculus, Xylosandrus germanus,* ornamentals, tree fruit, pecans

Ambrosia beetles are wood boring beetles that attack primarily young, stressed trees. Several species have been introduced into the United States through ports of entry (Rabaglia 2006). Some have been problematic in woody ornamental nurseries (Addesso et al. 2019, Ranger et al. 2016) and recently have become pests of fruit and nut orchards (Ree and Knutson 1997, Agnello et al. 2015, Reed et al. 2015). Several species of ambrosia beetles attack pecans (*Carya* spp.), chestnut (*Castanea mollissima* Blume), black walnut (*Juglans nigra* L.), apples (*Malus* spp.), peaches(*Prunus* spp.), and a wide host range of ornamentals (Mannion and Oliver 2001, Williams and Ginzel 2019). Infestations can cause severe losses of trees in nurseries (Ranger et al. 2016). Not all attacks lead to the death of the trees, but they can negatively impact growth and aesthetics as well as the economic value (Oliver and Mannion 2001, Ranger et al. 2016). The trees that are infested with ambrosia beetles may have toothpicks (compacted saw dust) emerging from entry holes on the trunks and branches. In some cases, the infestations lead to sap production and staining, tissue discoloration, wilting foliage, and branch dieback (Ranger et al. 2016). In addition, the ambrosia beetles introduce a symbiotic fungus that is cultivated by the beetles as a food source that can also contribute to damage and injury (Contarini et al. 2020).

In Georgia, woody ornamental commodities and orchards are vulnerable to the potential economic impacts of ambrosia beetles. Field nurseries are valued at $125 million USD where ambrosia beetles are considered a major problem (Wolfe and Stubbs 2019). Peaches and apples contribute to $46 million USD (Wolfe and Stubbs 2019). Although ambrosia beetles are currently not considered a major pest in peaches and apples, tree fruit orchards have been seeing increased attacks of ambrosia beetles and they may be associated with rapid apple decline phenomenon in North Carolina and other mid-Atlantic states (Agnello et al. 2015; Ranger et al. 2020). Pecan production is valued at $401 million USD and with the increasing hectarage of newly planted trees in recent years (Wells 2014), ambrosia beetles have re-emerged as an economic pest (Ree and Knutson 1997, Acebes-Doria 2019).

Two of the major exotic pest species found in Georgia are the granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) and the black stem borer, *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae) (Ranger et al. 2016, Addesso et al. 2019). Both species go through obligatory diapause and adult females overwinter in the galleries they bore inside the trees (Ranger et al. 2016). Previously, several studies investigated phenology of *X. crassiusculus* (Mizell et al. 1994) and *X. germanus* (Reding et al. 2013) throughout the United States. In Taiwan, *X. crassiusculus* undergoes four generations in a year (Wu et al. 1978). In Europe, *X. germanus* has shown to have one or two generations in a year (Galko et al. 2016). In the United States, studies have shown that *X. germanus* has two generations per year in Tennessee, Ohio, Illinois, North Carolina, and Virginia, and in favorable conditions in New Jersey, they can even have three generations (Hoffman 1941, Ghandi et al. 2010, Mannion and Oliver 2001, Reding et al. 2010, Ranger et al. 2016). In Tennessee, Ohio, and Virginia, first emergence of *X. crassiusculus* and *X. germanus* occurs in late March to mid-April (Reding et al. 2010). However, incidence of peak flight can vary depending on the species, location and year (Reding et al. 2010). More recently, high densities of the black twig borer, *Xylosandrus compactus* (Eichhoff), have been found in landscape ornamentals, nursey plants, and orchards such as coffee and macadamia crops (Chong et al. 2009, Greco and Wright 2015, Ranger et al. 2016), although the pest status has yet to be determined.

Knowing the species composition and phenology of ambrosia beetle species in an area is important information to develop targeted management strategies for ornamental nurseries, as well as nut and tree fruit orchards. Currently, the management of *Xylosandrus* spp. involves repeated applications of pyrethroid insecticides, such as bifenthrin and permethrin (Ranger et al. 2016, Reding and Ranger 2018). These insecticides reduce the attacks on tree trucks by repelling the invading females, especially during peak adult flight periods (VanDerLaan and Ginzel 2013, Ranger et al. 2016). Thus, a thorough understanding of ambrosia beetle phenology and flight activities through monitoring is critical for precise timing of management decisions (Reding and Ranger 2018).

In 1994, a survey for *X. crassiusculus* was conducted in nurseries in Florida and Georgia (Mizell et al. 1994). This study was only focused in ornamental nurseries and not in tree fruit and nut orchards. Moreover, the major focus was restricted to phenology of *X. crassiusculus* and other ambrosia beetles were not surveyed. There is no recent research on species complex and phenology of ambrosia beetles in various agricultural systems in Georgia.

Therefore, our major objective is to conduct a survey and monitor the phenology of the most common ambrosia beetle species in Georgia across three different production systems.

Materials and Methods

Study sites. In 2019 and 2020, three sites were selected in ornamental nursery, nut and tree fruit orchard for this study. However, in 2020, only one tree fruit site was monitored (Table 1). All sites except for the Univ. of Georgia Horticultural research farm were managed following commercial standard insecticide management guidelines. The ornamental nursery sites were located in central Georgia. The major ornamental tree species grown in the nursery sites were *Magnolia* spp., *Prunus* spp., *Cercis* spp., *Illex* spp., *Ginkgo* spp., *Lagerstroemia* spp., *Stewartia* spp., *Camellia* spp., *Acer* spp. and *Cupressus* spp. The planted nursery trees were spaced ~1.5 m apart between rows and trees at sites 1 and 2, and variable spacing was used in the container field nursery at site 3. The age of the majority of the growing trees ranged from 0 to 4 years. The apple orchards and peach orchard were located in North Georgia. The apple orchards only contained *Malus* spp. within the area that the traps were placed. The planted trees were spaced ~1.8 m apart (Blue Ridge) and spaced ~5.5 m apart (Ellijay). The peach sites only contained *Prunus* spp. within the area that the traps were set. The planted trees were spaced ~5.5 m apart and are 7 years old (Watkinsville). The pecan orchard sites only contained *Carya* spp. The planted trees were spaced ~12 m apart and the ages of the planted trees ranged from 4 to 7 years. At each site, six traps were deployed, three along the edge of the wood-line (~3 m from wood-line) and three traps were deployed about 30 m from the woodline in the interior of the ornamental nursery or orchard. Each trap was at least 20 m from any other.

Traps and monitoring. Previously, ethanol-baited traps were used to monitor ambrosia beetle populations (Ranger et al. 2010, Galko et al. 2019). At the ornamental sites, the bottle trap consisted of a clear rectangular-shaped 1774 mL plastic bottle (VTM LLC, Lexington, KY; Fig. 1A). The tree fruit and pecan sites used a clear square-shaped 1774 mL plastic bottle (Berlin Packaging, Chicago, IL; Fig. 1B, 1C). The plastic bottle traps were baited with one commercially available ethanol dispenser (AgBio Inc., Westminster, CO) and were used in all sites in the current study. The ethanol dispenser had 7-8 mL 95% ethanol and a 65 mg/day release rate at 30°C. The plastic bottles (Fig. 1) were modified by cutting the sides (~5 cm × ~9 cm) to create two (ornamental and pecan) or four (tree fruit) access windows across from each other. Two small holes (6 mm) were drilled on the bottom of bottle where one zip tie (152.4 mm × 4.76 mm) was inserted into the bottle to suspend the ethanol lure and a second zip tie (152.4 mm × 4.76 mm) was added around the wooden stake to attach the zip tie suspending the lure and bottle to the stake. The bottle was hung upside down and placed ~0.5m above the ground. The ethanol lure was hung inside the bottle. The incoming ambrosia beetles attracted to ethanol lure were drowned and trapped in the soapy water added to the bottle. The soap solution was prepared by adding 0.5 mL of dish washer soap (Dawn, P&G, Kansas City, KS) in 300 mL tap water. The soap solution and ethanol dispensers were changed at weekly and monthly intervals, respectively. One temperature logger (HOBO, Bourne, MA) was placed at each site to record the seasonal temperature fluctuations. However, there were malfunctions across several sites where data was not recorded, so some temperature information was obtained through the closest weather stations to each site (Ornamentals: Williamson; Fruits: Blue Ridge, Ellijay,Watkinsville-Hort.; Pecans: Dixie, Alapaha, Tifton-Bowen).

The trapping period was from early spring to late summer in both years (Table 1). The traps were emptied at weekly intervals by removing the bottle caps over a coffee filter and sieve and filtering the soapy water through (~300 mL water to ~0.5 mL of soap). The collected beetles were sorted and preserved in 70% ethanol vials for identification. During the winter months, propylene glycol was added to prevent water from freezing. The beetles were identified to the genus or species using a lucid key for Southeast Asian Xyleborini species (Smith et al. 2019) and Bateman and Hulcr's ambrosia beetle guide (Bateman and Hulcr 2017).

Data analysis. The total relative abundance of each species captured at each site was calculated for 2019 and 2020. Although there were higher numbers of other non-damaging pest species (*Hypothenemus*) present in some sites we omitted them from statistical analyses and focused on the main damaging pests. Captures of the two most abundant pest species, usually *X. crassiusculus,* and *X. germanus,* and a few sites with *X. compactus,* were subjected to further analyses. Average weekly captures of the two species at each site were examined and approximated following assumptions of parametric test, and data that did not satisfy normality assumptions were transformed using log and exponential transformations, accordingly. Data were analyzed using a two-way analysis of variance with trap location and sampling date as fixed effects. Subsequently, a Tukey-Kramer HSD test was used for post-hoc multiple mean comparisons. All analyses were conducted using JMP ver 15 (SAS Institute Inc., Cary, NC, 2007) at $\alpha = 0.05$.

Results

Relative Beetle Abundance per site

Ornamentals. Across all sites, the most abundant species in 2019 were *X. crassiusculus* and *X. germanus* constituting 82-94% and 3-10% of the overall captures, respectively (Fig. 2A). The other beetle species constituted 3-9% which included *Hypothenemus* spp., *Xyleborus* spp., and *Xyleborinus* spp. Although the total captures in 2020 was lesser than 2019 at all sites, the relative species abundance was similar with *X. crassiusculus* and *X. germanus* being the most abundant at 40- 46% and 9-21%, respectively (Fig. 2A). The other beetles contributed to 39-46% and consisted of *Hypothenemus* spp., *Ambrosiodmus rubricollis, Xylosandrus compactus, Xyleborus* spp.*, Xyleborinus* spp.*, Cyclorhipidion* sp., *Euwallacea* sp., and *Anisandrus* spp.

Tree fruit. The most abundant species in 2019 across all sites were *X. crassiusculus* and *X. germanus* constituting 27-96% and 2-67% of the overall captures, respectively. The other beetles constituted 1-5% and were *Hypothenemus* spp., *Ambrosiodmus rubricollis*. *Xylosandrus compactus*, *Xyleborus* spp.*, Xyleborinus* spp.*, Cyclorhipidion* sp., *Euwallacea* sp., and *Anisandrus* spp.

In 2020, at the Blue Ridge site, the most abundant species were *X. germanus* and *X. crassiusculus* contributing 47% and 42% of overall captures, respectively. The other beetles contributed 1-4% of the overall beetle captures (Fig 2B). The beetles accounting for 1-4% of overall captures were *Hypothenemus* spp., *Xyleborus* spp., *Xyleborinus* spp., *Euwallacea* sp., *Anisandrus* spp., and *Cnestus mutilatus.*

Pecans. In 2019, the Quitman site had *X. compactus* and *X. crassiusculus* being the most abundant species making up 45% and 28%, respectively. The Nashville site had *X. crassiusculus* and *X. compactus* as the most abundant pest species present constituting 21% and 7% of captures, respectively. The Irwin site had *X. crassisuculus* and *X. germanus,* as the most abundant species present making up 47% and 14% of captures, respectively. Across all sites, other beetles contributed to 1-41% of captures. At some sites, high numbers of *Hypothenemus* spp., were recorded. The other beetle species were *X. germanus*, *Xyleborus* spp, *Xyleborinus* spp., and *A. rubricollis*.

In 2020, the Quitman site's most abundant emerging pest and pest species were *X. compactus* and *X. crassiusculus*, making up 65% and 10%, respectively. The Nashville site's most abundant species (pest or emerging) were also *X. crassiusculus* and *X. compactus* constituting 7% and 6% of captures, respectively. The most abundant pest species at the Irwin site were *X. crassisuculus* and *X. germanus* making up 15% and 6% of captures, respectively. Across all sites, the other beetles present contributed to 2-39% of overall captures. Some sites had a high numbers of nonpest species such as *Hypothenemus* spp. and *Xyleborinus* spp. (Fig. 2C). The other beetles with lower numbers consisted of *X. germanus, Xyleborus* spp, *Xylosandrus amputatus,* and *A. rubricollis,* depending on the site.

Spatial and seasonal trends of *X. crassiusculus, X. germanus* and *X. compactus* trap captures across three cropping systems

Ornamental nurseries

Trap Location Effect. In 2019, the ornamental sites 1 and 3 showed higher overall mean captures of *X. crassiusculus* (Site 1: $F_{1, 190}$ = 11.83, $P = 0.0007$; Site 3: $F_{1, 184}$ = 6.83, $P = 0.0097$) and *X. germanus* in sites 1 and 2 (Site 1: $F_{1, 190}$ = 6.77, $P = 0.0100$; Site 2: $F_{1, 189}$ = 10.51, $P = 0.0014$) in the traps along the edge of the woodline relative to the interior traps, while no difference between trap locations were observed at ornamental site 2 for *X. crassiusculus* ($F_{1, 189}$ = 3.69, $P = 0.0561$) and site 3 for *X. germanus* ($F_{1, 184}$ = 3.40, $P = 0.0667$). Results for 2020 were similar to the 2019 data with higher overall mean captures of *X. crassiusculus* in site 1 and 3 (Site 1: $F_{1. 165}$ = 5.52, $P = 0.0199$; Site 3: $F_{1, 166}$ = 13.19, $P = 0.0004$) and *X. germanus* in sites 1 and 2 (Site 1: $F_{1, 165}$ = 4.03, $P = 0.0464$; Site 2: $F_{1, 166}$ = 11.87, $P = 0.0007$) in the traps along the edge of the woodline relative to the interior traps. Trap location did not show any significant effects on the captures of *X. crassiusculus* at site 2 (Site 2: $F_{1, 166}$ = 0.64, $P = 0.4265$), and of *X. germanus* at site 3 (Site 3: $F_{1, 166}$ = 3.88, $P = 0.0506$).

Seasonal Effects. In 2019, a significant interaction was found in *X. crassiusculus* captures between sampling date and trap location at sites 1 and 2 (Site1: $F_{31, 1}$ = 11.35, $P < 0.0001$; Site 2: $F_{31, 1}$ = 3.21, $P < 0.0001$); indicating both factors influenced the seasonal trends. Captures of *X. crassiusculus* were similar throughout the sampling period at all sites (Site 1: $F_{63, 128}$ = 25.16, $P < 0.0001$; Site 2: $F_{63, 127}$ = 9.92, $P < 0.0001$; Site 3: $F_{61, 124}$= 7.45, $P < 0.0001$; Figure 3). The first flight activity was observed on the week of 13 February at all sites, where the temperature was ~15.5°C . At site 1, the peak flight activity was observed in mid-March, mid-April, and early

May (Fig. 3A). Site 2 had peak flight activity observed in late March, mid-April, and early May (Fig. 3B). Peak flight activity observed in site 3 was seen in late March, mid-April, and mid-May (Fig. 3C). The peak flight activity in all sites was observed when the temperature was ~15.5°C or higher (Fig. 3).

In 2020, a significant interaction was found in *X. crassiusculus* between sampling date and trap location at sites 1 and 2 (Site 1: $F_{27} = 5.54$, $P < 0.0001$; Site 2: $F_{27} = 1.76$, $P = 0.0217$); indicating both factors influenced the flight activity. The captures of *X. crassiusculus* were significantly different throughout the sampling period at all sites (Site 1: $F_{55, 111} = 11.58$, $P < 0.0001$; Site 2: $F_{55, 112} = 10.18$, $P < 0.0001$; Site 3: $F_{55, 112} = 2.10$, $P = 0.0005$). The first flight activity of the season was seen on the weeks of 27 February, 6 February, and 18 March for sites 1, 2, and 3, respectively. During these differing weeks of activity, the first flight occurred when the average temperature reached ~15.5°C. The peak flight activity in site 1 was detected in mid-March and early May (Fig. 3A). Peak flight activity in site 2 was observed in mid-February, mid-March, and early May (Fig. 3B). Site 3 had peak flight activities in mid-March, early May, and late August (Fig. 3C). The peak activity coincided with temperatures reaching ~15.5°C or higher.

In 2019, there was a significant interaction for *X. germanus* captures between sampling date and trap location at site 1 and 2 (Site 1: $F_{31, 1} = 1.81$, $P = 0.0116$; Site 2: $F_{31, 1} = 2.85$, $P < 0.0001$) indicating both factors influenced the season-long flight activity. Captures of *X. germanus* showed higher overall mean captures throughout the sampling period at sites 1 and 2 (Site 1: $F_{63, 128} = 3.57$, $P < 0.0001$; Site 2: $F_{63, 127} = 3.97$, $P < 0.0001$). Similar to the first flight captures of *X. crassiusculus,* the first flight captures of the season occurred when temperatures reached ~15.5°C. The captures occurred on the weeks of 13 March for site 1 and 3, and 6 March for site 2. Site 1 had peak flight activity in mid-March, mid-April, and late May (Fig. 3A). For site 2, the peak flight activity was detected in late March, mid to late April, and late May (Fig. 3B). Site 3 peak flight activity occurred in mid-May and early June (Fig. 3C). Peak activity occurred with temperature reaching at least ~15.5°C.

In 2020, captures of *X. germanus* at site 2 were significantly influenced by sampling date and trap location (Site 2: $F_{27, 1} = 7.07$, $P < 0.0001$). The captures of *X. germanus* were significantly different throughout the sampling period at all sites (Site1: $F_{55, 111} = 2.96$, $P < 0.0001$; Site 2: $F_{55, 112} = 8.33$, $P < 0.0001$; Site 3: $F_{55, 112} = 1.48$, $P = 0.0401$). The first flight activity occurred on the weeks of 18 March for sites 1 and 3 and 11 March for site 2. Similar to 2019, the first flight activity occurred when the temperature reached ~15.5°C. The peak flight activity for site 1 was observed in early April, early May, and early June (Fig. 3A). The beetle's flight activity occurred in early April and early June at site 2 (Fig. 3B). Peak flight activity in site 3 happened in early April, early May, late May to early June and early July (Fig. 3C). Throughout the flight season, peak activity was observed when temperatures were ~15.5°C or higher.

Tree fruit orchards

Trap Location Effects. In 2019, the two apple sites showed higher overall mean captures of *X. crassiusculus* (Blue Ridge: $F_{1, 54} = 6.34$, $P = 0.0148$, Ellijay: $\mathrm{F}_{1, 57} = 4.41$, $P = 0.0401$) and *X. germanus* (Blue Ridge: $F_{1, 54} = 7.69$, $P = 0.0076$; Ellijay: $F_{1, 57} = 5.68$, $P = 0.0205$) in the exterior traps relative to the interior traps; while no difference between trap locations were observed in Athens (peach) site (*X. crassiusculus*: $F_{1, 45} = 1.04$, $P = 0.3114$; *X. germanus*: $F_{1, 45} = 1.84$, $P = 0.1882$). Similar results were observed in the 2020 tree fruit site with overall mean captures of *X.*

crassiusculus (Blue Ridge: $F_{1. 153} = 36.46$, $P < 0.0001$) and *X. germanus* (Blue Ridge: $F_{1, 153} = 28.06$, $P < 0.0001$) being higher by the exterior of the orchard than in the interior.

Seasonal Effects. In 2019, for *X. crassiusculus*, a significant interaction was found between sampling date and trap location at the Blue Ridge site ($F_{13, 1} = 5.43$, $P < 0.0001$) indicating both factors influenced flight activity. Captures of *X. crassiusculus* were different throughout the sampling period at all sites (Blue Ridge: $F_{27, 28} = 6.20$, $P < 0.0001$ Ellijay: $F_{29, 29} = 2.14$, $P = 0.0226$ Athens: $F_{23, 23}= 0.96$, $P = 0.5411$). First flight activity of *X. crassiusculus* at Blue Ridge was observed on the week of 2 April, in Ellijay on the week of 21 February, and in Athens on the week of 5 April. The first flight activity coincided with temperatures below ~15.5°C. The beetle's peak flight activity, in the Blue Ridge site occurred in early April, with another peak in mid-April (Fig. 4A). In Ellijay, the peak flight activities were observed mid-February, mid-April, mid-June and late August (Fig. 4B). For the Athens site, peak flight was observed in early April and mid-May (Fig. 4C). The highest peak activity was seen when temperatures reached ~15.5°C or higher.

The 2020 captures of *X. crassiusculus* were similar to the 2019 captures with a significant interaction between trap location and sampling period at Blue Ridge ($F_{25, 1} = 4.48$, $P < 0.0001$). The first flight activity in Blue Ridge in 2020 occurred on the week of 19 February. The first flight occurred with the average temperature being below ~15.5°C. The peak flight activity in Blue Ridge was seen in early April, late April into early May, and mid-August (Fig. 4A). The peak activity occurred when temperatures reached ~15.5°C or higher.

The captures of *X. germanus* in 2019 showed significant interaction between sampling date and trap location at the Blue Ridge and Ellijay site (Blue Ridge: $F_{13, 1} = 10.75$, $P < 0.0001$ Ellijay: $F_{14 ,1} = 5.17$, $P < 0.0001$). Captures of *X. germanus* differed throughout the sampling period at each site (Blue Ridge: $F_{27, 28} = 14.47$, $P = <0.0001$ Ellijay: $F_{29, 29} = 5.55$, $P < 0.0001$ Athens: $F_{23, 23} = 0.94$, $P = 0.94$). The first flight activity of *X. germanus* occurred on the weeks of 2 April, 19 March, and 5 April of Blue Ridge, Ellijay, and Athens, respectively. First flight activity was observed to occur with average temperature being higher than ~7.2°C, but below ~15.5°C. Peak flight activity in Blue Ridge was observed in early April, and another peak in early May (Fig. 4A). In Ellijay, peak activity was seen late March, mid-May, and late August (Fig. 4B). In Athens, peak activity was observed in early April (Fig. 4C). The highest peak of activity in the apple sites (Blue Ridge and Ellijay) occurred when temperatures were ~15.5°C or higher, but below ~15.5°C in the peach site (Athens).

Similar to 2019 results, the 2020 captures of *X. germanus* in Blue Ridge, showed a significant interaction between sampling date and treatment ($F_{25, 1} = 6.17$, $P < 0.0001$), indicating that both factors influenced seasonal flight activity. The first flight activity was observed on the week of 18 March with peak captures in early April, early to late May, and later in mid-August (Fig. 4A). The first flight activity coincided with temperatures below ~15.5°C, but above ~7.2°C, and similar to the *X. crassiusculus* captures regarding temperature in 2020, the highest peak activity was seen when temperatures reached ~15.5°C or higher.

Pecans.

Trap Location Effects. In 2019, significantly more *X. crassiusculus* were captured in the orchard exterior than in the interior at the Quitman and Nashville sites (Quitman: $F_{1, 195} = 31.43$, $P < 0.0001$; Nashville: $F_{1, 196} = 7.28$, $P = 0.0076$). Captures of *X. compactus* in exterior traps were significantly greater than in interior traps at Quitman ($F_{1, 195} = 27.16$, $P = <0.0001$) while no difference between trap locations was observed at Nashville for *X. compactus* ($F_{1, 196} = 0.32$, $P = 0.57$) and Irwin for *X. crassiusculus* ($F_{1, 190} = 0.02$, $P = 0.90$) or *X. germanus* ($F_{1, 190} = 0.06$, $P =$

0.8045). In 2020, *X. crassiusculus* ($F_{1. 202}$ = 20.19, $P < 0.0001$) and *X. compactus* ($F_{1, 202}$ = 38.80, $P < 0.0001$) were captured more at the orchard exterior than in the interior at Quitman. Significantly more *X. compactus* were captured along the edge of the orchard than inside the orchard at Irwin ($F_{1, 208}$ = 5.32, $P = 0.0221$). Trap location did not affect the captures of *X. crassiusculus* at Nashville ($F_{1, 202}$ = 1.04, $P = 0.3088$) or Irwin ($F_{1, 190}$ = 0.0161, $P = 0.8991$), and captures of *X. compactus* at Nashville ($F_{1, 202}$ = 0.00, $P = 1.0000$).

Seasonal Effects. In 2019, a significant interaction on captures of *X. crassiusculus* was found between sampling date and trap location at Quitman ($F_{32, 1}$ = 1.80, $P = 0.0116$) and Irwin ($F_{31, 1}$ = 1.86, $P = 0.0086$) indicating that both factors influenced the seasonal trends. At all sites, captures of *X. crassiusculus* were similar across the sampling period (Quitman: $F_{32, 1}$ = 4.18, $P < 0.0001$; Nashville: $F_{32, 1}$ = 2.90, $P < 0.0001$; Irwin: $F_{31, 1}$ = 3.50, $P < 0.0001$). The first flight activity was observed on the weeks of 25 January, 22 February and 20 February, at Quitman, Nashville, and Irwin, respectively with some of the flights occurring at temperatures below ~15.5°C and others occurring at or above 15.5°C. At Quitman, peak flight activity was observed late in February, in mid-April, and again by mid-June (Fig. 5A). In Nashville, flight activity also peaked late in February, increased again in late-May, and in early August (Fig. 5B). In Irwin, the peak of beetle activity was highest in early April and May (Fig. 5C). The peaks in activity across the sites coincided with temperatures at or above ~15.5°C.

The 2020 captures of *X. crassiusculus* showed that a significant interaction was found between sampling date and trap location at Quitman ($F_{33, 1}$ = 2.85, $P < 0.0001$) and Nashville ($F_{33, 1}$ = 4.27, $P < 0.0001$) indicating both factors influenced the seasonal trends. All sites differed in captures of *X. crassiusculus* across the sampling period (Quitman: $F_{33, 1}$ = 9.94, $P < 0.0001$; Nashville: $F_{33, 1}$ = 6.30, $P < 0.0001$; Irwin: $F_{34, 1}$ = 2.50, $P < 0.0001$). The onset of flight activity was observed during the weeks of 24 January at Quitman, 23 January at Nashville, and 14 February at Irwin with some first activity occurring in temperatures above ~7.2°C but below ~15.5°C. At Quitman, peak flight activity was first seen in mid-February and mid-March, with another slight peak in late May and mid-August (Fig. 5A). At Nashville, peak flight activity of *X. crassiusculus* was observed in late January and by mid-March, with a final peak in mid-May (Fig. 5B). Increased flight activity of *X. crassiusculus* at Irwin was recorded in mid- February, mid-March and again early to mid-July (Fig. 5C). Peak flight activity occurred in temperatures that were at or above ~15.5°C.

The 2019 captures of *X. compactus* and *X. germanus*, respectively showed that there was a significant interaction between sampling date and trap location at Quitman ($F_{32, 1} = 4.13$, $P < 0.0001$) and Irwin ($F_{31, 1} = 2.79$, $P < 0.0001$) indicating both factors influenced seasonal captures. All sites had differing captures of *X. compactus* and *X. germanus* across the sampling period (Quitman: $F_{31, 1} = 5.73$, $P < 0.0001$, *X. compactus*; Nashville: $F_{33, 1} = 1.57$, $P = 0.0388$l, *X. compactus*; Irwin: $F_{31, 1} = 2.62$, $P < 0.0001$, *X. germanus*). The first flight activity was observed in Quitman and Nashville on the week of 22 February (*X. compactus*), and Irwin on the week of 20 February (*X. germanus*). For both *X. compactus* and *X. germanus*, the first flight activity occurred with average temperatures being ~15.5°C. At Quitman, peak flight activity of *X. compactus* occurred in February, early to mid-May, and late in August (Fig. 5A). Nashville had peak flight activity of *X. compactus* in mid-March and once again in early August (Fig. 5B). Irwin showed highest captures of *X. germanus* in mid-February and mid-March (Fig. 5C). The peak flight activity at all sites occurred at temperatures at or above ~15.5°C.

The 2020 results were similar to 2019 in relation to a significant interaction between sampling date and trap location of *X. compactus* only at Quitman ($F_{33, 1} = 5.29$, $P < 0.0001$)

indicating that both factors influenced beetle flight activity. All sites differed in captures of *X. compactus* across the sampling period (Quitman: $F_{33, 1} = 20.44$, $P < 0.0001$; Nashville: $F_{33, 1} = 1.11$, $P = 0.3335$; Irwin: $F_{34, 1} = 2.99$, $P < 0.0001$). In Quitman, the beetles first became active in mid-February with peak flights observed in mid-March and finally, in mid- to late August (Fig. 5A). At Nashville, the first flight activity was recorded on the week of 19 March with highest flight activity occurring from mid-March to early April and in early August (Fig. 5B). At Irwin, beetles were first detected on the week of 11 March with captures peaking in late March, mid-June and mid-August (Fig. 5C). Similar to the 2019 results, the first flight activity and peak flight activity occurred at or above ~15.5°C.

Discussion

To date, this is the first comprehensive survey to investigate relative abundance and spatial and seasonal trends of ambrosia beetles in three different agricultural systems: woody ornamentals, tree fruits, and tree nuts in the southern United States. It is known that beetle distribution and abundance differs depending on region due to climate and weather (Oliver and Mannion 2001, Reding et al. 2011). The pest species, *X*. *crassiusculus*, *X*, *germanus*, and emerging pest species, *X*. *compactus* are all non-native species that were introduced into the United States from subtropical regions (Ranger et al. 2016). The climate in the regions where the beetles are established has an effect on the reproductive habits of ambrosia beetles as well as their seasonal activity (Ranger et al. 2016). In some states, such as Tennessee and North Carolina, some species have two generations per year whereas in Taiwan they are thought to have up to four generations (Weber and McPherson 1983, Ranger et al. 2016). Ambrosia beetle phenology in Georgia has not been well studied in recent decades. Thus, to gain knowledge of ambrosia beetle phenology in Georgia, this study spanned across three agricultural systems to determine the differences in abundance and seasonal trends throughout different regions in the state. This is crucial when it comes to monitoring and management efforts of these invasive beetles in the affected production systems.

All of the species found had previously been recorded throughout Georgia. The main pest species found in all the ornamental and tree fruit orchard sites were *X. germanus* and *X. crassiusculus*. At the pecan sites in southern part of the state, the main pest species present were *X. crassiusculus* and *X. compactus* in the Quitman and Nashville sites for 2019 and 2020. In Irwin, *X. crassiusculus* and *X. compactus* were the most abundant in 2019 and *X. crassiusculus* and *X. germanus* were abundant in 2020. Many species found within the state have been present within North America for years (Rabaglia 2005, Gandhi et al. 2010), such as *Hypothenemus* spp. and *Xyleborus* spp., however these particular genera are not considered economically important in North America. The species composition remained mostly the same throughout both years with one of the differences being *X. compactus* appearing in 2020 in site 2 of the ornamental nursery where it was not found the previous year. Similarly, in the tree fruit system *C. mutilatus* was recorded in 2019, but not in 2020. Similar to the findings in the tree fruit, the tree nut (pecan) sites also had *C. mutilatus* present in low numbers in 2020 where none had been found in 2019. Increased finding of *X. compactus* and *C. mutilatus* could potentially impact nurseries and orchards in the next few years (Olatinwo et al. 2014). Both of these species are polyphagous, with low host specificity (Olatinwo et al. 2014). *X. compactus* has been observed to be an emerging pest in ornamentals (Ranger et al. 2016). There were no new species recorded that had not previously been found in Georgia.

Ambrosia beetle flight activity has previously been established to occur when the temperature reaches about 20°C (68°F; Reding et al. 2013). This current study showed that the average temperature for flight activity can be as low as 7.2°C, however the flight activity during those lower temperatures occured during first emergence where flight activity ceased until temperatures reached ~15.5°C or higher and peak activity occurred. Some studies have suggested that trap captures, nor attacks have been observed before this temperature (20°C) has been reached, but that trap captures and attack increase the more consecutive days the temperature stays around 20°C (Reding et al. 2013, Addesso et al. 2019). This study shows that trap captures have been observed throughout Georgia in temperatures lower than 20°C. In this study we see that peak activity was seen on days that the temperature was at ~15.5°C or higher. Flight activity was observed throughout the season as temperatures increased and remained at or above 15.5°C (Figs. 2, 3, 4). Similar to the previous phenology data in Georgia (Mizell et al. 1994), first emergence was in February, as a few days reached close to spring temperatures, then activity ceased after cold weather returned, and then again as temperature increased in the spring (March) activity resumed.

Flight activity fluctuated throughout the study period, however ambrosia beetles are known to attack trees in spring time after first emergence (Addesso et al. 2019). In early spring, trees naturally produce ethanol during bud break, along with dealing with other potential stressors such as saturated water conditions, which can cause ethanol levels to spike (Ranger et al. 2010, Ranger et al. 2016). At around the same time that the bud break occurs, the increased spring temperatures cause the ambrosia beetles to emerge after overwintering and begin their search for increased ethanol in the trees (Ranger et al. 2016). Since beetle activity is observed throughout the season and is influenced by the increased temperatures in spring, monitoring for beetle activity can more accurately inform better timing for management purposes in ornamental nurseries and tree crop orchards. Either plastic bottle traps or bolt traps can be used for monitoring purposes. Ethanol-infused bolt traps are grower friendly options for monitoring beetle activity (primarily, *Xylosandrus* spp.) by checking the attacks on the traps. Bolt traps can be made by using logs about 0.3-0.6 m long and drilling a hole down the center to fill with alcohol. Ethanol-baited bottle traps are used for capturing a wider range of ambrosia beetles (Ranger et al. 2020) and are more suitable for researchers aiming to identify captured beetles. Sources of ethanol used in bolt and bottle traps can vary from store-bought denatured alcohol to commercially manufactured ethanol lures. The AgBio lures were proven to be effective at capturing beetles for up to 8 weeks (Monterrosa et al. Accepted 2021).

In summary, this study was the first to observe relative abundance and phenology of ambrosia beetles in three different agricultural systems. This study compiled the captured data separated by region and commodity This study did not find many differences in beetle species relative abundance across the different locations across Georgia. Similar to previous studies, this study found that flight activity is sporadic throughout the season. However, it found that flight activity can occur in temperatures lower than ~20°C. Some initial flights in the winter months were seen to occur in temperatures slightly above ~7.2°C. Continuing assessments throughout different agricultural commodities and regions in Georgia can help growers refine management strategies throughout the state as well as help gain more understanding on population trends of pest species.

Acknowledgements

We thank C. Julian, M H. Hardin, P. Halliday, M. Hayden, L. Pool, J. Bailey, B. Wassel, M. Dowdy, J. Morgan, D. Tucker, and B. Reeves for assistance in the deployment of traps and the regular maintenance of those traps at the grower sites. Additionally, we thank the growers for their help with the research sites. This research was funded through Georgia Specialty Crop Block Grant # AM190100XXXXG033 and Georgia Farm Bureau Grant.

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Table 1. Summary of the trapping locations and trapping period across the three cropping systems.

			Trapping Period	
CROPPING SYSTEM	**Location**	**GPS Coordinates**	**2019**	**2020**
Apple	Mercier	34°53'11.1"N 84°20'34.9"W	7 Feb - 23 Jul	3 Feb - 30 Aug
	R&A	34°39'08.0"N 84°25'24.1"W	8 Feb - 23 Jul	.
Peach	UGA Hort Farm	33°53'12.9"N 83°25'21.7"W	12 Feb - 23 Jul	.
Pecan	Quitman, GA	30°48'10.7"N 83°32'21.6"W	18 Jan - 30 Aug	8 Jan - 30 Aug
	Nashville, GA	31°10'25.8"N 83°14'10.3"W	18 Jan - 30 Aug	8 Jan - 30 Aug
	Irwinville, GA	31°40'05.0"N 83°29'32.3"W	18 Jan - 30 Aug	8 Jan - 30 Aug
Ornamental	Mid-Georgia Site 1	33°02'18.1"N 84°20'03.5"W	16 Jan - 28 Aug	30 Jan - 26 Aug
	Mid-Georgia Site 2	33°04'04.5"N 84°18'03.9"W	16 Jan - 28 Aug	30 Jan - 26 Aug
	Dorsey Farms	33°03'28.1"N 84°14'49.7"W	16 Jan - 28 Aug	30 Jan - 26 Aug

Fig. 1. Plastic bottle trap A) used in ornamental nurseries B) tree fruit orchards and C) pecan orchards.

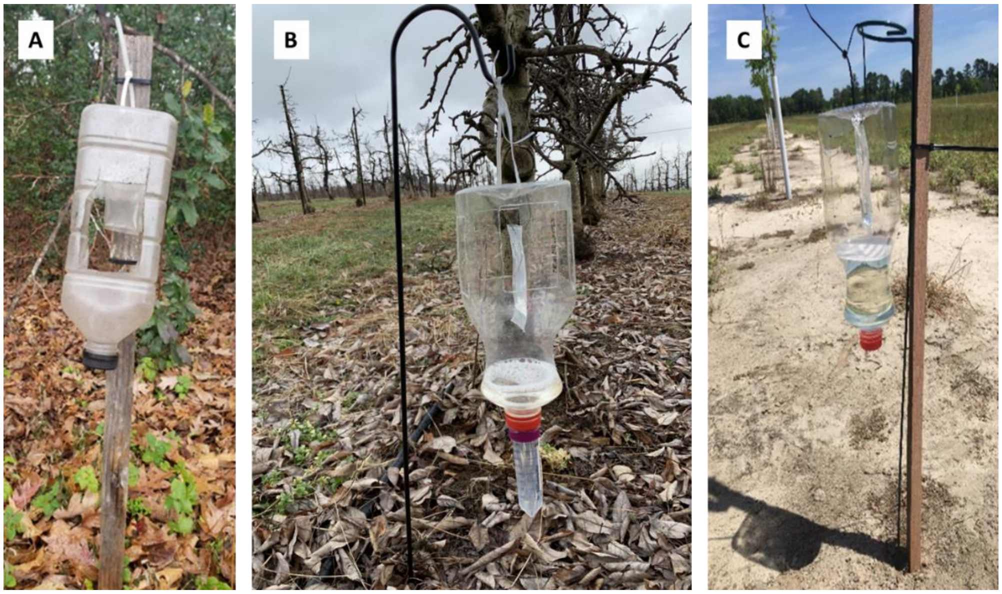

A
B
C

Fig. 2. Relative species abundance of ambrosia beetles captured in ethanol-baited bottle traps at ornamental (A), tree fruit (B) and pecan (C) sites in 2019 and 2020.

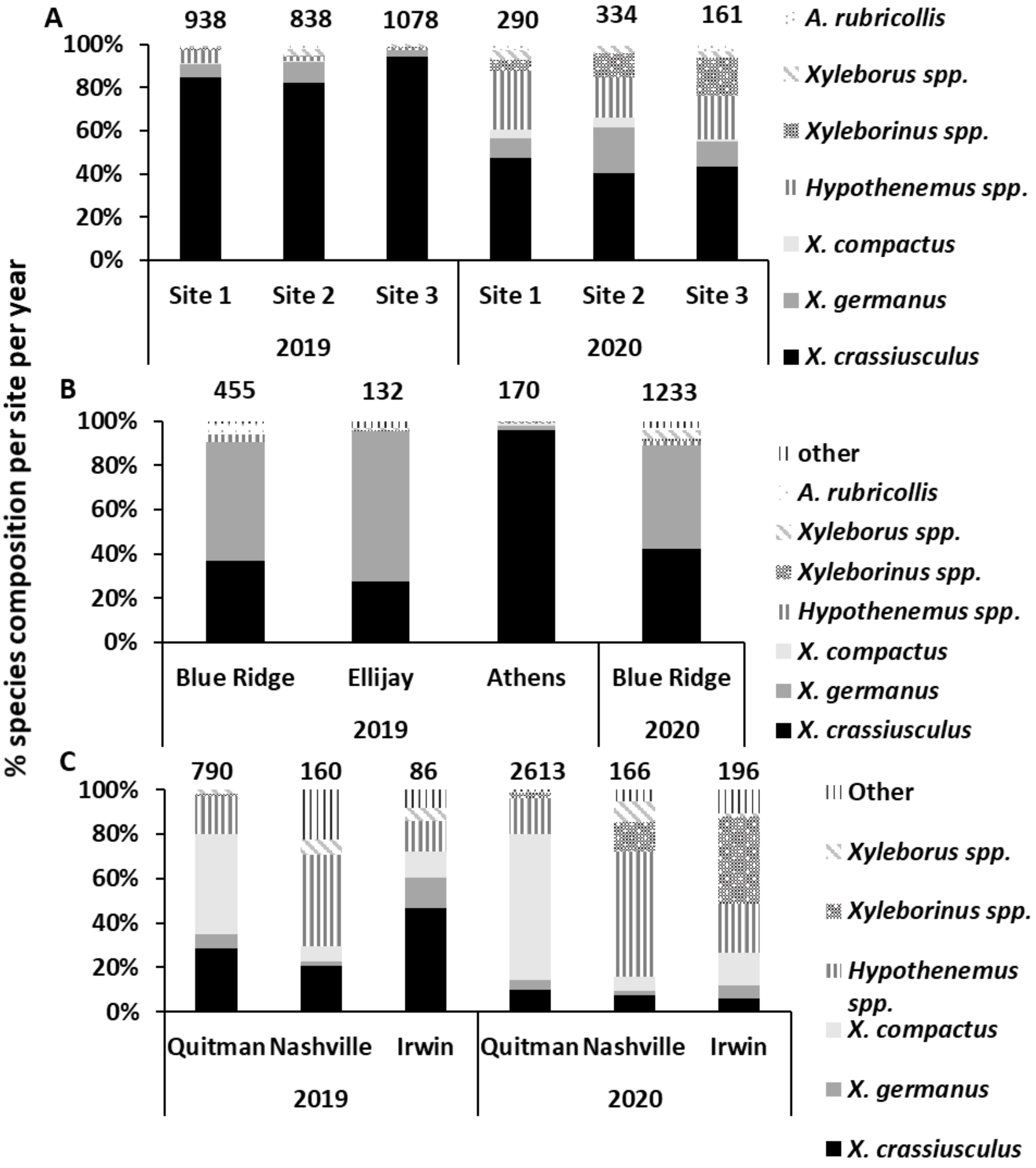
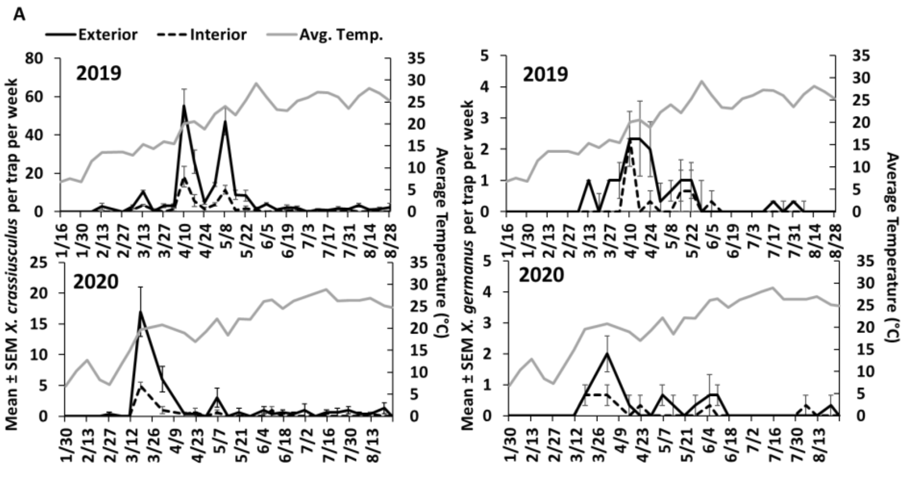
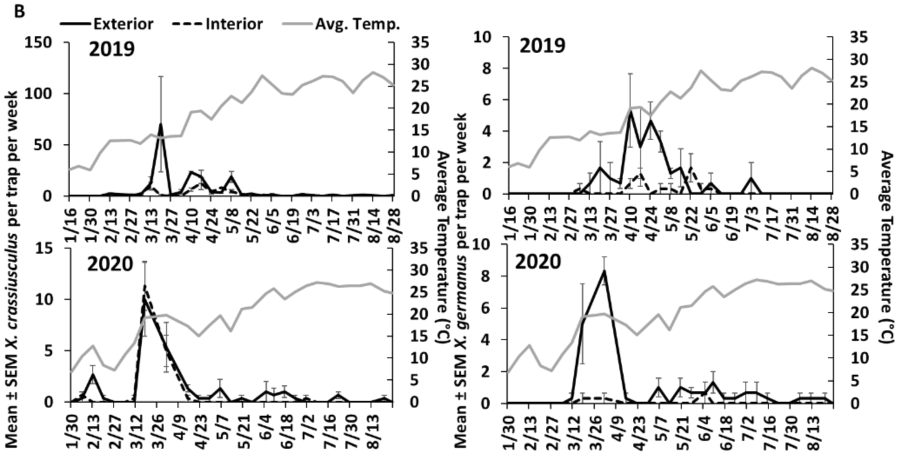

% species composition per site per year
A
938
838
1078
290
334
161
100%
80%
60%
40%
20%
0%
Site 1
Site 2
Site 3
Site 1
Site 2
Site 3
2019
2020
A. rubricollis
Xyleborus spp.
Xyleborinus spp.
Hypothenemus spp.
X. compactus
X. germanus
X. crassiusculus
B
455
132
170
1233
Blue Ridge
Ellijay
Athens
Blue Ridge
2019
2020
other
A. rubricollis
Xyleborus spp.
Xyleborinus spp.
Hypothenemus spp.
X. compactus
X. germanus
X. crassiusculus
C
790
160
86
2613
166
196
Quitman
Nashville
Irwin
Quitman
Nashville
Irwin
2019
2020
Other
Xyleborus spp.
Xyleborinus spp.
Hypothenemus spp.
X. compactus
X. germanus
X. crassiusculus

Fig. 3: Mean ± SEM, and temperature data (°C) of *X. crassiusculus* and *X. germanus* captures per trap per week in 2019 and 2020 at ornamental nursery site 1 (A), site 2 (B), and site 3 (C). Some 2020 sampling dates were missed due to severe weather and COVID (3/5, 3/25, 4/8).


A
Exterior
Interior
Avg. Temp.
2019
2020
Mean ± SEM X. crassiusculus per trap per week
Mean ± SEM X. germanus per trap per week
Average Temperature (°C)
0
20
40
60
80
0
5
10
15
20
25
0
1
2
3
4
5
0
5
10
15
20
25
30
35
1/16
1/30
2/13
2/27
3/13
3/27
4/10
4/24
5/8
5/22
6/5
6/19
7/3
7/17
7/31
8/14
8/28
1/30
2/13
2/27
3/12
3/26
4/9
4/23
5/7
5/21
6/4
6/18
7/2
7/16
7/30
8/13


B
Exterior
Interior
Avg. Temp.
2019
2020
Mean ± SEM X. crassiusculus per trap per week
Mean ± SEM X. germanus per trap per week
Average Temperature (°C)
0
50
100
150
0
5
10
15
0
2
4
6
8
10
0
5
10
15
20
25
30
35
1/16
1/30
2/13
2/27
3/13
3/27
4/10
4/24
5/8
5/22
6/5
6/19
7/3
7/17
7/31
8/14
8/28
1/30
2/13
2/27
3/12
3/26
4/9
4/23
5/7
5/21
6/4
6/18
7/2
7/16
7/30
8/13

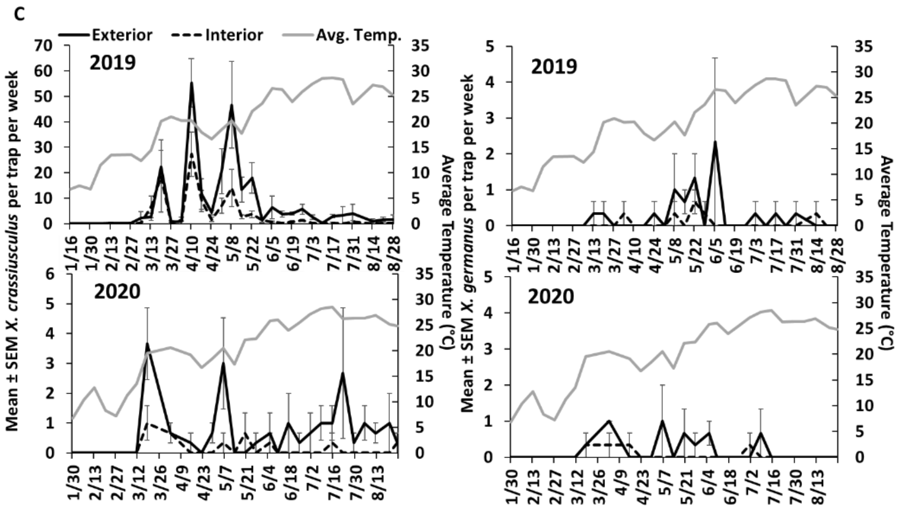
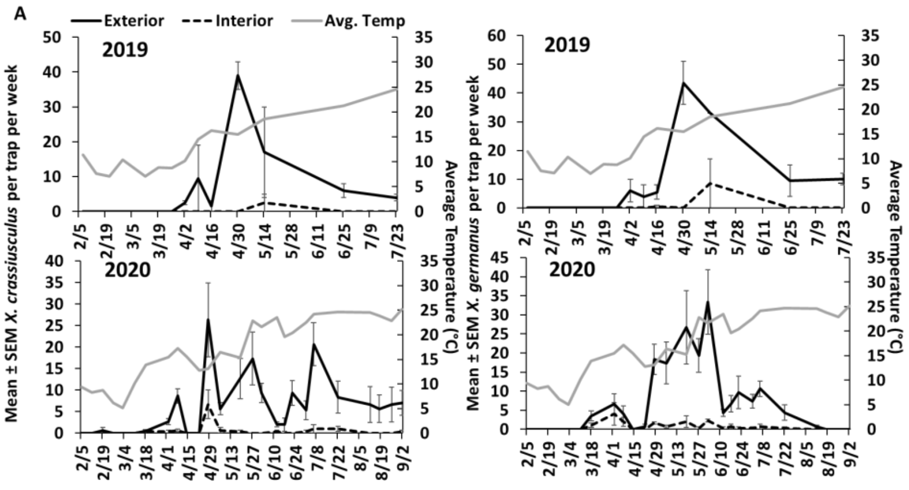

C
Exterior
Interior
Avg. Temp.
2019
2020
Mean ± SEM X. crassiusculus per trap per week
Mean ± SEM X. germanus per trap per week
Average Temperature (°C)
0
10
20
30
40
50
60
70
0
1
2
3
4
5
6
0
1
2
3
4
5
0
5
10
15
20
25
30
35
1/16
1/30
2/13
2/27
3/13
3/27
4/10
4/24
5/8
5/22
6/5
6/19
7/3
7/17
7/31
8/14
8/28
1/30
2/13
2/27
3/12
3/26
4/9
4/23
5/7
5/21
6/4
6/18
7/2
7/16
7/30
8/13

Fig. 4: Mean ± SEM, and temperature data (°C) of *X. crassiusculus* and *X. germanus* captures per trap per week in 2019 and 2020 at apple orchard site in Blue Ridge (A). Mean ± SEM of *X. crassiusculus* and *X. germanus* captures per trap per week in 2019 at Ellijay (B) apple site, and Athens (C) peach site.


A
Exterior
Interior
Avg. Temp
2019
2020
Mean ± SEM *X. crassiusculus* per trap per week
Mean ± SEM *X. germanus* per trap per week
Average Temperature (°C)
0
5
10
15
20
25
30
35
40
45
50
60
2/5
2/19
3/5
3/19
4/2
4/16
4/30
5/14
5/28
6/11
6/25
7/9
7/23
3/4
3/18
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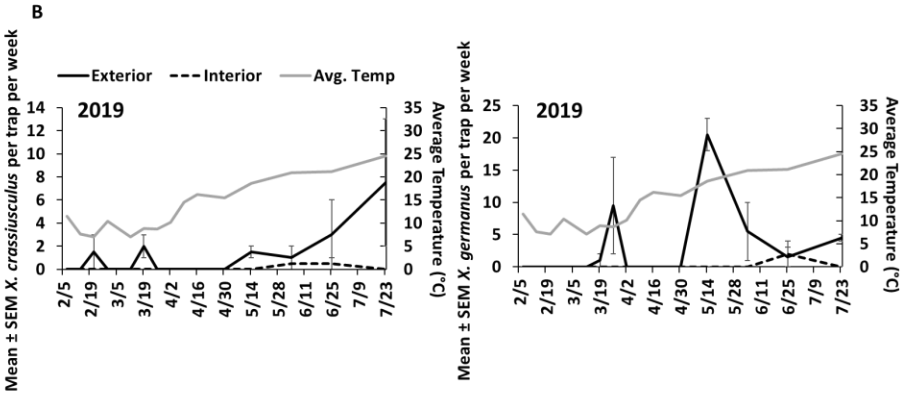
7/8
7/22
8/5
8/19
9/2


B
Exterior
Interior
Avg. Temp
2019
Mean ± SEM *X. crassiusculus* per trap per week
Mean ± SEM *X. germanus* per trap per week
Average Temperature (°C)
0
2
4
6
8
10
12
14
5
15
20
25
30
35
2/5
2/19
3/5
3/19
4/2
4/16
4/30
5/14
5/28
6/11
6/25
7/9
7/23

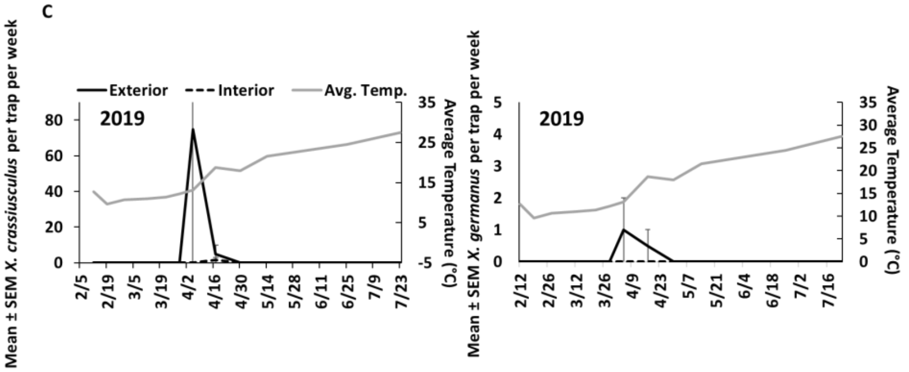
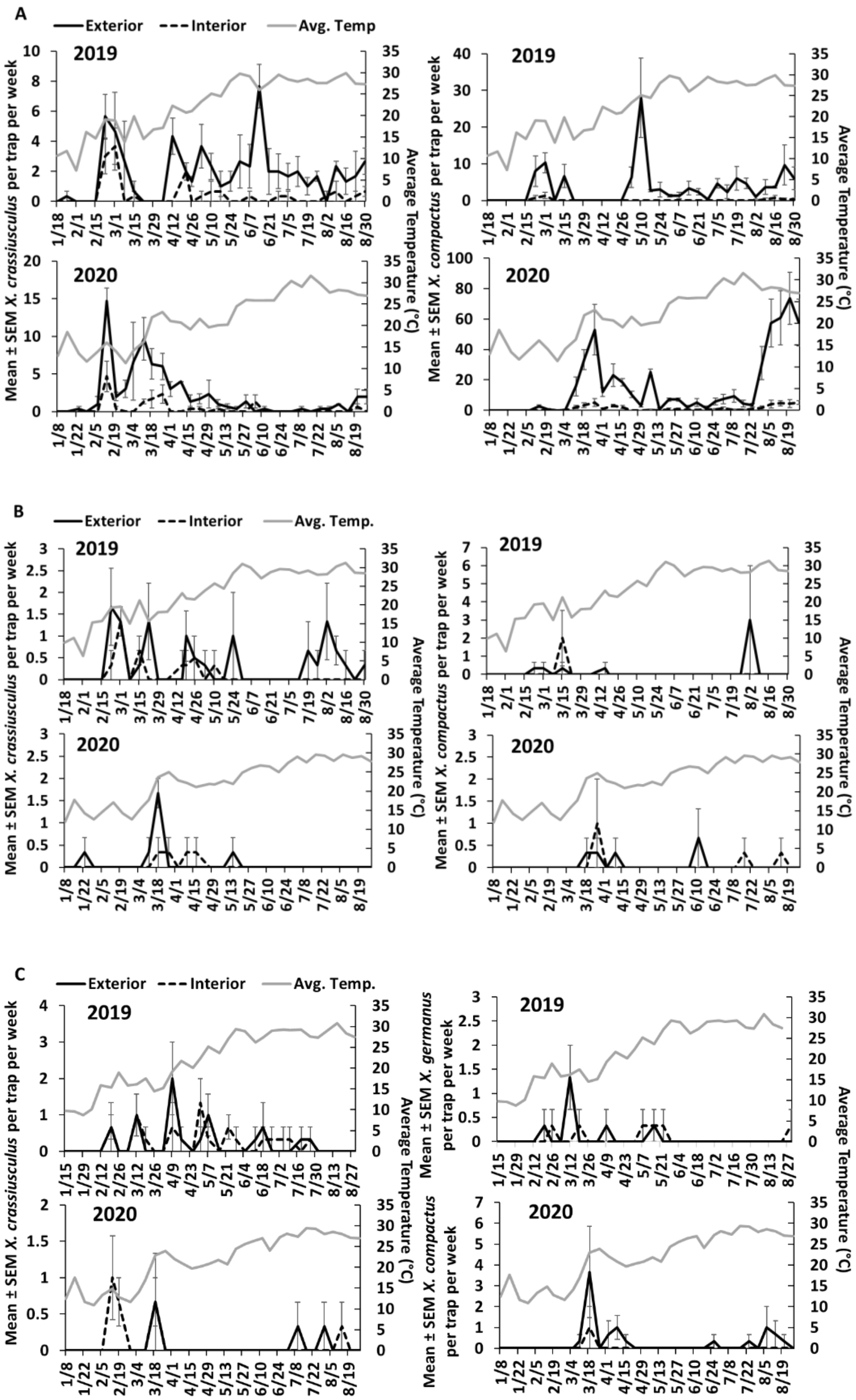

C
Exterior
Interior
Avg. Temp.
2019
Mean ± SEM *X. crassiusculus* per trap per week
Mean ± SEM *X. germanus* per trap per week
Average Temperature (°C)
0
20
40
60
80
-5
5
15
25
35
1
2
3
4
10
30
2/5
2/19
3/5
3/19
4/2
4/16
4/30
5/14
5/28
6/11
6/25
7/9
7/23
2/12
2/26
3/12
3/26
4/9
4/23
5/7
5/21
6/4
6/18
7/2
7/16

Fig. 5: Mean ± SEM, and temperature data (°C) of *X. crassiusculus* and *X. compactus* captures per trap per week for 2019 and 2020 at pecan orchard sites: Quitman (A), Nashville (B). Irwin site (C) shows mean ± SEM of *X. crassiusculus* and *X. germanus* captures per trap per week (2019) and *X. crassiusculus* and *X. compactus* (2020).

A
Exterior
Interior
Avg. Temp
2019
2020
Mean ± SEM X. crassiusculus per trap per week
Mean ± SEM X. compactus per trap per week
Average Temperature (°C)
B
Exterior
Interior
Avg. Temp.
2019
2020
Mean ± SEM X. crassiusculus per trap per week
Mean ± SEM X. compactus per trap per week
Average Temperature (°C)
C
Exterior
Interior
Avg. Temp.
2019
2020
Mean ± SEM X. crassiusculus per trap per week
Mean ± SEM X. germanus per trap per week
Mean ± SEM X. compactus per trap per week
Average Temperature (°C)

CHAPTER 3

INFLUENCE OF PLACEMENT AND AGE ON ETHANOL BAIT ATTRACTIVENESS TO AMBROSIA BEETLES (COLEOPTERA: CURCULIONIDAE)

A. Monterrosa, S. V. Joseph, B. R. Blaauw, and A. L. Acebes,

Accepted for publication in the Journal of Economic Entomology

Abstract

Ambrosia beetles (*Xylosandrus* spp.) are pests of woody ornamental nurseries and tree nut orchards. Growers use ethanol- infused bolts and ethanol-mediated bottle traps for monitoring ambrosia beetles in spring. To refine these monitoring tools, we investigated the 1) sensitivity of the commercially available ethanol-pouches placed on bolts compared to that of ethanol- infused bolts for ambrosia beetle attacks; and 2) the effect of aging of ethanol-pouches on ambrosia beetle captures in bottle traps. Experiments were conducted in ornamental nurseries and pecan orchards in 2019 and 2020. For objective one, the treatments included a bolt infused with locally purchased ethanol, an ethanol-pouch attached to a bolt, and a control (bolt without ethanol). For objective two, ethanol-pouches were initially field-aged for 0, 1, 2, 3, and 4 weeks and ambrosia beetle captures were documented weekly for four weeks. Ethanol-pouches older than five weeks were replaced with fresh ones at the pecan sites while ethanol-pouches at nursery sites were aged up to eight weeks. The ethanol-pouch comparison study showed that the number of ambrosia beetle attacks was significantly greater on ethanol-infused bolt traps than with the ethanol-pouches on bolts at the nursery and pecan sites in both years. The age of the ethanol-pouches did not affect captures of *Xylosandrus crassiusculus* (Motschulsky) and *X. germanus* (Blandford) in bottle traps within eight weeks of deployment. Results provide practical implications into the sensitivity of the different ethanol lure types in association with bolt traps and the longevity of the ethanol-pouches for grower use.

Keywords *Xylosandrus crassiusculus, Xylosandrus germanus*, monitoring, nursery, pecan,

Ambrosia beetles (mostly, *Xylosandrus* spp.) (Coleoptera: Curculionidae) are serious pests of woody ornamental nurseries (Ranger et al. 2016, Addesso et al. 2019) and tree nut orchards (Ree and Knutson 1997). In 2018, the value of ornamental horticultural crops was estimated at $888 million USD, with field nurseries accounting for 14.1%, and the value of tree fruits and nuts was estimated at $614 million USD, with pecan accounting for 35.5% (Wolfe & Stubbs 2019). The major ambrosia beetle species affecting woody ornamentals are the granulate ambrosia beetle *Xylosandrus crassiusculus* (Motschulsky) and the black stem borer *Xylosandrus germanus* (Blandford) (Reding et al. 2010, Ranger et al. 2016, Addesso et al. 2019). In winter, beetles mate and overwinter as mated adults within trees (Addesso et al. 2019). When temperatures spike in spring, females emerge and seek new host trees to infest (Ranger et al. 2016, Addesso et al. 2019). When the females land on a new host, they bore into the tree trunk and create galleries and brood chambers in the heartwood regions of trees. Females oviposit and farm a symbiotic fungus to feed developing larvae and adults (Ranger et al. 2016). Flightless male beetles remain in the galleries of previous hosts (Ranger et al. 2016). Females that colonize new tree hosts pose a serious threat to woody ornamental and pecan trees (Ranger et al. 2016, Addesso et al. 2019). The affected trees appear wilted with branch dieback (Hara and Beardsley 1979, Ranger et al. 2016). In extreme cases, damage can lead to tree death. However, not all affected trees perish due to infestation. In ornamental nurseries, damage symptoms can reduce the marketability of trees (Ranger et al. 2016).

Trees naturally produce ethanol during bud break in spring, and the levels of ethanol production spike in young, stressed trees, which makes them attractive to ambrosia beetles (Ranger et al. 2010). Trees can become stressed due to winter freezing, frequent flooding, or diseases (Ranger et al. 2010). Stress causes trees to increase acetaldehyde production, which is then converted to ethanol and transported through the vascular tissue (xylem) of the tree (Ranger et al. 2010).

Ambrosia beetles are difficult to manage once trees are infested, and even a single gallery can be detrimental to seedlings and twigs (Hara and Beardsley 1979). The population of ambrosia beetles that colonize the heartwood of the trees is difficult to reach even using systemic insecticides. Trunk spray applications rarely penetrate the colony because the bark provides a protective barrier for developing beetles (Reding et al. 2010, VanDerLaan and Ginzel 2013). Thus, the current management practices involve keeping the invading females off the trunk using repellent insecticides, such as pyrethroids, especially during peak adult flight periods (VanDerLaan and Ginzel 2013, Ranger et al. 2016). Thus, monitoring strategies should be precise to determine peak flight activity.

Ambrosia beetle populations are mainly monitored using ethanol-baited trapping mechanisms (Galko et al. 2019). Ranger et al. (2010) identified ethanol as the most attractive compound to ambrosia beetles among several organic compounds including acetaldehyde, acetone, and methanol. However, adding additives, such as α-pinene and conophthorin, to ethanol has not consistently improved ambrosia beetle captures (Addesso et al. 2019). Several trap types, including bottle traps, wooden bolt traps, and Lindgren multiple funnel traps, have been tested (Coyle et al. 2005). However, Reding et al. (2011) showed that bottle traps caught more *X. crassiusculus* than Lindgren funnel traps. For bottle traps, soda bottles are cut to generate multiple vents, baited with ethanol lure pouches, inverted and hung. The ambrosia beetles lured to ethanol are drowned in soapy water added to the bottle. Similarly, nursery growers use wooden bolt traps for monitoring ambrosia beetles (Reding and Ranger 2020). The wooden bolt traps are prepared by cutting approximately 10-cm diameter logs at variable lengths of any tree species, drilling a hole down the center of the bolt and filling it with ethanol, and then hanging the log. Although ornamental nursery and pecan growers use bottle and wooden bolt traps, the efficiency of these traps can be improved. Growers buy ethanol from local stores for the wooden bolt trap. Before deploying the bolts, growers need to prepare the logs by drilling holes in the center. These holes should be sealed using a cork cap and regularly refilled to maintain attractancy. Commercially available ethanol pouches can be used instead, and they can be placed on wooden bolt traps for monitoring ambrosia beetle flight activity. Ethanol pouches are commonly used in bottle traps following the manufacturer’s directions, especially when the lures should be replaced. The recommended length of exposure could be extended beyond a one-month interval. However, the length of time the ethanol pouches remain attractive is not documented. Thus, the major objectives of the current study were to determine the 1) attractivity of locally purchased ethanol versus commercially available ethanol pouches and 2) duration of the attractiveness of commercially available ethanol pouches for ambrosia beetle captures.

Materials and Methods

Study sites. The experiments were conducted in woody ornamental nurseries in central Georgia and pecan nurseries or orchards in southern Georgia in 2019 and 2020 (Table 2). The major ornamental tree species grown in the nursery sites (sites 1 and 2) were *Magnolia* spp., *Prunus* spp., *Cercis* spp., *Illex* spp., *Ginkgo* spp., *Lagerstroemia* spp., *Stewartia* spp., *Camellia* spp., *Acer* spp. and *Cupressus* spp. The planted nursery trees were spaced ~1.5 m apart between rows and trees at site 1, and variable spacing was used in the container field nursery at site 2. The age of the majority of the growing trees ranged from 0 to 4 years. Most of the tree crops grown in ornamental nurseries are susceptible to *X. crassiusculus* and *X. germanus* attacks (Ranger et al. 2010, 2016). The woody areas outside the nurseries had a variety of trees and shrubs, such as

Liquidambar styraciflua L., *Acer* spp., *Quercus* spp., *Pinus* spp., and *Ligustrum* spp. All woody ornamental trees in the nurseries were drip irrigated and pruned as needed throughout the year. In spring of both years, the nursery managers at both sites preventatively sprayed bifenthrin and permethrin (pyrethroids) to protect the trees from ambrosia beetle attacks and dinotefuran (neonicotinoid) for scale management.

At the pecan sites, the orchards only contained *Carya illinoinensis*. The planted trees were spaced ~12 m apart and the ages of the planted trees ranged from 4 to 7 years. Pecan trees are susceptible to ambrosia beetles, particularly *X. germanus* (Hoffman 1941, Ree and Knutson 1997) and *X. crassiusculus* (Acebes-Doria, unpublished data). The orchard was bordered by several types of coniferous and deciduous trees, such as *Liquidambar styraciflua* L., *Acer* spp., *Quercus* spp., and mainly, *Pinus* spp. The planted trees within the orchard were irrigated using microjets and pruned as needed throughout the year. Throughout both years, the orchard managers sprayed general purpose insecticides and miticides for a broad range of arthropod pests.

Traps and lures. For this experiment, wooden bolts and bottle traps were used. For the experiments at ornamental sites, fresh red maple (*Acer rubrum* L.) was used to make wooden bolt traps. Bolts were ~0.5 m length and ~0.06 m diameter. At the pecan sites, fresh pecan (*Carya illinoinensis* (Wangenh.) K. Koch logs were used to make the wooden bolt traps. The bolts were used within three weeks after cutting from the tree branches. Ethanol (70%) was the attractant used for the bolts at the ornamental and pecan sites. For the treatment where ethanol was added to the core of the bolt traps (Fig. 6A), locally purchased ethanol (Klean Strip Denatured Alcohol, Memphis, TN, for the ornamental sites; and Jasco Denatured Alcohol, Memphis, TN, for the pecan sites) was used. The same brand of ethanol was used in both years at the ornamental and pecan sites. A 10-cm deep hole was drilled in the center of the bolt traps, and 10 mL of ethanol was poured into the logs deployed at the ornamental site while 100 mL of ethanol added into the logs at the pecan site. The hole was closed using a cork (Fig. 6A, indicated by white arrow). Each week, ethanol was replenished in each bolt and each bolt was assessed for the number of attacks. For the treatment with the ethanol pouch attached to the bolt trap, commercially available pouches with a 65 mg/day release rate at 30°C were purchased (AgBio Inc., Westminster, CO). The pouches included 7-8 mL 95% ethanol. The pouch was attached by screwing it to the bolt trap ~2 cm from the top edge of the bolt (Fig. 6B). The wooden bolt was hung ~0.61 m off of the ground surface using a metal stake.

At the ornamental sites, the bottle trap consisted of a clear rectangular-shaped 1774 mL plastic bottle (VTM LLC, Lexington, KY). Two ~5 cm × ~9 cm rectangular windows were cut and removed on the opposite sides of the bottle to allow access to the AgBio lure (Fig. 7A). Two holes were drilled into the bottom of the bottle to hang an AgBio lure using a zip tie. The bottles were hung upside down on a wooden stake using zip ties to attach the bottle to the wooden stake. The bottle trap used at the pecan site was constructed using a clear plastic 1774 mL square decanter with the same sized windows cut out as the ornamental bottle traps (Fig. 7B). Soap water solution (2 mL dawn dish soap per 10 mL water, P&G, Kansas City, KS) was added to the bottle trap to drown and trap the visiting insects. The lid of the bottle was unscrewed to empty the collected insects. The traps were emptied into a coffee filter placed on a mesh strainer.

Lure type experiment. This experiment was conducted at ornamental and pecan sites in 2019 and 2020 (Table 2). At the ornamental sites, red maple bolts were used in the experiment, whereas at the pecan site, pecan bolts were used. The treatments were 1) ethanol added into the bolt, 2) an AgBio lure attached to the bolt, and 3) a nonbaited control bolt. Bottle traps with

AgBio lures were added to the experiment to help identify the species of ambrosia beetles in the area. The treatments were replicated four times in a randomized complete block design (RCBD). Traps were deployed along the wood lines, ~9.1 m from the border rows of nursery or orchard and deployed 20 m apart using metal stakes. The experiments were conducted during known periods of active ambrosia beetle activity at the nursery and pecan sites (Table 2).

Aged lure experiments. Two types of experiments were conducted in 2019 and 2020 to determine the effects of AgBio lure longevity on ambrosia beetle captures.

Experiment 1. The AgBio lures were aged within bottle traps and deployed in horticultural gardens (33.266503, -84.293293) at the University of Georgia in Griffin, GA. A set of AgBio lures were set out in the field every week. When the first set of lures reached four weeks old, the AgBio lures plus bottles were transferred to an ornamental nursery site in Lamar County and the experiment was initiated. The treatments at the beginning of the study included 0-, 1-, 2-, 3-, and 4-week-old lures, which were replicated four times using an RCBD. Once the traps were deployed, the AgBio lures were not changed; thus, the treatments were aged by a week every 7 days. The experiment was set up ~9.1 m from the wood line of the nursery. The bottle traps were 20 m apart and deployed using wooden stakes as described above. The experiments were conducted during known periods of active ambrosia beetle activity at the nursery and pecan sites (Table 2).

Experiment 2. This experiment was similar to experiment 1 but with few exceptions, and it was conducted at pecan sites in Cook County. The AgBio lures were aged in bottle traps at the University of Georgia in Tifton, GA (31.3048251, -83.4951191) by deploying them along a wood line. Once the first set of AgBio lures reached four weeks, the lures and bottles were transferred to the pecan orchard for the initiation of the experiment. The treatments included 0-, 1-, 2-, 3-, and 4-week-old AgBio lures and were replicated four times following an RCBD. Starting one week after initiation of the experiment, the AgBio lures in a treatment that passed 4 weeks were replaced with new AgBio lures (0 weeks old), and this process continued for the next three weeks. Thus, the age of the treatments was maintained at 0, 1, 2, 3, and 4 weeks of age during the entire study period. The bottle traps were deployed 20 m apart using garden stakes as described above. The experiments were conducted during known periods of active ambrosia beetle activity at the nursery and pecan sites (Table 2).

Evaluation. The traps in all the experiments were assessed weekly. For the wooden bolts, the number of entry holes and toothpicks were marked and quantified. For the bottle traps, all the contents in the bottle were emptied and transferred to entomology labs (University of Georgia, Griffin or Tifton) every week. The emptied bottles were replaced with fresh soapy water. The curculionid beetles were sorted in 70% ethanol and identified to the genus or species using a lucid key for Southeast Asian Xyleborini species (Smith et al. 2019) and Bateman and Hulcr's ambrosia beetle guide (Bateman and Hulcr 2017).

Statistical analyses. The statistical analyses for all of the experiments were conducted using SAS software (SAS Institute 2012). For the lure placement experiment in ornamental and pecan sites for 2019 and 2020, the number of holes caused by ambrosia beetle attacks with each sample date was subjected to two-way ANOVA using a generalized linear mixed model analysis, where the sampling date was considered a repeated measure (PROC GLIMMIX) in SAS with a log link function and a negative binomial distribution. The lure type, sampling date, and their interactions served as the treatments, and each bolt was the replication. Although the bottle traps were part of the experimental design, they were not included in the ANOVA because they collected various species of ambrosia beetles. The various genera or species of ambrosia beetles collected served as an inventory of beetles active during the experiment because *X. crassiusculus* and *X. germanus* were the only known pest species of ornamental trees and pecan. To further understand the effect of lure placement, data from each sample date were subjected to a one-way ANOVA using the generalized linear model procedure (PROC GLIMMIX) using a slice function in SAS. The means were separated post-ANOVA using Tukey's HSD method ($\alpha = 0.05$). To determine the association between ambrosia beetle attack holes on the bolts and captures in the bottle traps, Pearson's correlation analysis was performed (PROC CORR) in SAS for ornamental and pecan sites in 2019 and 2020. For this analysis, the number of beetle attack holes on bolts from all three treatments (No ethanol on bolt, ethanol pouch on bolt, ethanol infused bolt) were combined for each block per sample date. Similarly, number of *X. crassiusculus* and *X. germanus* captured were combined per sample date. To obtain a robust association, data from 2019 and 2020 were analyzed together for ornamental (eight sample dates) and pecan sites (nine sample dates).

For the aged lure experiment in ornamental sites in 2019 and 2020, the number of *X. crassiusculus* and *X. germanus* captured were subjected to a one-way ANOVA using the generalized linear model procedure (PROC GLIMMIX) with a log link function and a negative binomial distribution. The treatments were lure age, and the bottle traps were the replications. The data were not analyzed as repeated measures because each sampling date was considered a separate experiment as the lures in all treatments aged with time. For the pecan sites in 2019 and 2020, to determine the effect of lure longevity, the numbers of *X. crassiusculus* and *X. germanus* captured on each sampling date were combined. The numbers of *X. crassiusculus* and *X. germanus* captured in 2019 were subjected to a one-way ANOVA using the generalized linear model procedure (PROC GLIMMIX) with a log link function and a negative binomial distribution. The *X. crassiusculus* and *X. germanus* captures were very low in 2020; thus, data analysis was not performed. The means were separated post-ANOVA using Tukey's HSD method ($\alpha = 0.05$).

Results

Lure type experiment. At the ornamental sites, significant interaction was observed between sampling date and treatment in 2019 ($F = 2.6$; df = 6, 33; $P = 0.036$) and in 2020 ($F = 5.3$; df = 6, 33; $P = 0.007$) indicating that both factors influenced ambrosia beetle attacks. Throughout the sampling period, ambrosia beetle attacks differed significantly among treatments in 2019 ($F = 22.0$; df = 2, 33; $P < 0.001$) and in 2020 ($F = 24.9$; df = 2, 33; $P < 0.001$). Ambrosia beetle attacks were also different across the sampling period in 2019 ($F = 6.8$; df = 3, 33; $P = 0.001$) and 2020 ($F = 5.9$; df = 3, 33; $P = 0.002$).

The data were then analyzed by sampling date. In 2019, attacks were statistically similar among treatments on 20 March ($F = 1.4$; df = 2, 33; $P = 0.267$) and 29 March ($F = 3.1$; df = 2, 33; $P = 0.059$; Fig. 8A). On 4 April, the number of attacks was significantly greater in the bolt treatment with ethanol than that without ethanol ($F = 3.8$; df = 2, 33; $P = 0.034$). On 11 April, a significantly greater number of holes was found in the bolt treatment with ethanol than that with the Agbio lure and the no ethanol treatment ($F = 29.9$; df = 2, 33; $P < 0.001$; Fig. 8A). *Xylosandrus crassiusculus* and *X. germanus* were present during the sampling period based on the ambrosia beetles collected in the bottle traps (Fig. 8B). In 2020, no holes were found on the bolts on 17 April (Fig. 8C). On 1 May, both the bolt with ethanol and AgBio lure bolt treatments had a significantly more holes compared with the no ethanol treatment ($F = 7.7$; df = 2, 33; $P = 0.002$). The number of holes was significantly greater in the ethanol bolt treatment than in the AgBio lures on the bolt and no ethanol treatments on 8 May ($F = 33.6$; df = 2, 33; $P < 0.001$) and

15 May ($F = 6.3$; df = 2, 33; $P = 0.005$; Fig. 8C). During the sampling period, *X. crassiusculus* adults were collected in bottle traps (Fig. 8D).

At the pecan sites, no significant interaction was observed between sampling date and lure placement in 2019 ($F = 0.9$; df = 4, 24; $P = 0.459$) or in 2020 ($F = 1.1$; df = 4, 24; $P = 0.387$). The lure type treatment alone significantly affected the severity of ambrosia beetle attacks on the bolts in 2019 ($F = 20.0$; df = 2, 24; $P < 0.001$) and in 2020 ($F = 5.4$; df = 2, 24; $P = 0.011$). Attacks did not differ significantly across the sampling date in 2019 ($F = 0.1$; df = 2, 24; $P = 0.001$) or 2020 ($F = 2.2$; df = 2, 24; $P = 0.134$).

The data were then analyzed by the sampling date. In 2019, a significantly greater number of attack holes was found in the bolt treatment with ethanol than that without ethanol on 14 March ($F = 4.6$; df = 2, 24; $P = 0.021$; Fig. 9A); the number of holes was greater in the ethanol bolt treatment than in the ethanol pouch on the bolt and no ethanol treatments on 21 March ($F = 11.9$; df = 2, 24; $P = 0.002$); the number of holes was greater in the ethanol bolt treatment than the no ethanol treatment on 28 March ($F = 5.5$; df = 2, 24; $P = 0.011$; Fig. 9A). Adult *X. crassiusculus* were collected in bottle traps during the sampling period (Fig. 9B). In 2020, no ambrosia beetle attacks were observed on 6 March (Fig. 9C); and a significantly greater number of attack holes was observed in ethanol bolt treatment than in the ethanol pouch on the bolt and no ethanol treatments on 13 March ($F = 6.4$; df = 2, 24; $P = 0.006$) and 20 March ($F = 5.4$; df = 2, 24; $P = 0.011$; Fig. 9C). Low numbers of *X. crassiusculus* and *X. germanus* were collected in the bottle traps during the sampling period (Fig. 9D).

The Pearson's correlation analysis showed that there was a significant association between numbers of beetles captured in the bottle traps and attack holes on the bolt ($r = 0.412$; $P = 0.019$; n = 32) in ornamental sites. However, the association between numbers of beetles captured in the bottle traps and attack holes on the bolt was not significantly different (r = -0.008; P = 0.961; n = 36) in pecan sites.

Aged lure experiments. *Experiment 1.* In 2019, captures of *X. crassiusculus* and *X. germanus* in bottle traps were not significantly affected by lure longevity across the sampling period (Table 2). Similar to 2019, significant differences were not observed among the aged lure treatments on any sampling date for *X. crassiusculus* or for *X. germanus* in 2020 (Table 3).

Experiment 2. In 2019, the number of *X. crassiusculus* and *X. germanus* captures were not significantly different among the aged lure treatments (Table 4).

Discussion

The results show that *X. crassiusculus* and *X. germanus* attacks were greater on ethanol-infused wooden bolts than on bolts with AgBio lures attached to them. Ethanol- infused wooden bolt traps are recommended to monitor the spring flight of ambrosia beetles in woody nurseries (Joseph and Hudson 2019). Growers build this trap after cutting 30-60 cm long, 5 cm diameter maple, magnolia or other common tree bolts then drilling a 1.5 cm diameter hole in the center, which is filled with locally purchased ethanol (Fig. 6A). In the current study, we attempted to determine the utility of commercially available ethanol pouches along with wooden bolts for ambrosia beetle attraction and attacks. We were also interested in determining whether there are differences in the number of ambrosia beetle attacks on bolts with AgBio ethanol lures compared with wooden bolts filled with locally purchased ethanol. Placement of an Agbio lure on the surface of the bolt did not increase the ambrosia beetle attacks on the bolt compared to that on the bolt with ethanol in the core.

Xylosandrus crassiusculus and *X. germanus* are pest species that attack trees in ornamental nurseries and pecan orchards (Ree and Knutson 1997, Ranger et al. 2016, Addesso et al. 2019, A. Monterrosa unpublished data). The onset of flight activity and peak flight activities are relevant for growers in order to time their control decisions for ambrosia beetles. Bolt traps are used to monitor the flight activity of beetles by using ethanol as an attractant. Nursery and pecan growers in rural areas have limited access to reagent-grade ethanol for monitoring ambrosia beetles, although most have access to ethanol products sold in local hardware stores. The bolts with locally purchased ethanol added to the core were attacked by ambrosia beetles, which suggests that ethanol-infused wooden bolt traps using ethanol purchased from local stores can be used to monitor ambrosia beetle activity. The easy accessibility of store-bought ethanol may be attractive to growers, especially for unexpected early or late flights of ambrosia beetles. It can be argued that growers may not be interested in knowing the species of beetles captured in the traps, as long as they have an indication when those beetles are active. Therefore, bolt traps are more useful for monitoring ambrosia beetle attacks.

The choice of the Agbio ethanol lures with the standard release rate is due to it being a commonly used brand and lure type (standard release) in previous studies regarding ambrosia beetles (Reding et al. 2010, Reding et al. 2011, Ranger et al. 2012). The use of the AgBio ethanol lures reduces trap maintenance because ethanol does not need to be added into the core of the bolt at regular intervals. However, why the AgBio lures attached to the bolts did not result in as many attacks as ethanol added inside the bolt is not clear. Perhaps the AgBio lures on the bolt attracted the ambrosia beetles but did not clearly direct them to the bolt, resulting in fewer attack holes. In the current study, the attack holes were only found immediately around the ethanol pouches. Future studies are warranted to determine the attractiveness and resulting attacks after placing the pouches inside the bolts.

The use of ethanol with bolt traps is essential for ambrosia beetle attacks due to the attractancy of the volatile compound to the beetles (Ranger et al. 2010, 2012). Ranger et al. (2010, 2012) found that trees injected with ethanol caught 5 times more beetles than trees injected with other volatile materials. Adding α-pinene and conophthorin with ethanol lures did not improve ambrosia beetle capture compared with the ethanol treatments alone (Miller and Ragbalia 2009, VanDerlaan and Ginzel 2013). When we conducted the experiments in nursery and pecan sites, the amount of ethanol used to bait ambrosia beetles varied. At the nursery sites, we initially used 10 mL and then added 10 mL at weekly intervals in the holes in the bolts. At the pecan sites, we initially used 100 mL of ethanol and then added 100 mL at weekly intervals. Previously, Reding et al. (2011) showed that ambrosia beetle captures were not influenced by increasing the amount of ethanol, although it was not clear if high amounts repelled the approaching ambrosia beetles. This information could be useful to growers because increasing the amount of ethanol may not necessarily increase the attacks of ambrosia beetles on wooden bolts.

Currently, ethanol pouches are only recommended as a lure in bottle traps (Cole 2008, Frank et al. 2019; Fig. 2). The results from the current study show that AgBio ethanol lures aged up to eight weeks did not reduce *X. crassiusculus* and *X. germanus* captures, although the manufacturer recommended AgBio lures to be used for up to four weeks. The manufacture recommendations can vary by the release rate of the ethanol lure. This result suggests that AgBio lures can be used for at least eight weeks in bottle traps before replacing with fresh pouches for ambrosia beetle monitoring. The peak ambrosia beetle flight activity timing and number of peaks in spring vary by cropping system and geographical region (Reding et al. 2010, Ranger et al. 2016, A. Monterrosa unpublished data). Understanding the length of attractiveness of AgBio lures is critical for determining peak flights in ornamental nurseries, tree fruit, and tree nut orchards where the early flights that coincide with the bud break of trees result in serious attacks on the trees. Therefore, the AgBio lures only need to be changed once to capture the peak activity of beetle flights from February to April.

In summary, the results show that ethanol-infused wooden bolt traps using locally purchased ethanol were an effective method in attracting ambrosia beetles while ethanol pouches on the bolt surface were not as effective. Oliver and Mannion (2001) showed that ambrosia beetle attacks on bolts coincide with beetle attacks on trees in nurseries and thus can be used by growers to determine beetle flight activity for management decisions. For those growers who use bottle traps for monitoring, the ethanol pouches in the bottle do not need to be replaced for up to eight weeks. This new information reduces the need for weekly trap maintenance and decreases the costs for growers purchasing ethanol pouches. Future research should investigate how to better integrate the ethanol pouch on the bolt for better ambrosia beetle attacks, which will reduce the need for frequent trap maintenance and improve the ability to monitor ambrosia beetle activity.

Acknowledgments

We thank C. Julian, M. Hayden, L. Pool, and J. Bailey for assistance in the deployment of traps and the regular maintenance of those traps at the grower sites. Additionally, we thank the growers for their help with the research sites. This research was funded through Georgia Specialty Crop Block Grant # AM190100XXXXG033 and Georgia Farm Bureau Grant.

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Table 2. Experimental site locations, experiment types, and trapping periods for 2019 and 2020.

Cropping system	Location (County)	Experiment type	GPS coordinates	Trapping period	
				2019	2020
Ornamental nursery	Lamar	Aged ethanol	33.058399, -84.245416	From 21 Mar to 18 Apr	From 10 Apr to 15 May
	Pike	Ethanol placement	33.067127, -84.300102	From 15 Mar to 11 Apr	From 17 Apr to 15 May
Pecan	Cook	Aged ethanol	31.3048251, -83.4951191	From 28 Mar to 25 Apr	From 1 May to 5 Jun
	Cook	Ethanol placement	31.2797208, -83.4898506	From 7 Mar to 18 Apr	From 28 Feb to 20 Mar

Table 3. Mean (± SE) number of *X. crassiusculus* and *X. germanus* captures in bottle traps when lures were aged up to 8 weeks in experiment 1 (ornamental sites) in 2019 and 2020. Ambrosia beetle captures marked without any letters indicate no significant differences between treatments (pairwise *t*-test, α = 0.05).

Weeks aged	2019		2020	
	X. crassiusculus	*X. germanus*	*X. crassiusculus*	*X. germanus*
0	0.8 ± 0.5	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
1	0.5 ± 0.5	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
2	0.5 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3	1.3 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.9	0.8 ± 0.5
	F = 0.3; df = 4, 57; P = 0.871	F = 0.1; df = 4, 57; P = 0.991	F = 1.8; df = 4, 72; P = 0.130	F = 0.4; df = 4, 72; P = 0.821
1	0.8 ± 0.3	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
2	1.0 ± 0.7	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0
3	0.8 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	1.0 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

5	0.3 ± 0.3	0.0 ± 0.0	0.3 ± 0.3	0.3 ± 0.3
	F = 0.2; df = 4, 57; P = 0.949	F = 0.1; df = 4, 57; P = 0.996	F = 0.1; df = 4, 72; P = 0.993	F = *0.1*; df = 4, 72; P = 0.99
2	16.0 ± 7.4	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0
3	17.8 ± 5.8	1.0 ± 1.0	0.8 ± 0.5	0.0 ± 0.0
4	8.0 ± 1.4	1.3 ± 0.5	0.8 ± 0.3	0.3 ± 0.3
5	20.5 ± 8.5	1.5 ± 0.6	0.8 ± 0.8	0.3 ± 0.3
6	23.0 ± 6.4	0.8 ± 0.3	0.0 ± 0.0	0.3 ± 0.0
	F = 1.4; df = 4, 57; P = 0.242	F = 0.7; df = 4, 57; P = 0.618	F = 0.3; df = 4, 72; P = 0.873	F = 0.1; df = 4, 72; P = 0.991
3	10.8 ± 7.4	1.3 ± 0.5	3.8 ± 0.9	0.0 ± 0.0
4	14.8 ± 4.8	1.0 ± 0.4	5.0 ± 2.1	0.5 ± 0.5
5	8.8 ± 5.5	0.3 ± 0.3	5.0 ± 1.4	2.3 ± 1.0

6	13.8 ± 6.8	0.8 ± 0.5	3.0 ± 1.0	0.3 ± 0.3
7	18.8 ± 5.4	1.0 ± 0.7	4.3 ± 2.9	1.3 ± 0.3
	F = 1.2; df = 4, 57; *P* = 0.322	*F* = 0.3; df = 4, 57; *P* = 0.873	*F* = 0.5; df = 4, 72; *P* = 0.774	*F* = 1.7; df = 4, 72; *P* = 0.165
4	-	-	0.3 ± 0.3	0.0 ± 0.0
5	-	-	0.8 ± 0.5	0.5 ± 0.3
6	-	-	0.3 ± 0.3	0.5 ± 0.3
7	-	-	0.5 ± 0.5	0.5 ± 0.3
8	-	-	0.8 ± 0.5	0.5 ± 0.3
			F = 0.2; df = 4, 72; *P* = 0.962	*F* = 0.1; df = 4, 72; *P* = 0.966

Table 4. Mean (± SE) number of *X. crassiusculus* and *X. germanus* captures in bottle traps when lured were aged up to 4 weeks in experiment 2 (pecan sites) in 2019 and 2020. Ambrosia beetle captures without any marked with letters indicate no significant differences between treatments (pairwise *t*-test, α = 0.05).

Weeks aged	2019		2020	
	X. crassiusculus	*X. germanus*	*X. crassiusculus*	*X. germanus*
0	6.5 ± 1.5	1.8 ± 0.8	0.0 ± 0.0	0.5 ± 0.5
1	5.0 ± 1.2	0.8 ± 0.5	0.0 ± 0.0	0.5 ± 0.3
2	4.8 ± 2.8	2.0 ± 1.4	0.8 ± 0.5	0.5 ± 0.5
3	5.5 ± 1.7	3.3 ± 0.8	0.0 ± 0.0	0.5 ± 0.3
4	5.8 ± 1.3	3.0 ± 0.7	0.3 ± 0.3	0.5 ± 0.3
F	0.1	1.2	-	-
df1, df2	4, 12	4, 12	-	-
P	0.964	0.344	-	

Fig. 6. A) Wooden bolt trap with the cork covering the hole with ethanol (indicated with arrow) and B) ethanol pouch attached to the bolt trap.

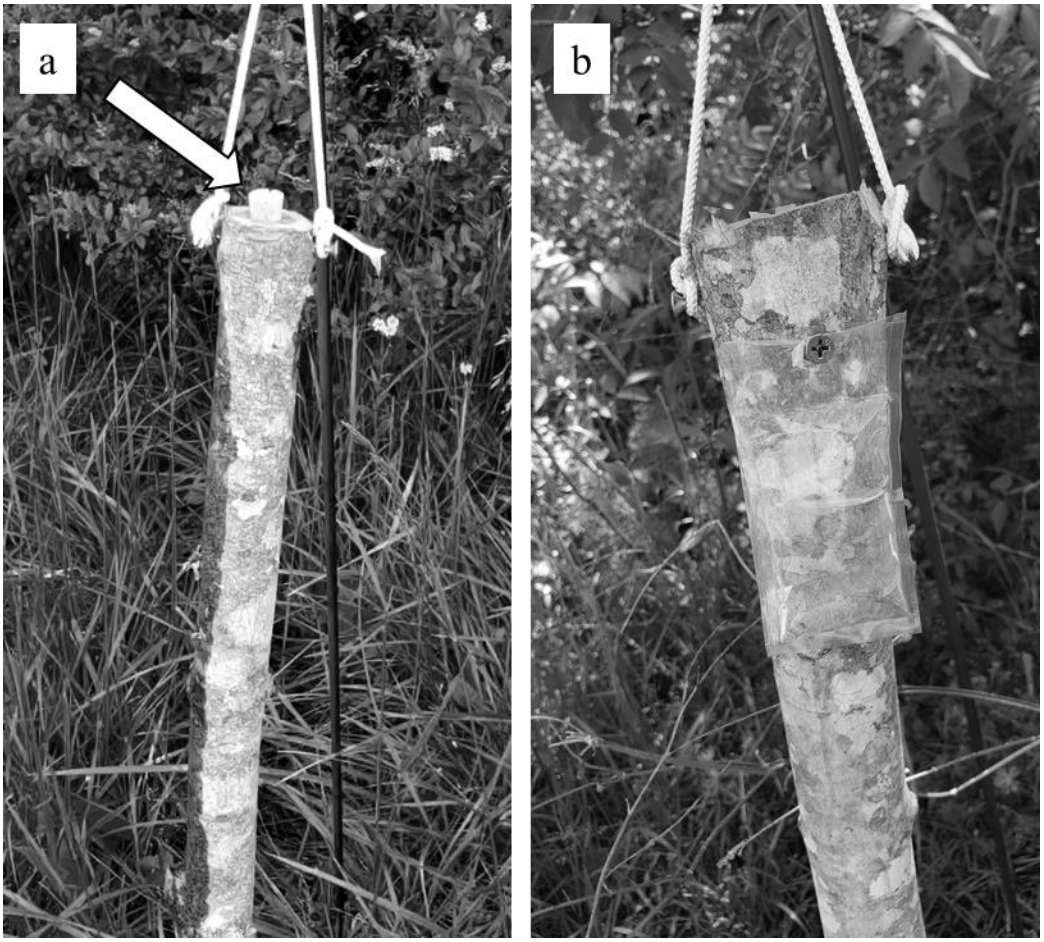

a
b

Fig. 7. Bottle trap used in a) ornamental and b) pecan sites with two rectangular access windows and ethanol pouch attached in the center of the trap using twist tie.

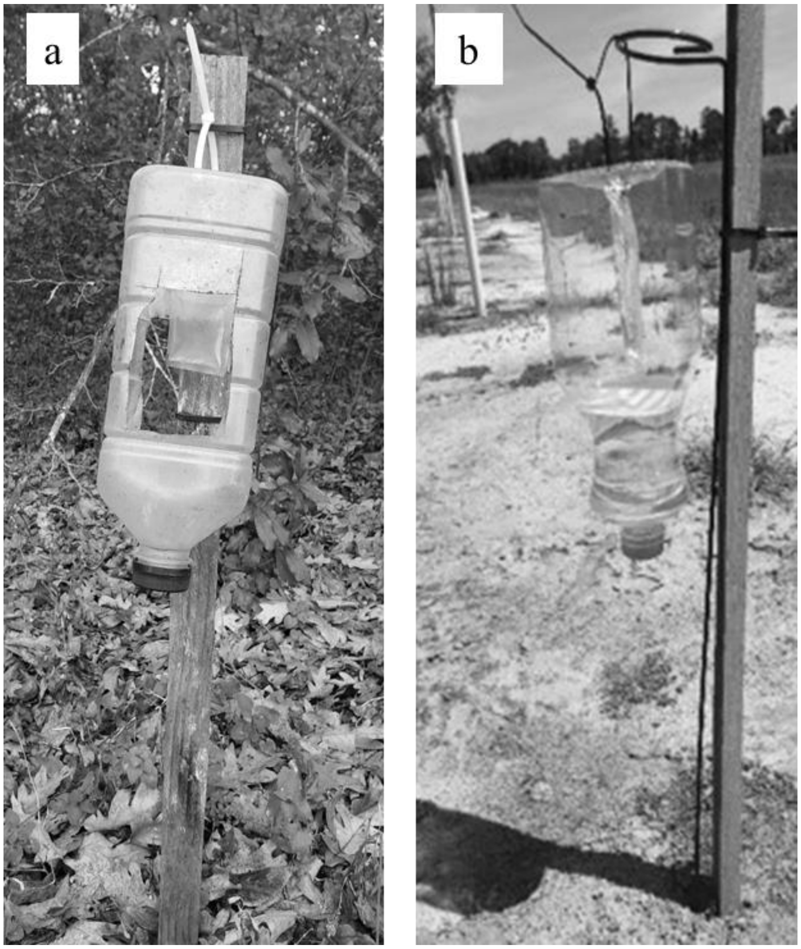

a
b

Fig. 8. Mean (± SE) number of (A and C) ambrosia beetle attack holes per bolt and (B and D) taxa captured in bottle traps at ornamental sites in 2019 and 2020, respectively. Sampling dates marked with the same letters are not significantly different (pairwise *t*-test, $\alpha = 0.05$), and sampling dates without letters are not significantly different at $\alpha = 0.05$.

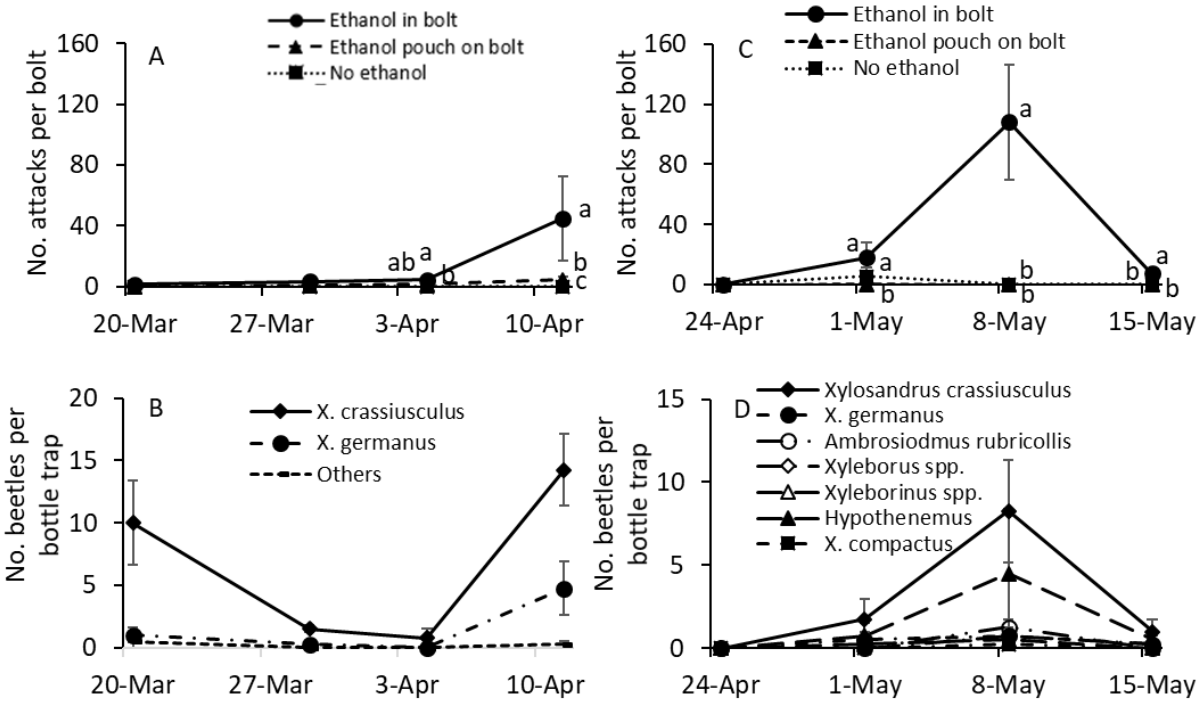
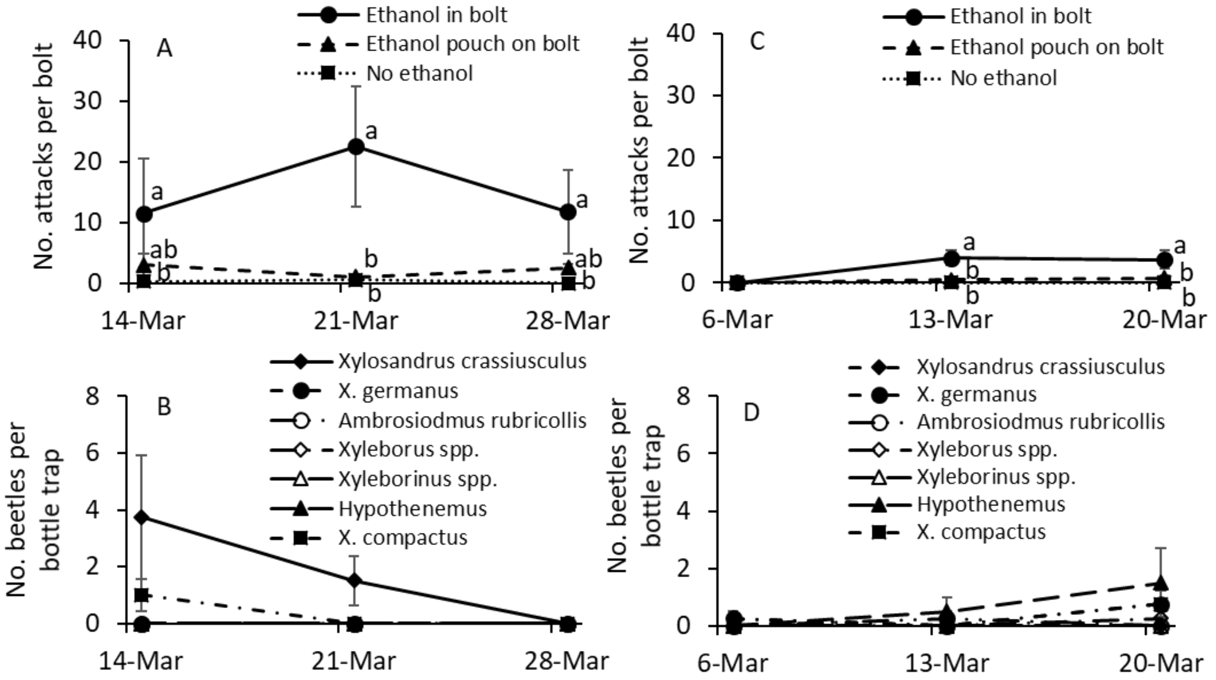

A
No. attacks per bolt
160
120
80
40
0
20-Mar
27-Mar
3-Apr
10-Apr
Ethanol in bolt
Ethanol pouch on bolt
No ethanol
ab
a
b
a
b
c
C
No. attacks per bolt
24-Apr
1-May
8-May
15-May
a
a
b
a
b
b
b
a
b
B
No. beetles per bottle trap
20
15
10
5
0
X. crassiusculus
X. germanus
Others
D
No. beetles per bottle trap
Xylosandrus crassiusculus
X. germanus
Ambrosiodmus rubricollis
Xyleborus spp.
Xyleborinus spp.
Hypothenemus
X. compactus

Fig. 9. Mean (± SE) number of (A and C) ambrosia beetle attack holes per bolt and (B and D) taxa captured in bottle traps at pecan sites in 2019 and 2020, respectively. Sampling dates marked with the same letters are not significantly different (pairwise *t*-test, α = 0.05), and sampling dates without any letters are not significantly different at α = 0.05.


A
No. attacks per bolt
40
30
20
10
0
14-Mar
21-Mar
28-Mar
Ethanol in bolt
Ethanol pouch on bolt
No ethanol
a
a
a
ab
b
b
b
ab
b
C
No. attacks per bolt
6-Mar
13-Mar
20-Mar
a
b
b
a
b
b
B
No. beetles per bottle trap
8
6
4
2
0
Xylosandrus crassiusculus
X. germanus
Ambrosiodmus rubricollis
Xyleborus spp.
Xyleborinus spp.
Hypothenemus
X. compactus
D
No. beetles per bottle trap
6-Mar
13-Mar
20-Mar

CHAPTER 5

CONCLUSIONS

Wood-boring ambrosia beetles have become economically important pest species throughout much of the United States. Limited studies have been conducted on ambrosia beetles in Georgia. The ambrosia beetles species abundance and phenology were investigated in ornamental nursery as well as tree fruit and tree nut orchards in 2019 and 2020. Six plastic bottle traps baited with ethanol dispensers were deployed in each site, with three placed in the interior of the nursery or orchard and three traps along the wood-line. There were nine sites in 2019, three in North Georgia, three in Middle Georgia, and three in South Georgia. In 2020, the sites were the same, except in North Georgia, where we only had one site. The ambrosia beetles were trapped from January to August at most sites. A number of species of ambrosia beetles were collected in 2019 and 2020 throughout Georgia. *X. crassiusculus* and *X. germanus* were the major species collected. A potential pest, *X. compactus,* was also collected from certain sites. The phenology data showed that first flight and peak activity are dependent on the region and subject to local temperatures. The flight activity was sporadic within most sites, with several peaks and dips throughout the growing season. This study found that flight activity can occur in temperatures lower than ~20°C. Ambrosia beetles flight activity was also noticed in the winter months when the temperatures were slightly above ~7.2°C.

To improve the monitoring tool for ambrosia beetles, alcohol type and their placement were studied in 2019 and 2020 in ornamental nurseries and pecan orchards. The treatments were 1) wooden bolts infused with locally purchased ethanol, 2) bolts with a nailed ethanol pouch and

3), and bolts with no ethanol bait. Plastic bottle traps baited with ethanol dispensers were added to the design to determine active ambrosia beetle species present during the sampling period. Bolt traps infused with the locally purchased ethanol attracted more ambrosia beetles than other two traps in both years and cropping systems.

In 2019 and 2020, attractancy of aged ethanol pouch lures to ambrosia beetles was tested in ornamental nurseries and pecan orchards. In the ornamental nursey, the treatments were a fresh lure, a 1-, 2-, 3-, and 4-week-old lure, and the lures continuously aged by week from 4 to 8-week old lure treatments. In the pecan orchard the 5-week old lures were replaced every week for up to four weeks. The results of both experiments showed that there was no significant difference in ambrosia beetles, mainly *X. crassiusculus* and *X. germanus*, captures among the treatments. These data suggest that growers do not need to change the ethanol dispensers for up to 8 weeks , which will reduce costs and traps maintenance.