EXPLORING THE ROLE AND IMPACT OF FLATHEADED BORERS (COLEOPTERA: BUPRESTIDAE) AND AMBROSIA BEETLES (COLEOPTERA: CURCULIONIDAE) IN GEORGIA SPECIALTY TREE CROP SYSTEMS AND URBAN LANDSCAPES

by

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ABSTRACT

Trunk boring beetles, such as flatheaded borers (Coleoptera: Buprestidae) and ambrosia beetles (Coleoptera: Curculionidae), are devastating pests of ornamentals, pecan, and tree fruit production. Three projects were conducted in 2021 and 2022 to explore their role in Georgia. On-site trapping of buprestids in specialty crop systems show that a wide range of species and genera are present, with flight occurring from early March to mid-June. The impact of trunk boring beetles in urban landscapes was also examined, revealing that increased high or low temperatures, tree species, and the reduced pervious area surrounding trees may all play a role in driving attacks in these areas. In addition, the mechanism of how permethrin prevents ambrosia beetle damage was explored, with results showing that contact repellency is the primary underlying mechanism.

KEYWORDS: borer, urban insect, heat island, specialty tree crop, ornamental nursery, orchard

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B.S.E.S., The University of Georgia, 2021

B.S.A., The University of Georgia, 2021

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

2022

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December 2022

ACKNOWLEDGEMENTS

I am deeply grateful to my major advisors, Dr. Shimat V. Joseph and Dr. Brett R. Blaauw, for their ongoing encouragement and support throughout my degree program. I also appreciate my committee member Dr. William G. Hudson for his invaluable advice and support. I wish to thank Jordan Bailey and Chris Hardin for their help and technical assistance in my studies, as well as express thanks to labmates and lab assistants who helped to make this work possible.

My appreciation also goes out to my family and friends for their support over the past year and a half.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Specialty crops are commonly known as horticultural crops. Systems involved in this group include fruit, nut, vegetable, and sod production, as well as the ornamental horticulture industry, ranging from nursery to greenhouse production. Together, these industries made up a combined 21.6% of Georgia's 2019 farm gate value (Stubbs 2020). As a result, these industries have a major impact on Georgia's overall economy, providing not only gross income but jobs and resources as well. Thus, it is important to effectively manage the disease and insect risks present in these systems (Frank et al. 2013).

The pecan industry

The pecan industry was established in Georgia in the early 1900s. In 2014, Georgia produced approximately 30% of the nation's pecans (Wells 2014). As the largest pecan producer in the United States, pecan trees, or *Carya illinoinensis* (Wangenh.) K. Koch, are a major specialty crop within Georgia. Nationally, pecan exports have continued to grow, with most pecans produced in the United States being exported to other countries (Asci and Devadoss 2021). Exports have grown by over 2000% since 1980, with the most significant period of increase beginning in 2009 (Wells 2014). Because of this, the pecan industry currently is, and likely will remain, a major source of income in Georgia and the United States.

The apple industry

As a native of central Asia, with the believed center of origin to be in Kazakhstan, apples, or *Malus pumila* (Mill.), have been consumed as early as 6500 bc, where remains characteristic of apple consumption were discovered. As early as 3500 bc, apples were widely circulated throughout Asia and Europe, establishing their role as a popular horticultural crop (Ferree and Warrington 2003). While apples are produced to some degree in all 50 states, production is primarily aggregated in the states of Washington, New York, and Michigan (Slattery et al. 2011). Within Georgia, almost all apple production is in the northern region of the state (Stubbs, 2020). Despite the popularity of apples, national production has been falling in recent decades (Slattery et al. 2011). Apples remain an essential part of Georgia agriculture and agritourism, however, with value going beyond that of fruit production itself.

The peach industry

Peach, or *Prunus persica* (L.) Batsch, is a fruiting woody perennial crop native to China currently grown worldwide (Johnson et al. 2021). Peach production in the United States is primarily confined to the coastal states of California, South Carolina, Georgia, New Jersey, and Washington. A vast majority of Georgia's peaches are used as fresh market crops, with little being used for processing purposes (Scherm et al. 2004). While the peach industry peaked in the 1920s, there have been major declines in production due to problems such as disease and insect issues, as well as labor shortages. Despite this, Georgia currently ranks third in peach production, behind California and South Carolina, and the peach industry remains an important part of the southeastern agricultural economy (Johnson et al. 2021).

Ornamental tree production in Georgia

Ornamental tree production and its associated businesses are a major industry in Georgia, which provides over 50,000 jobs to workers in Georgia, creating a major economic impact felt throughout the state (Kane and Kent 2012). The 2017 USDA Census of Agriculture reported that there are 7,559 acres of nursery stock crop production in Georgia, with a sale value of $160,850,424 (USDA 2019). Production scale ranges from small container-based operations to larger in-ground field operations (Chappell et al. 2012). In the ornamental tree industry, there is an emphasis on plant aesthetics, making insect or disease damage a potentially devastating prospect (Fulcher et al. 2012)

Trees in urban landscapes

With continued growth of human population, urbanization of areas is inevitable, resulting in rapid changes in the landscapes in these expanding areas (Andersson, 2006). Since urban areas are constructed primarily to suit human needs, these spaces have high population density and increased structural development, such as roads and buildings (Pickett et al. 2001). As a result of increased structures and impervious surfaces, such as roads, urban areas often experience warmer temperatures in the form of “heat island” effects. Furthermore, water runoff is increased in these settings due to increased pervious cover, leading to less soil infiltration, a process important in detoxifying pollutants (Pickett et al. 2001, Dale et al. 2016).

Urban vegetation serves an important role in urban ecosystems where trees are vital in improving air quality by reducing temperature through shade and radiation absorption, as well as through purifying air via the uptake of pollutants through stomata (Roy et al. 2012). Urban forests also play a fundamental part in carbon sequestration, storing atmospheric carbon within

their tissues (Nowak, 1993). Furthermore, trees in urban areas also drive increased property values (Pandit et al. 2013).

While trees benefit those living within urban areas, urban forests are often exposed to increased environmental stressors. Increased impervious surface increases temperature, inducing "heat island" effects, limited water uptake, and reduced rootable soil volume (Dale et al. 2016). Furthermore, road salt that is applied in areas with frequent snow may lead to increased soil salinity (Cregg and Dix, 2001). This stress, in turn, makes trees more susceptible to insect and disease attacks, damaging aesthetic values and causing potential tree deaths (Dale and Frank, 2014; Lowe et al. 2019). Despite this, landscapes are often held to a high standard of aesthetic appeal (Braman et al. 1998).

The effect of heat island effects and percent impervious cover on insect abundance

The phenomenon known as the "heat island" effect occurs due to the rapid heating and radiation absorption of urban structures, such as buildings, paved areas, bare soil, and areas with short grass (Kim 2007). As a result, trees are often exposed to increased stress factors, such as moisture stress due to reduced water infiltration or temperature-related stress caused directly by the heat island effect (Cregg and Dix 2001). As insects such as borers and scale insects are more attracted to stressed trees, there is the potential for trees exposed to urban stresses to experience higher rates of insect attack and herbivory (Evans et al. 2004, Frank et al. 2013). Meineke et al. (2013) found that increased urban temperatures lead to higher numbers of the Hemipteran scale insect pest species oak lecanium, *Parthenolecanium quercifex* (Fitch 1859). Furthermore, Dale and Frank (2014) posit that trees in warmer urban areas had a greater incidence of the Hemipteran pest gloomy scale, *Melanaspis tenebricosa* (Comstock 1881), than cooler urban

sites. This idea is expanded upon in Dale et al. (2016), where the effects of impervious surface cover are more directly related to heat and insect effects on urban trees. This study found that while temperature did predict *M. tenebricosa* abundance, percent impervious cover did so more strongly. As a result, this lends credibility to the theory that impervious surfaces in urban areas do more than simply increase temperatures but may also contribute to tree stress by increasing temperature and water stress.

Flatheaded borers in Georgia specialty crop systems

Flatheaded borers (Coleoptera: Buprestidae) are typically wood boring pests, feeding on the bark and wood of trees, whereas some species are leaf miners, feeding within leaf tissues. Among wood-feeding species are those that feed on phloem, residing entirely within the bark, and those that feed on both the xylem and the phloem, moving deeper into the wood of the tree to feed on the sapwood (Evans et al. 2007).

Buprestids are characterized by their distinct larvae. The larvae of these beetles are cream-colored and dorsoventrally flattened, having an enlarged sclerotized thoracic segment that gives them their distinct “flatheaded” appearance (Evans et al. 2007). Adult borers are often bullet-shaped with distinct shiny spots or patterns on the elytra. Below the elytra, the abdomen is also frequently shiny (Frank et al. 2013). The life cycle of all buprestid beetles starts when an egg is laid on the host plant by a female beetle. This egg then hatches, with the larvae mining into the tissue where it will feed, pupate, and subsequently emerge as an adult (Burke 1910).

Flatheaded borers typically attack stressed or damage trees (Evans et al. 2004, Frank et al. 2013). Attacks often start in wounded areas, such as sunburned tissues (Burke 1919). Damage to trees largely occurs due to larval feeding within the tree and the subsequent formation of

feeding galleries (Beddes et al. 2014). This damages the cambium layer, disrupting the transport of water and nutrients throughout the tree's vascular system. While girdling is more likely to occur over consecutive years, one larva has the potential to girdle a small tree in the span of one season (Frank et al. 2013). Damage caused by buprestid beetles varies; however, the most serious injury resulting from their colonization of a tree is the appearance of cankers associated with the presence of larvae, posing both a health risk through possible girdling as well as impacting aesthetics (Seagraves et al. 2012).

Signs of damage resulting from flatheaded borers are characteristic. Cankers formed from larval feeding may be present, as well as exit holes caused by the emergence of adult beetles (Seagraves et al. 2012). Exit holes formed by adult borers are "D" shaped but may sometimes appear as more of a flattened oval (Burke, 1910, Seagraves et al. 2012, Frank et al. 2013). Among flatheaded borers, *Chrysobothris* species are often considered the most serious pests (Hansen et al. 2015).

The *C. femorata* species complex

The *Chrysobothris femorata* (Olivier, 1790) group of flatheaded borer species includes 12 recognized species. As a species complex, beetles within the group are often morphologically indistinguishable. Because of this, the number of *Chrysobothris* species within the group is continuously being amended (Hansen et al. 2015). In 2007, Wellso and Manley published a revision of the group, expanding what was previously 5 recognized species to 12 species. Among those species, eight occur in the southeastern United States, with three remaining species being confined to the western United States, and the remaining *Chrysobothris sloicola* (Manley and Wellso 1976) being found only in Michigan (Wellso and Manley 2007).

Identification of the *Chrysobothris femorata* group is often difficult (Fisher 1942); however, members have a clypeus with a semicircular appearance, forelegs with a row of small teeth in males, and the presence of a defined carina on the female tergite (Hansen et al. 2015). The male aedeagus is often the most reliable characteristic for distinguishing different species of the complex (Hansen et al. 2015). Other phenotypic variation includes differences in antenna shape and color, the overall appearance of the female pygidium, the carina of the female's terminal tergite, and clypeus color, among other variations (Fisher 1942, Wellso and Manley 2007). Furthermore, group members often overlap in range and host preference, further confounding the identification of *Chrysobothris* specimens.

The life cycle of *Chrysobothris femorata*

Like all other members of the *Chrysobothris femorata* species group, the actual singular species *C. femorata,* commonly known as the flatheaded appletree borer, possesses a semicircular clypeus, a toothed margin of the male foreleg, and carina on the female's terminal tergite (Hansen et al. 2015). The larvae of *C. femorata*, similar to many other buprestid beetles, feed on the wood beneath the bark, where it damages the vascular cambium, phloem, and outer sapwood of trees. There is one *C. femorata* generation per year (Potter et al. 1988). While the timing of the life cycle of *C. femorata* varies slightly by region, eggs are typically deposited in crevices in the bark of host trees. Upon hatching, the larva then bores directly into the trunk of the tree (Burke et al. 1919). Larvae overwinter in the wood of the tree, pupating the following spring to ultimately emerge and take flight as adults (Potter et al. 1988, Oliver et al. 2010). *C. femorata* has a wide host range, with Dawadi et al. (2019) reporting that more than 30 tree species are attacked by *C. femorata*.

Ambrosia beetles in Georgia specialty crop systems

Ambrosia beetles (Coleoptera: Curculionidae) are trunk boring beetles that colonize host plants with a symbiotic fungus. This associated fungus is carried in a groove known as a mycangium and subsequently introduced into the walls of brood chambers to serve as food for larval and adult ambrosia beetles (Ngoan et al. 1976). As nearly 60 species of ambrosia beetles are present in the United States, the group possesses a wide range of potential hosts (Miller et al. 2018). Host plants of ambrosia beetle species encountered in Georgia are incredibly numerous, with common hosts including pecan, *Carya illinoinensis*, maples, *Acer* spp., and dogwoods, *Cornus* spp. (Ngoan et al. 1976). Because of this, ambrosia beetles are a pest of particular concern to Georgia's specialty crop production.

Mated adult female beetles bore into the trunks of host trees, excavating galleries that will then become inoculated with spores of the fungal *Ambrosiella* spp. symbiont that is carried in the mycangia of the beetles (Ngoan et al. 1976). Eggs are then laid in the brood chambers where the larval stages develop, feeding on the fungus before pupation. The mature females mate with their brothers within the galleries, and then overwinter as mated adults (Addesso et al. 2019). These female beetles then emerge from the trees to find a new host tree, with the first emergence occurring in spring (Mizell and Riddle 2004, Frank et al. 2013).

As with many wood-boring insect pests, the introduction of exotic ambrosia beetle species commonly occurs at Ports of Entry, particularly due to the transport of wood packing material (Hanula et al. 2008, Rabaglia et al. 2008). Among those introductions are some of the ambrosia beetles most impactful to Georgia specialty crop systems, including *Xylosandrus crassiusculus* (Motschulsky, 1866) and *X. germanus* (Blandford, 1894) (Reding et al. 2011, Monterrosa et al. 2021).

Ambrosia beetles prefer to attack stressed trees, with ethanol serving as a primary attractant (Ranger et al. 2010, Reding et al. 2011). Because of this, even trees that may appear healthy can be attacked due to the release of stress volatiles (Reding et al. 2016). As is typical with ambrosia beetle feeding and colonization, damage of ambrosia beetles is evident through appearance of "toothpicks" of frass and sawdust that have been extruded from the trunk of the tree, as well as the presence of small entrance/exit holes on the trunk of the tree (Monterrosa et al. 2021).

Longhorned beetles in Georgia specialty crop systems

Longhorned beetles (Coleoptera: Cerambycidae) are phytophagous insects that most often feed on the wood of trees. Some feed solely on the bark, while others move into the wood of the tree while feeding (Evans et al. 2004). Longhorn beetles colonize a wide range of hosts, including conifers, deciduous trees, fruit trees, and even bushes or herbaceous plants. Some species may also bore into other plant components, such as twigs or roots (Cocquempot and Lindelöw 2010). While cerambycid beetles are often forest pests, they may also have impacts on specialty crop systems (Miller and Asaro 2005).

Among the most newsworthy longhorned beetles in the southeast is the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky, 1853). As an exotic pest native to mainland China and Korea, this insect likely arrived through wood packing materials associated with global trade (Hajek 2007, Hoebeke 2007). These insects were first discovered in the summer of 1996 in Brooklyn, NY, USA. However, it is believed that they existed undetected in the United States before that time. (Hoebeke 2007). The adult female lays an egg on the trunk of the tree, with larvae boring and overwintering in that wood. Upon the completion of pupation, adults

emerge from the tree around mid-summer, creating large round exit holes (Collinge et al. 2001, Hajek 2007). Like many other longhorned beetles, *A. glabripennis* typically have a relatively prolonged life cycle of about a year; however, they may need two years to reach full maturity (Hoebeke 2007). Typical damage includes exudation of sap from oviposition sites, bark splitting as a result of larval feeding, round exit holes from adults, and scraping or scratching of bark resulting from adult feeding. Among those damage types, the bark splitting from larvae is typically the most serious threat that trees may experience due to *A. glabripennis* (Haack et al. 2010, Coyle et al. 2021).

As with other cerambycids*, A. glabripennis* are attracted to plant stress volatiles. A highly polyphagous species, there are dozens of reported hosts (Haack et al. 2010). Preferred hosts include common street trees, such as elm, ash, willow, popular, birch, and maple (Hajek 2007, Wang 2017). While these insects are not currently known to be present in Georgia, they have been found in the southeast. As the southernmost incident of infestation, the discovery of *A. glabripennis* beetle in May 2020 in Hollywood, South Carolina, USA, may pose a serious threat to southeastern specialty crops due to the potential to spread to nearby states (Coyle et al. 2021).

Management of borers in Georgia specialty crop systems

Early detection of borers is one of the most important facets of management (Miller et al. 2015). As flatheaded borers, ambrosia beetles, and longhorned beetles all attack stressed trees, limiting tree stress is essential to mitigate attacks on trees (Ranger et al. 2010, Frank et al. 2013, Reding et al. 2016). Because of this, proper irrigation and tree management are critical (Cregg and Dix 2001). Furthermore, site selection to avoid flooding should be a priority when establishing new plantings (Frank and Ranger, 2016).

Monitoring for flatheaded borers is an important component of efficient management (Frank et al. 2013). Sticky traps, often in the form of a panel or "tree" shape, prove effective in capturing adult beetles in flight (Oliver et al. 2004). After the detection of adults, trunk sprays of chemicals, such as pyrethroids, may be used to kill adults, preventing oviposition, however this may be impractical due to concerns regarding adequate coverage (LeBude and Adkins 2014, Dawadi et al. 2019). The application should take place within one week of the detection of adults (Potter et al. 1988). Systemic insecticide drenches may prove more effective, killing larvae within the tree. Once an immature flatheaded borer is established within a tree, contact insecticides will have no activity on the insect (Frank et al. 2013).

As ambrosia beetles feed within the tree, management most occur before colonization of the host tree (Monterrosa et al. 2021). In addition, systemic insecticides have little effect as the beetles feed on the *Ambrosiella* fungus rather than tree tissue. If colonization has occurred, there are few available options in terms of effective interventions (Mizell and Riddle 2004). Because of this, it is recommended to monitor adult flight by utilizing ethanol-baited traps such as Baker soda-bottle traps (Frank et al. 2013). By understanding adult flight times, targeted applications of contact insecticides such as pyrethroids may have utility through the prevention of female colonization of host trees (Frank and Sadof 2011, Reding et al. 2016). Semiochemicals like verbenone have been demonstrated to have some degree of repellent activity against *Xyleborus glabratus* (Eichhoff, 1877), the laurel wilt vectoring ambrosia beetle, particularly using the "Push-Pull" strategy. Through pushing ambrosia beetles away using the anti-aggregation inducing verbenone, beetles are pulled toward ethanol-baited traps (Rivera et al. 2012).

Management of longhorned beetles is closely aligned with that of other insect borers. Interventions largely depend on the use of contact insecticides to prevent colonization, with the

only activity on larval stages achieved through systemic insecticide drenches to the soil surrounding trees (Grosman et al. 2006, Poland et al. 2006). In terms of the Asian longhorned beetle, quarantine is one of the recommended methods for preventing the spread of the insect to new areas (Haack et al. 2010).

Objectives

Because of the economic and aesthetic implications associated with various trunk boring insects, the objectives of the specific graduate research program are as follows: 1) Explore the incidence and abundance of adult flatheaded borers in woody ornamental nurseries, pecan production, and tree fruit orchards. 2) Identify the risk factors associated with the incidence of various borers attacking trees in urban landscapes. 3) Determine the action and potential repellecy of pyrethroids in management of ambrosia beetles in ornamental nurseries. Through exploration of these specific topics, it is proposed that improved management and understanding of these insect pests and the specific specialty crop systems they are involved with may be achieved.

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CHAPTER 2

INCIDENCE AND ABUNDANCE OF FLATHEADED BORERS IN GEORGIA SPECIALTY TREE CROP SYSTEMS[1]

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Abstract

Flatheaded borers (Coleoptera: Buprestidae) are wood boring insects that utilize a wide range of host trees.. Larvae of flatheaded borers tunnel into the trunks of trees, with boring and feeding damage appearing as large cankers. These insect pests pose a serious risk to specialty tree crops in Georgia, with *Chrysobothris* species, such as *Chrysobothris femorata* (Olivier), damaging various tree species. Despite this, studies relating to buprestids in Georgia are limited, with no previous studies focusing on their presence in specialty crop systems. In 2021 and 2022, a study was conducted to trap buprestids flying in or near ornamental nurseries, pecan orchards, and tree fruit orchards. A total of 79 and 119 buprestid beetles were captured in the 2021 and 2022 trapping seasons, respectively. Flatheaded borer genera collected include *Acmaeodera, Agrilaxia, Agrilus, Anthaxia, Brachys, Chrysobothris*, and *Ptosima* species. Nine species of *Chrysobothris* were collected throughout the study. A greater number of buprestids were collected from ornamental and pecan sites than from tree fruit sites in 2022; however, this was not true for the 2021 trapping season. Adult beetles emerge by early March to mid-June, as evident from 2021 and 2022 trapping data. Results suggest that flatheaded borers are widespread in Georgia specialty crops, and growers should consider managing flatheaded borers in the early spring.

Keywords: Buprestidae, *Chrysobothris*, *Chrysobothris femorata*, specialty crops, pecan, tree fruit, ornamental nursery

Introduction

Specialty crops, commonly known as horticultural crops, made up a combined 21.6% of Georgia's 2019 farm gate value, with ornamental nurseries, pecan, peach, and apple production having farmgate values at ~ $360, $263, $71, and $11 million USD in 2019, respectively, and together valued at ~ $706 million USD in 2019 (Stubbs 2020). Flatheaded borers (Coleoptera: Buprestidae) are an economically important insect pest group of fruit, nut, and ornamental trees (Burke 1919, Wellso and Manley 2007). Stressed trees are particularly at risk for *Chrysobothris femorata* (Olivier) attack, especially trees that have recently been planted, are experiencing drought stress, or possess wounds to the trunk (Oliver et al. 2010). Buprestid adults do not directly feed and cause economic damage. Larval feeding instead creates tunnels in the trunk, which may cause tree mortality (Dawadi et al. 2019a). Scarring and cankers are often formed on the attacked tree trunk, and tree mortality may occur after successive years of flatheaded borer larval feeding and tunneling (Dawadi et al. 2019a, Oliver et al. 2010). However, a single larva can girdle and kill a tree in one season. Damage is typically observed in the following fall and early spring (Dawadi et al. 2019b).

The adult flatheaded borer female singly oviposit 20 to 170 eggs in cracks or sunscald spots on the tree trunk (Maxwell 1937). The emerging larvae burrow into the tree, consuming the cambium, xylem, and phloem tissues (Davis et al. 1968, Lewis 1987). The larvae are dorsoventrally flattened with an enlarged and sclerotized thoracic segment arising behind the head capsule (Evans et al. 2007). Larvae overwinter in the trunk of the tree and pupate in the following spring (Burke 1919, Potter et al. 1988). The adults emerge out of the tree trunk after chewing the bark, leaving characteristic "D" or oval-shaped exit holes (Burke 1919, Potter et al. 1988). They undergo one generation per year (Dawadi et al. 2019a).

Although *C. femorata* has been reported in Georgia (Wellso and Manley 2007), most other species of buprestids are insufficiently reported from Georgia specialty crop systems. Furthemore, the phenology of adult flights of buprestids has not been systematically studied in Georgia specialty crops. This information is critical to determine risk periods for management programs to combat flatheaded borers. Currently, managing adults and larvae of buprestids involves trunk sprays of pyrethroids and drench application of neonicotinoids such as imidacloprid in field nurseries (Addesso et al. 2020). Similarly, little is known about buprestids that actively attack trees in Georgia nurseries. Because the aesthetic appeal is the central component of ornamental trees in nurseries, understanding the phenology of buprestids is essential and the first step to developing monitoring and management options (Dawadi et al. 2019b). Thus, the objectives were to determine 1) the species complex of buprestids present and 2) their phenology in ornamental tree nurseries, pecan production areas, and tree fruit orchards.

Materials and Methods

Study sites. To determine buprestid occurence in specialty crops, 18 and 17 sites were selected in 2021 and 2022, respectively. Of the 18 sites, six were in ornamental tree nurseries, five were in fruit tree orchards, one in tree fruit-pecan mixed orchards, and six in pecan orchards. Similarly, of 17 sites selected in 2022, six sites were in ornamental tree nurseries, four in fruit orchards, five in pecan orchards, and two in pecan-tree fruit mixed orchards. Two pecan sites used in 2021 were not accessible in 2022 but were replaced with two new ones (Table 2.1, Fig. 2.1). Furthermore, one tree fruit site was relocated to a pecan--tree fruit mixed site between the 2021 and 2022 trapping season. Sites were selected based on commodity. The ages of trees in selected sites were mostly < 5 years old. The tree fruit sites were primarily located in northern

Georgia, USA, whereas pecan sites were mostly in southern Georgia, USA (Fig 2.1). The ornamental nurseries were mostly located in mid-Georgia, USA. Most of the sites were adjacent to wood lots. The tree species in woodlots were red maple, *Acer rubrum* L., pignut hickory, *Carya glabra* (Mill.), American sweetgum, *Liquidambar styraciflua* L., loblolly pine, *Pinus taeda* L., and post oak, *Quercus stellata* Wangenh. Longleaf pine, *Pinus palustris* Mill., was common in the surrounding sites in the southern region of the study. The sites were subdivided by the zones of North, middle, and South, as abiotic factors such as daily temperature and precipitation varied from North to South during the growing season.

The crops in most of the sites were managed according to standard production recommendations specific to the commodity. However, two tree fruit and one pecan-tree fruit mixed site in 2021, and one tree fruit and two pecan-tree fruit mixed sites in 2022 did not receive any standard management input, such as pesticide and fertilizer applications.

Sampling plan. Three purple sticky panel traps (AgBio Inc. Westminster, Colorado, USA; Product Code P577-Trap) were deployed in each site from the second or third week of March to the third or fourth week of July in 2021 and 2022. The traps were installed with the support of two 1.8 m long wooden stakes. About 0.3 m of the stake was buried into the ground. The purple sticky traps were attached between the top of the two wooden stakes using large binder clips; thus, the traps were 1.5 m above the ground. The purple trap was sticky on both sides. The trap was 20.3 cm ×25.4 cm (width and length) with ~883cm^2 of sticky surface (Fig. 2.2). These sticky panel traps were deployed along the wood line using 20 m spacing between traps. The purple sticky panel traps were selected because previous studies demonstrated increased attraction and *Chrysobothris* beetle captures to shades of purple (Perkovich et al. 2022). Moreover, purple

sticky panel traps are commercially available for purchase to growers and researchers for monitoring. The traps were oriented facing the wood lot and the production area. The purple sticky panel traps were exposed for two weeks and replaced with new sticky panels after being deployed for those two weeks.

The purple sticky panel trap was used along with a benzaldehyde lure (100% benzaldehyde, Product Code P065-LureB, AgBio Inc. Westminster, CO). The benzaldehyde lure is commercially available and was previously used as an attractant for *Chrysobothris* (JO, unpublished data). The benzaldehyde lure was fastened to the purple sticky panel trap using a twist tie affixed to the binder clips. The lure consisted of 740-750 mg of benzaldehyde loaded onto fiberboard before being enclosed in a controlled release membrane. These lures can be effective up to 60 d, but in the current study, they were only used for 30 d before being replaced with a fresh lure.

The panel traps were monitored for buprestid beetles at biweekly intervals. At biweekly intervals, the exposed trap was removed from the wooden stakes and wrapped using cling wrap (GLAD, The Glad Products Company, Oakland, California, USA) and stored until final processing at -18 °C. The traps were viewed weekly for large contaminants, such as leaves or larger insects, such as moths and butterflies (Lepidoptera). If necessary, contaminated traps were replaced with new panel traps. The benzaldehyde lures were replaced monthly at all sites.

Evaluation and Identification. Panel traps were evaluated for the presence of buprestid beetles at 14 d intervals as they were exposed for 14 d between replacements. While processing panels for buprestids, they were removed as we attempted identification to genus or species. To remove beetles from the glue of the sticky panel, 5 mL of Histo-Clear (Electron Microscopy Sciences,

Hatfield, Pennsylvania, USA) was applied. After 10 min of exposure, beetles were easily detached from the glue, carefully extracted from the panel traps using pointed forceps, and placed in vials with 30 mL of Histo-Clear. The vials were placed on an orbital shaker overnight at 125 RPM for 15 h. After removing them from the shaker, beetles were rinsed using molecular grade 100% anhydrous ethanol (Decon Laboratories, Inc. King of Prussia, Pennsylvania, USA) and stored in ethanol for identification.

All buprestid beetles were identified to genus using Paiero et al.'s field guide to jewel beetles (2012). As *Chrysobothris* is considered the most economically important genus (Hansen et al. 2015), they were identified to species using Wellso and Manley's revision to the *Chrysobothris femorata* (Olivier) complex (2007). The sex of the *Chrysobothris* specimens was confirmed with the presence of the male aedeagus or female pygidium. Species-level identification was confirmed by a coleopteran taxonomist at the Georgia Museum of Natural History, Athens, Georgia.

As trapping was initiated over two weeks, with different start days between sites, results are presented as pairs of weeks corresponding with trapping initiation. These weeks are based on the standard week numbering system of the Julian calendar.

Statistical analyses. All statistical analyses were conducted utilizing R software (R Core Team 2022). Cumulative Buprestid captures were analyzed separately for 2021 and 2022 by zone (North, Central, and South) and production system (Tree fruits, pecan, and ornamentals). Sites with tree fruit-pecan mixed production were omitted from the production system analysis. Because data were not normally distributed, the Kruskal Wallis test was conducted with the "kruskal.test" function of R. Analysis was conducted using cumulative buprestid captures as the

response variable and either zone or production system as the dependent variable. Post hoc pairwise comparison of groups was then conducted when the Kruskal Wallis test was found to be significant using Dunn's Test through the "FSA" package and "dunnTest()" function of R using the "holm" method of p-adjustment. An α value of 0.05 was used for all analyses.

Results

Buprestid captures by zone and commodity. In 2021, a total of 79 buprestid beetles were captured in the 2021 trapping season from 10 sites. Northern sites had no buprestid captures (Fig. 2.3a). Central sites had 39 buprestid captures with 25 *Acmaeodera*, 1 *Agrilaxia*, 3 *Agrilus*, 1 *Brachys*, and 9 *Chrysobothris* (Fig. 2.3b). Southern sites had 40 captures, with 7 *Acmaeodera*, 13 *Agrilus*, and 20 *Chrysobothris* (Fig. 2.3c). The number of buprestids collected was significantly greater for the central zone than for the northern zone ($\chi^2 = 8.7$, df = 2, $P = 0.012$; Fig 2.4a), The buprestid count collected in the southern zone was not significantly different from either the northern or central zones.

Among cropping systems in 2021, ornamental nursery sites had 36 buprestid captures with 24 *Acmaeodera*, 1 *Agrilaxia,* 3 *Agrilus*, 1 *Brachys*, and 7 *Chrysobothris* collected from traps (Fig. 2.3d). Pecan sites captured40 buprestid**s** ,including 7 *Acmaeodera*, 13 *Agrilus*, and 20 *Chrysobothris* (Fig 2.3e). Tree fruit production sites had no buprestid captures (Fig. 2.3f). The effect of commodity was not significantly different among cropping system ($\chi^2 = 4.3$, df = 2, $P = 0.117$; Fig 2.4b).

In 2022, a total of 119 buprestid beetles were trapped from 12 sites. Northern sites had 13 buprestid captures with 10 *Agrilus*, 2 *Chrysobothris*, and 1 *Ptosima* (Fig. 2.3a). Central sites had 56 buprestid captures with 19 *Acmaeodera*, 1 *Agrilaxia*, 12 *Agrilus*, 4 *Anthaxia*, and 20

Chrysobothris (Fig. 2.3b). Southern sites had 50 buprestid captures with 17 *Acmaeodera*, 2 *Agrilus*, and 31 *Chrysobothris* (Fig. 2.3c). The effect of zone on buprestid captures was significantly different ($\chi^2 = 6.6$, df = 2, $P = 0.036$; Fig 2.4a). However, pairwise comparisons from Dunn Test were not significantly different ($P > 0.05$), although nonadjusted *P*-values displayed significant differences between groups. This may be caused by low sample size and corrections for pairwise comparisons, as only three southern sites existed. Therefore, we cannot conclusively determine which zones were different.

Ornamental nursery sites in 2022 had 34 buprestid captures in the following genera: 9 *Acmaeodera*, 12 *Agrilus*, 1 *Anthhaxia*, 11 *Chrysobothris*, and 1 *Ptosima* (Fig. 2.3d), whereas 66 buprestids were collected from pecan sites with 18 *Acmaeodera*, 9 *Agrilus*, 2 *Anthaxia*, and 37 *Chrysobothris* (Fig. 2.3e). From tree fruit sites, only one *Agrilus* was captured (Fig 2.4f). The numbers of buprestid captures from pecan and ornamental commodities were significantly greater than those from tree fruit ($\chi^2 = 8.5$, df = 2, $P = 0.014$; Fig 2.4b).

***Chrysobothris* captures by species and sex.** Of 29 *Chrysobothris* specimens captured in 2021, they were 3 male *Chrysobothris adelpha* (Harold), 6 female *Chrysobothris chrysoela* (Illiger), 1 female *Chrysobothris cribraria* (Mannerheim), 2 male and 3 female *Chrysobothris femorata* (Olivier), 3 male and 2 female *Chrysobothris quadriimpressa* (Gory and Laporte), 1 male *Chrysobothris rugosiceps* (Melsheimer), 4 female *Chrysobothris sexsignata* (Say), and 1 male and 2 female *Chrysobothris viridiceps* (Melsheimer) (Fig 2.4). In 2022, among 53 specimens of *Chrysobothris* captured, they were 4 male and 7 female *C. adelpha*, 6 female *C. chrysoela*, 3 female *C. cribaria*, 12 female and 1 male *C. femorata*, 2 female and 4 male *C. quadriimpressa*, 1

male and 1 female *C. rugosiceps*, 4 female *C. scitula* Glory, 1841, 4 female and 1 male *C. sexsignata*, and 3 female *C. viridiceps*.

Buprestid captures by time and zone. In 2021, captures of buprestids first peaked around weeks 15-16 and 17-18 (mid-April to early May) of the year in central and southern sites, respectively. A second lower peak occurred at around 21-22 and 23-24 weeks (late May to mid-June) of the year in both central and southern sites. Some buprestids were captured at the initial sampling date in the southern and central sites in late March. No captures were recorded in northern sites (Fig. 2.6a). *Chrysobothris* captures were similar to overall buprestid captures, with peak captures at 15-16 and 17-18 weeks in central and southern sites (mid-April to early May), then a follow-up peak at 21-22 and 23-24 weeks (late May to mid-June) in central and southern sites (Fig. 2.6c).

In 2022, captures of buprestids in the central zone peaked in weeks 15-16 (mid-April), and captures continued at the same level up to weeks 21-22 (Late May to early June). Central sites noticed the second peak at weeks 27-28 (early to mid-July). In southern sites, the pattern of buprestid captures was similar between 2021 and 2022. The buprestids captures were initially high at 13-14 weeks, then tapered off by 15-16 weeks (late March to mid-April). At weeks 17-18 and 23-24 (late April to early May and early to mid-June), buprestid captures peaked for a second and third time in southern sites. Far fewer buprestids were captured in northern sites relative to central or southern sites, with one peak at weeks 15-16 through 19-20 (mid-April to mid-May; Fig. 2.6b). In 2022, *Chrysobothris* captures in the central region peaked in weeks 19-20 (mid-May) and then held at the same level in the following weeks up to weeks 27-28 (early to mid-July). The number of *Chrysobothris* captures was comparatively lower in northern sites,

with one beetle being collected in weeks 17-18 and one in weeks 19-20 (late April to mid-May). Southern *Chrysobothris* captures were similar between 2021 and 2022. Captures were initially high at weeks 13-14 (late March to early April), indicating beetles were already flying, before tapering off in weeks 15-16 (mid-April). Captures of *Chrysobothris* peaked again at weeks 17-18 (late April to early May), with another final peak in weeks 23-24 (mid-June; Fig. 2.6d).

Buprestid captures by time and commodity. In 2021, buprestid captures in pecan and ornamental sites experienced a peak in weeks 15-16 through 17-18 (mid-April to mid-May; Fig. 2.7a). A second peak was observed in weeks 21-22 through 23-24 (late May to mid-June). *Chrysobothris* captures in both pecan and ornamental sites had two distinct peaks, one in weeks 17-18 (late April to early May) and another in weeks 21-22 (late May to early June). However, captures were relatively lower in ornamental sites than in pecan sites. Pecan sites appear to have an earlier emergence before the trapping period began in 2021 (Fig. 2.7c).

In 2022, buprestids were captured at the first sampling date of weeks 13-14 (late March to early May) in pecan. Follow-up peaks were observed at weeks 17-18 and 23-24 (late April to early May and early to mid-June). Trap captures of buprestids in tree fruit sites were much lower, with only one beetle captured at weeks 19-20 (mid-May). Buprestid captures in ornamentals sites were consistent from weeks 15-16 (mid-April) and captures slowly tapered off by weeks 25-26 (mid-June to early July; Fig. 2.7b). In 2022, the first *Chrysobothris* capture in pecan sites occurred during the first sampling weeks. After that, captures were recorded in weeks 17-18 and 23-24 (late April to early May and mid-June). In ornamental sites, *Chrysobothris* captures were relatively constant throughout the season, with captures occurring in weeks 15-16 through 27-28 (mid-April to mid-July; Fig. 2.7d).

Discussion

Seven genera of buprestid adults were trapped from various cropping systems in Georgia, and they were *Acmaeodera* spp., *Agrilaxia* spp., *Agrilus* spp., *Brachys* spp., *Chrysobothris* spp., *Ptosima* spp. and *Anthaxia* spp.. Among them, *Acmaeodera* spp., *Agrilus* spp., and *Chrysobothris* spp. were collected the most. *Acmaeodera* spp. is not considered a pest on specialty crops and was previously collected in funnel traps from southeastern wood lots (Miller and Crowe 2011). Two *Acmaeodera* species have been reported in the eastern US,and they were *Acmaeodera pulchella* Herbst and *Acmaeodera tubulus* Fabricius (Miller and Crowe 2011, Paiero et al. 2012). Despite this, larval hosts have only been recorded for *A. tubulus,* which colonizes a wide variety of tree species, including *Acer* spp., *Ulmus* spp., and *Carya illinoinensis* (Wangenh) K. Koch (Paiero et al. 2012). This genus is largely considered as borers of dead wood and small twigs, thus is likely of minimal concern in any specialty tree crop production system, despite frequent captures (Westcott et al. 1979). *Agrilus* species have historically been regarded as pests of forest trees. With some exceptions, this genus infests woodland trees such as *Acer* spp*., Quercus* spp*.*, *Aesculus* spp*.*, *Carya* spp., *Ulmus* spp., *Celtis* spp., *Diospyros* spp*., Juglans* spp*., Cercis* spp., and *Gleditsia* spp*.* as larval hosts. Few *Agrilus* species have been recorded on *Malus domestica* (Suckow) Borkh, *C. illinoinesis*, or *Prunus persica* (L.) Batsch as larval hosts (Paiero et al. 2012). Therefore, their impact is likely greater in nursery production systems where some of thesetrees are in sub-urban and urban landscapes; however, the economic damage is likely limited due to their larval feeding primarily occurring in stems of small trees and shrubs (Kajihiro and Millspaugh 1950). Notable species in this group include the native bronze birch borer, *Agrilus anxius* Gory, which serves as a serious pest of *Betula* spp. (Akers and Nielsen 1984) and the exotic emerald ash borer, *Agrilus planipennis* Fairmaire, which has served as a

devastating pest of *Fraxinus* in the eastern USA (Sydnor et al. 2007). Other less frequently captured genera include *Anthaxia, Agrilaxia, Brachys*, and *Ptosima*. *Agrilaxia* and *Anthaxia* species share a similar host range as *Agrilus*, with larvae primarily impacting forest trees (Paiero et al. 2012). Although only one specimen was captured in the current study, *Brachys* species typically serve as leaf miners of various *Quercus* species (Paiero et al. 2012). As long-term trunk damage is limited, this group is of little concern in specialty tree crop settings. However, some aesthetic damage may become evident due to larval feeding. Finally, only one *Ptosima* specimen was collected. *Ptosima* larvae have previously been described to feed on *Cercis candensis* L., *Sassafras albidium* Nutt., *Prunus serotinia* Lindl., and *Robinia pseudoacacia* L. (Paiero et al. 2012).

Nine species of *Chrysobothris* were collected from the Georgia cropping systems, making it one of the most widely captured groups. *Chrysobothris* species, particularly those within the *Chrysobothris femorata* complex, have a wider host range than the aforementioned buprestid genera (Paiero et al. 2012). Larvae of the *Chrysobothris femorata* species group captured in the study have been shown to utilize the commonly grown ornamental tree genera of oak, *Quercus*, maple, *Acer*, elm, *Ulmus,* redbud, *Cercis*, beech, *Fagus,* and birch, *Betula,* species in addition to pecan, *C. illinoinensis, Prunus,* the genus containing stonefruits such as peaches and plums, and *Malus* species, the genus containing apples (Hansen et al. 2011, Paiero et al. 2012). Thus, they are among the most damaging Buprestids captured in our trapping efforts. Larval hosts remain unknown for many other *Chrysobothris* species, with records for *C. scitula* and *C. chrysoela* primarily being collected by the buprestid preying wasp *Cerceris fumipennis* Say or on purple prism traps (Nalepa et al. 2015, Westcott and Thomas 2015). *Chrysobothris sexsignata, C. viridiceps, and C. adelpha* all share similar larval hosts with the *Femorata*

complex. *Chrysobothris rugosiceps* has been shown to attack *Quercus* spp. and some *Pinus* spp. but has not been recorded on other specialty crop trees (Paiero et al. 2012).

Among *Chrysobothris* species, *Chrysobothris femorata* is considered the most economically important of the species captured, serving as widely polyphagous pests of specialty tree crops (Paiero et al. 2012, Frank et al. 2013, Addesso et al. 2020). Other members of the *Chrysobothris femorata* group, *C. rugosiceps* Melsheimer, *C. viridiceps* Melsheimer, *C. quadriimpressa* Gory and Laporte, *C. adelpha* Harold, and *C. femorata* are hard to determine through morphological characters as limited identification keys are available. Features are often indistinct and subjective, with the *C. femorata* species complex recently undergoing an expansion of species in 2007 (Wellso and Manley 2007). Because certain key characters, overlapping hosts, and ranges are shared among *Chrysobothris* species, there is a high probability of interbreeding between species currently considered distinct (Hansen et al. 2015, Klingeman et al. 2015). Thus, the collection of *Chrysobothris* species may help to play an important role in species-level characterization through molecular techniques (Klingeman et al. 2015). Overall, the results suggest that a diverse complex of genera and species of buprestids occur in tree fruit, pecan, and ornamental cropping systems in Georgia. Buprestid species are not widely reported because monitoring of buprestids has been difficult due to a lack of response to commercially available attractants (Oliver et al. 2004). They are still important in specialty tree crops despite rarely being studied extensively. Stakeholders indicated that among commonly experienced damage insect pests, wood boring insects, such as buprestid beetles, are the second most important cause of concern in nurseries (Fulcher et al. 2012). Wounds from flatheaded borers are not only unsightly, potentially deeming ornamental trees nonsellable, but may also

cause vascular damage or allow secondary infection by pathogens, which may lead to eventual tree death (Adkins et al. 2010).

Although buprestid captures were similar among cropping systems in 2021, in 2022, more beetles were collected in pecan and ornamental sites. Similarly, the central and southern regions of the state captured more buprestids than the northern region in 2021, but this was not true in 2022. These results are consistent with previous research where considerable variation of flatheaded borer captures between nurseries and years was observed (Potter et al. 1988). Although the exact reasons for this pattern are unclear, there could be a possible explanation. Wood borers are particularly attracted to stress signals or plant volatiles (Evans et al. 2004, Grossnickle 2005, Frank et al. 2013). The pecan sites included in the current study were primarily located in the southern region of the state. This region was heavily impacted in 2018 by hurricane Michael, and a large proportion of the state's pecan trees were either damaged or destroyed. Recently, more young pecan trees have been planted in the state, perhaps related to replacing destroyed or damaged trees (Dorfman et al. 2018). Usually, newly planted trees undergo severe stress. Because many orchards are planted with young trees in a relatively small spatial range in a short time frame, stress volatiles released by these trees likely attracted more buprestids to the area, especially *Chrysobothris* (Evans et al. 2004, Grossnickle 2005, Frank et al. 2013). Similarly, field and container ornamental nurseries continuously plant liner or bare root trees, and these young trees that are likely under stress could contribute to greater buprestid activity, and trap captures in ornamental systems (Grossnickle 2005). Young trees in container nurseries are also susceptible to stress from water deprivation. Similarly, the dark containers absorb more heat, increasing root and soil temperatures and releasing stress volatiles (Mathers 2003).

In general, trends in buprestid adult emergence and *Chrysobothris* species were similar. In southern sites, where pecans were primarily grown, buprestid emergence was noticed in early March, with a few subsequent peaks. This indicates that buprestids may be active earlier in the season, pre-dating the initiation of the trapping period. In the central region, where ornamental nurseries were primarily found, the first buprestid emergence occurred in mid-March, followed by another subsequent peak. In contrast, in the northern region, where tree fruit is primarily grown, the first emergence occurred in late April to early May. This is likely due to differences in local weather, with the temperature being lower in northern areas of the state where tree fruit production is primarily located, potentially shortening their period of activity (Fig. 2.6). Potter et al. (1988) found that *Chrysobothris femorata* emergences in Kentucky occurred in May or June, depending on the year, with differences in emergence being attributed to local weather. Similarly, *Agrilus macer* LeConte was captured in Augusta, Georgia, mid to late June of 2016 (Poole et al. 2019). *Agrilus ruficollis* (Fabricius) emergence ranged from late April to early June in 1985 through 1987, depending on the year, with temperature and growing degree days impacting emergence (Johnson and Mayes 1989). These results were corroborated by a 1981-1983 study by Akers and Nielsen (1984), where temperature and heat unit accumulation was used to accurately time the emergence of *Agrilus anxius*. The results from the current and prior studies suggest that the emergence of flatheaded borers and *Chrysobothris* are subject at least in part to local weather, with species likely playing a role as well. Timing of adult emergence is important information for specialty crop growers as management for flatheaded borers is largely based on preventative applications by preventing the oviposition and subsequent larval development (Addesso et al. 2020).

Although the current study is the first to explore flatheaded borers in specialty tree crops, buprestids have been sampled from various regions within Georgia, primarily in forest systems. Those studies did not cover agricultural production areas in Georgia. Clearly, a comprehensive survey and intensive sampling would help us understand the broader range of buprestids in the state's agricultural zones. Also, the traps in the current study were monitored at biweekly intervals, where the precision of adult flight is not narrowed down to a specific week. Additionally, in the current study, we did not obtain any specific buprestid species and host-plant associations for accurately determining economically important species of buprestid and *Chrysobothris* species, which warrants future research. The purple panel traps were only used to sample emerging adults of buprestids in the current study. It is unclear if this trapping method creates any unknown bias in the species sampled. Thus, more effective monitoring tools should be developed so that they can be used alongside panel traps to sample buprestid and *Chrysobothris* species in specialty crop settings.

In summary, the study sampled seven genera of buprestid adults and nine species of *Chrysobothris* from tree fruit, pecan, and ornamental cropping systems. The temporal sampling indicated that the first peak flight of buprestids was initiated by early March in the southern region, followed by the central and northern regions of Georgia. Buprestid captures tend to be more abundant in central and southern than in northern regions, perhaps associated with local temperatures. Because buprestids, such as those in the *Chrysobothris femorata* complex, remain a formidable pest group to specialty tree crops (Burke 1910), more research is warranted to determine the species-host associations to develop and refine integrated pest management tailored to specific cropping systems. Effective trapping tools are much need to nullify any potential bias associated with existing trapping tools in determining the flight pattern of specific

buprestid species for monitoring and pest management decisions. Future studies may include surveillance of actual damage to trees to determine the severity of buprestids in Georgia specialty tree crops rather than adult emergence alone. Based on the current study, a diverse species complex of buprestids occurs and emerges in spring or early summer in most of the specialty crop systems. The production sites in central and southern regions are particularly at risk from buprestid infestations, and management tactics should be applied in spring targeting new emerging buprestids.

Acknowledgments

We thank J. Bailey, A. Burnett, J. Carter, J. Fuder, P. Halliday, C. Hardin, L. Ibanez, M. Mitcham, J. Morgan, K. Phillips, A. Sawyer, K. Slusher, B. Wassel, T. Williams, and J. Zabik for assistance in field trapping efforts. We also thank E. R. Hoebeke for assistance and confirmation of specimen identifications. This work is funded by the USDA Specialty Crop Research Initiative under award # 2020-51181-32199.

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Table 2.1. Details on sites, location, cropping system, and hectarage used in 2021 and 2022 trapping seasons. Sites are classified into three zones (North, Central, and South), as shown in Fig. 2.1.

Coordinates	County	Cropping system	Crop Spacing (m)	Production Area (ha)	Year(s)	Zone
33.040510, -84.328305	Pike	Ornamental Field Nursery	2	100	2021, 2022	Central
34.159218, -84.410688	Cherokee	Ornamental Container Nursery	variable	4	2021, 2022	North
34.286743, -84.271187	Cherokee	Ornamental Field Nursery	2	22	2021, 2022	North
33.4710278, -82.1489117	Columbia	Ornamental Container Nursery	variable	9	2021, 2022	Central
32.969398, -84.122629	Upson	Ornamental Field Nursery	2	48	2021, 2022	Central
33.209981, -84.692714	Meriweather	Ornamental Container Nursery	variable	9	2021, 2022	Central
32.080268, -82.094620	Tattnall	Pecan Orchard	9	25	2021	South
30.804375, -83.538736	Brooks	Pecan Orchard	9	27	2021	South
31.311484, -83.487616	Cook	Pecan Orchard	8	270	2021	South
32.022266, -82.216840	Toombs	Pecan Orchard	9	2	2021, 2022	South
31.327939, -82.573681	Ware	Pecan Orchard	10	40	2021, 2022	South
31.518675, -84.318196	Dougherty	Pecan Orchard	8	50	2021, 2022	South
33.128767,-84.373197	Pike	Pecan Orchard	10	25	2022	Central
33.8810393, -83.2922207	Clarke	Pecan Orchard	9	9	2022	Central
33.886765, -83.416613 +	Oconee	Mixed Pecan + Tree Fruit	variable	5	2021, 2022	Central

34.890474, -84.341876 *	Fannin	Tree Fruit	3	48	2021, 2022	North
34.838725, -83.922612 *	Union	Tree Fruit	3	5	2021, 2022	North
34.656847, -84.424138 +	Gilmer	Tree Fruit	3	40	2021, 2022	North

+ one site in 2021 and two sites in 2022; *two sites in 2021 and one site in 2022

Table 2.2. Sampling weeks are denoted as numbers from the beginning of the year and corresponding dates for 2021 and 2022.

Week[a]	2021	2022
10	March 8 - March 14	March 7 - March 13
11	March 15 - March 21	March 14 - March 20
12	March 22 - March 28	March 21 - March 27
13	March 29 – April 4	March 28 – April 3
14	April 5 – April 11	April 4 – April 10
15	April 12 – April 18	April 11 – April 17
16	April 19 – April 25	April 18 – April 24
17	April 26 – May 2	April 25 – May 1
18	May 3 - May 9	May 2 - May 8
19	May 10 – May 16	May 9 – May 15
20	May 17 – May 23	May 16 – May 22
21	May 24 – May 30	May 23 – May 29
22	May 31 – June 6	May 30 – June 5
23	June 7– June 13	June 6 – June 12
24	June 14– June 20	June 14 – June 19
25	June 21 – June 27	June 20 – June 26
26	June 27 – July 3	June 27 – July 3
27	July 5 – July 11	July 4 – July 10
28	July 12 – July 18	July 11 – July 17
29	July 19 – July 26	July 18 – July 24

30	July 25 – August 1	July 25 – July 31

[a]Count from the beginning of the year

Figure 2.1. Map of sites in Georgia where sampling was conducted in 2021 and 2022 trapping Seasons. Regions are subdivided into (A) north, (B) central, and (C) south Georgia.

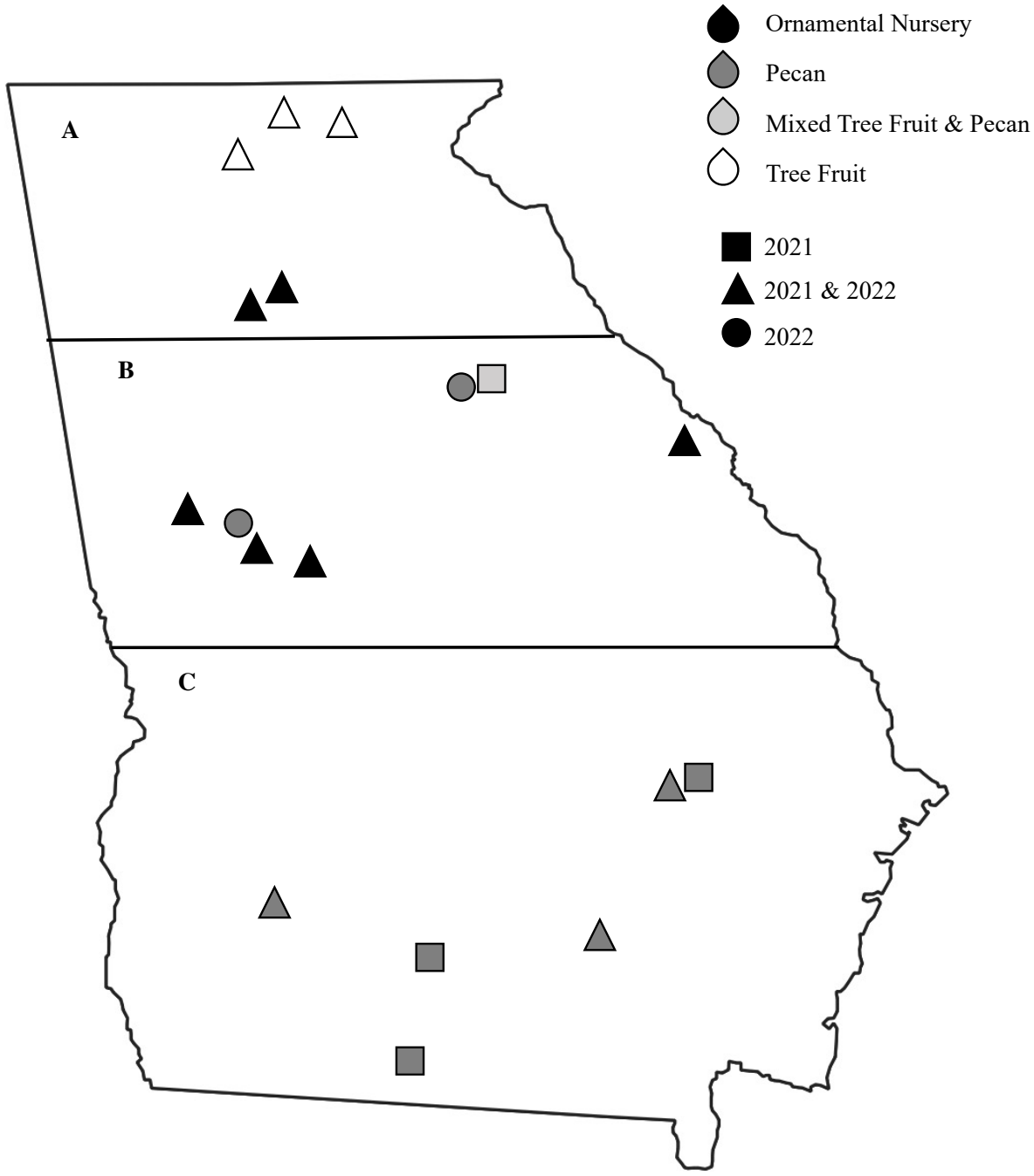
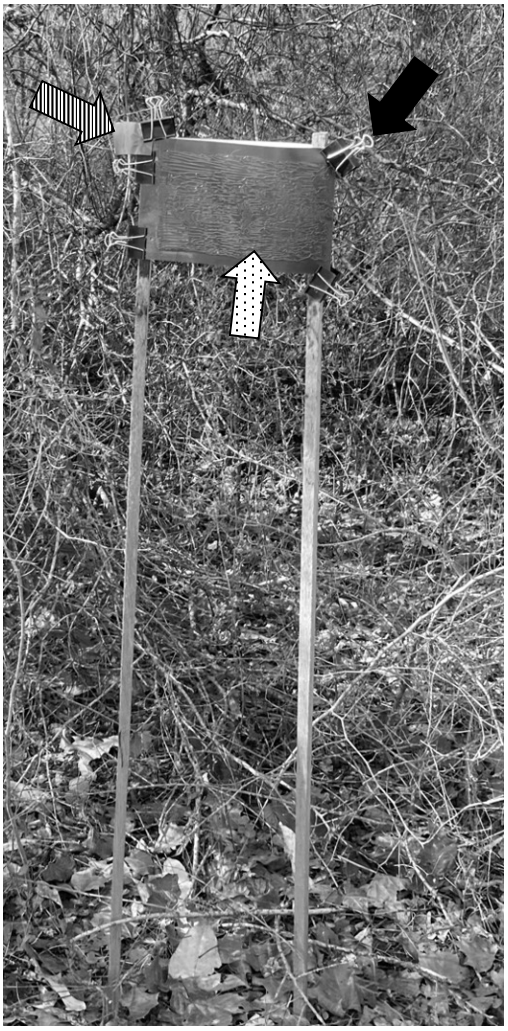

Ornamental Nursery
Pecan
Mixed Tree Fruit & Pecan
Tree Fruit
2021
2021 & 2022
2022
A
B
C

Figure 2.2. Set up of the purple sticky panel trap in 2021 and 2022. 1.8 m stakes were utilized, with 0.3 m buried in the ground. The figure shows the placement of a 25.4 cm by 20.3 cm purple sticky panel trap (dotted arrow) and a benzaldehyde lure (striped arrow) on the stake. The trap was affixed to the stakes using binder clips (black arrow).



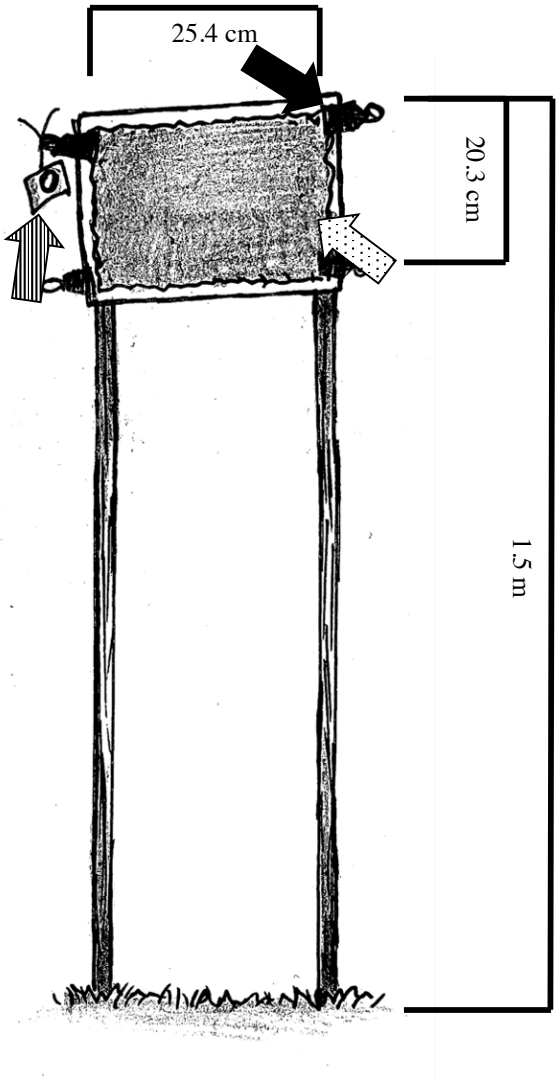

25.4 cm
20.3 cm
1.5 m

Figure 2.3. Mean (± SE) buprestids captured by (A) zone (North, Central, and South regions of Georgia) and (B) cropping system are presented. Buprestids captured in the tree fruit and pecan mixed system were omitted from the analysis by cropping system only but included in the analysis by zone. Bars with the same letter types (italics and bold fonts) were compared within the zone or cropping system, and the same letters on treatment bars (specific zone or cropping system) are not significantly different (Dunn's Test, $\alpha = 0.05$).

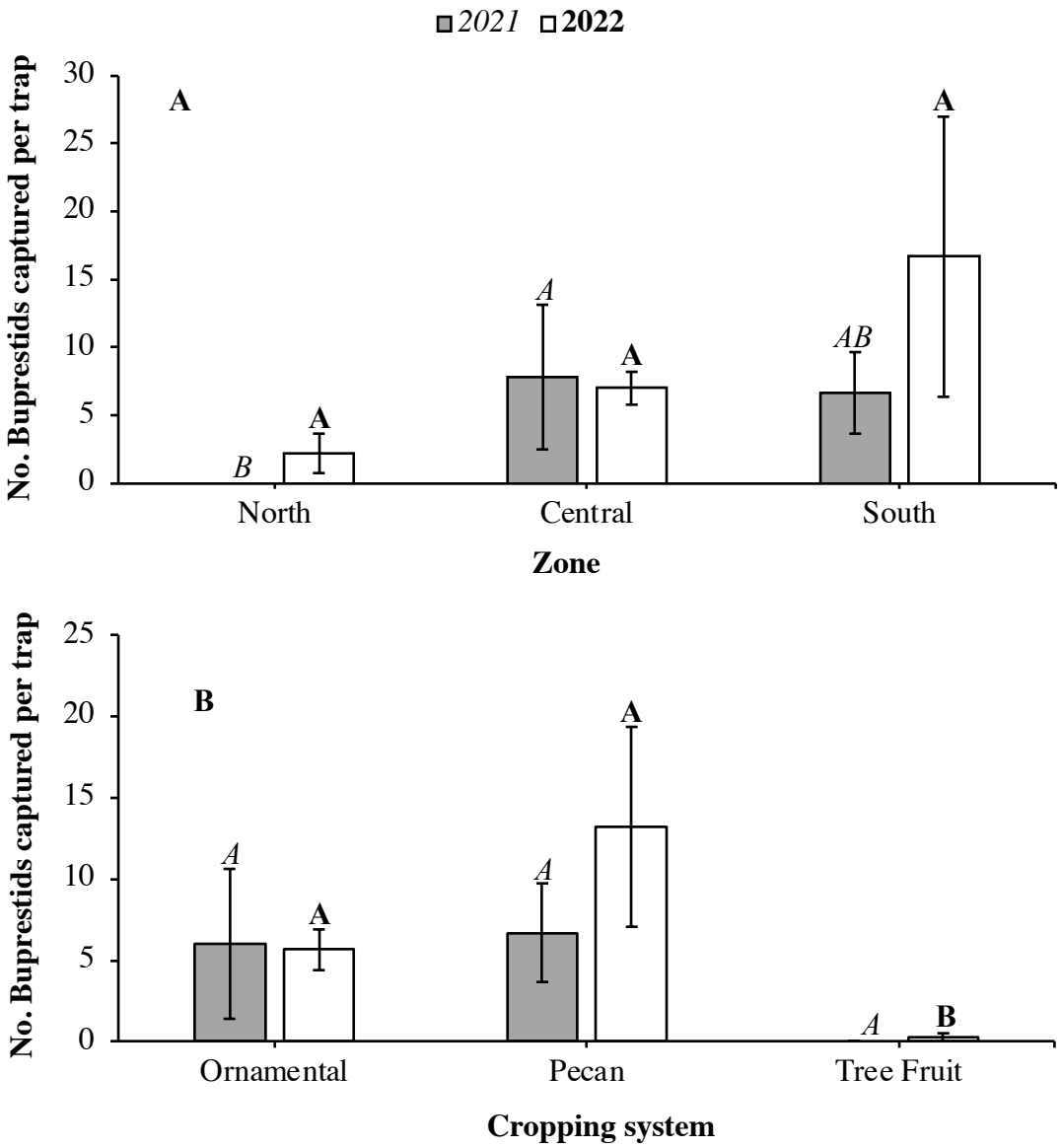

2021
2022
No. Buprestids captured per trap
30
25
20
15
10
5
0
A
A
A
B
A
AB
North
Central
South
Zone
No. Buprestids captured per trap
25
20
15
10
5
0
B
A
A
A
A
A
B
Ornamental
Pecan
Tree Fruit
Cropping system

Figure 2.4. Buprestid genera collected from the (A) north, (B) central, and (C) south zone and from (D) ornamental tree nursery, (E) pecan orchard, and (F) tree fruit orchard are presented. Captures from mixed tree fruit and pecan production sites were omitted from figure panels D, E, and F.

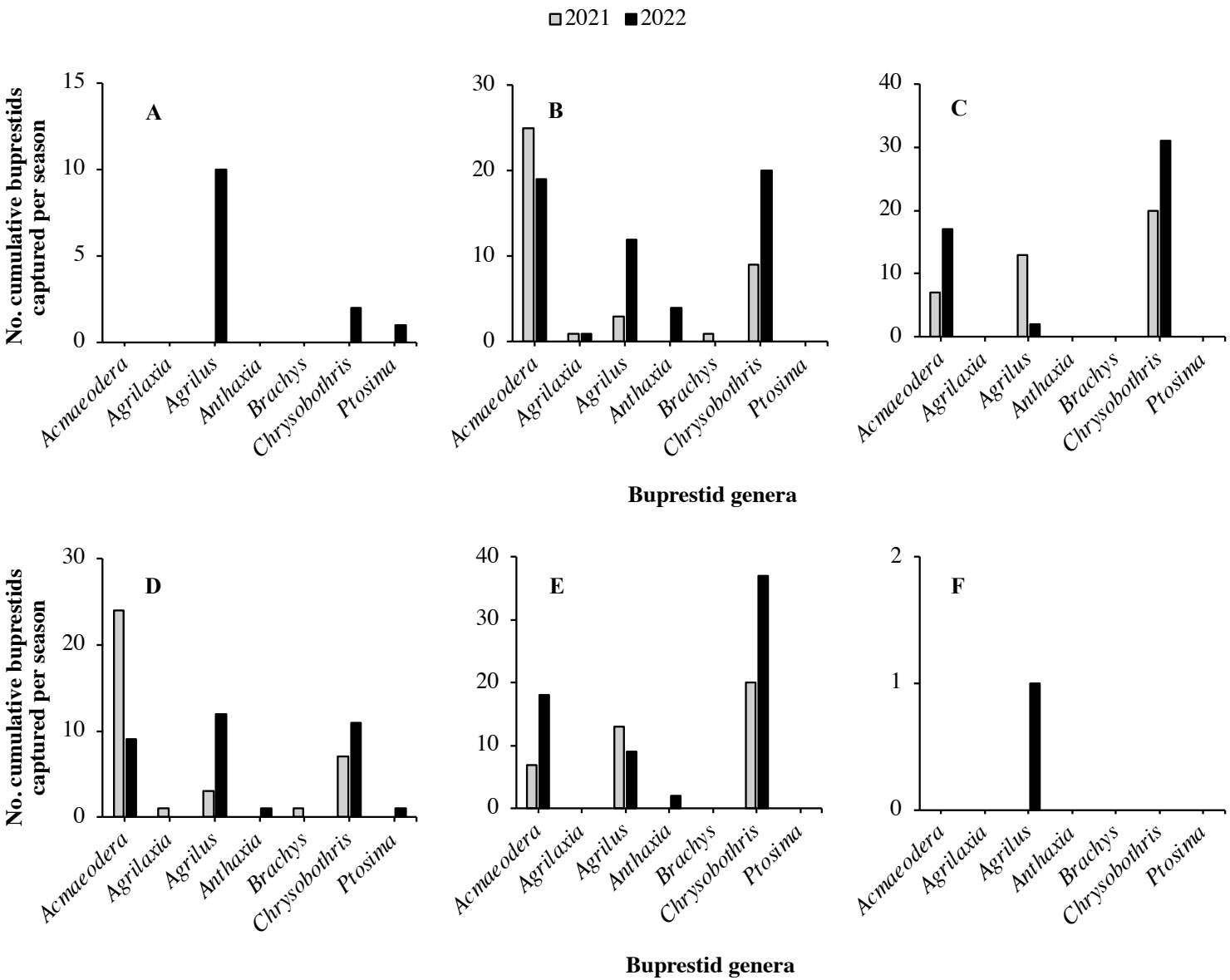

2021
2022
No. cumulative buprestids captured per season
A
B
C
D
E
F
Acmaeodera
Agrilaxia
Agrilus
Anthaxia
Brachys
Chrysobothris
Ptosima
Buprestid genera

Figure 2.5. Captures of *Chrysobothris* spp. and gender were collected in purple sticky panel traps in (A) 2021 and (B) 2022.

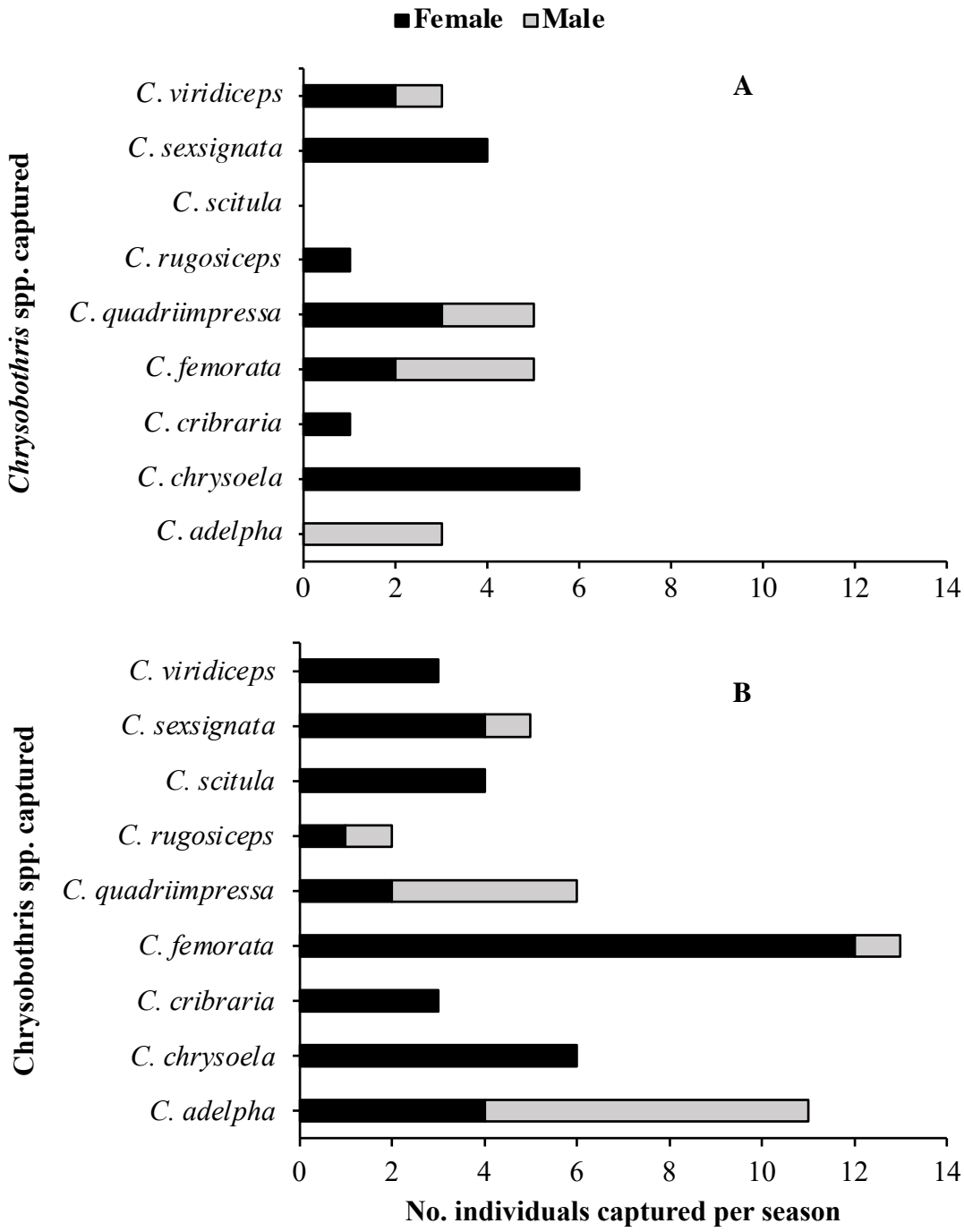

Female
Male
A
B
Chrysobothris spp. captured
C. viridiceps
C. sexsignata
C. scitula
C. rugosiceps
C. quadriimpressa
C. femorata
C. cribraria
C. chrysoela
C. adelpha
0
2
4
6
8
10
12
14
No. individuals captured per season

Figure 2.6. (A, B) The number of buprestids and (C, D) *Chrysobothris* spp. captured by zone (North, Central, South Georgia) in (A, C) 2021 and (B, D) 2022. Traps were placed in the nursery or orchard at 11 or 12 weeks each year, with the evaluation for buprestid captures beginning at 13 or 14 weeks. Week 13 corresponds with the middle of March, whereas week 30 corresponds with the end of July.

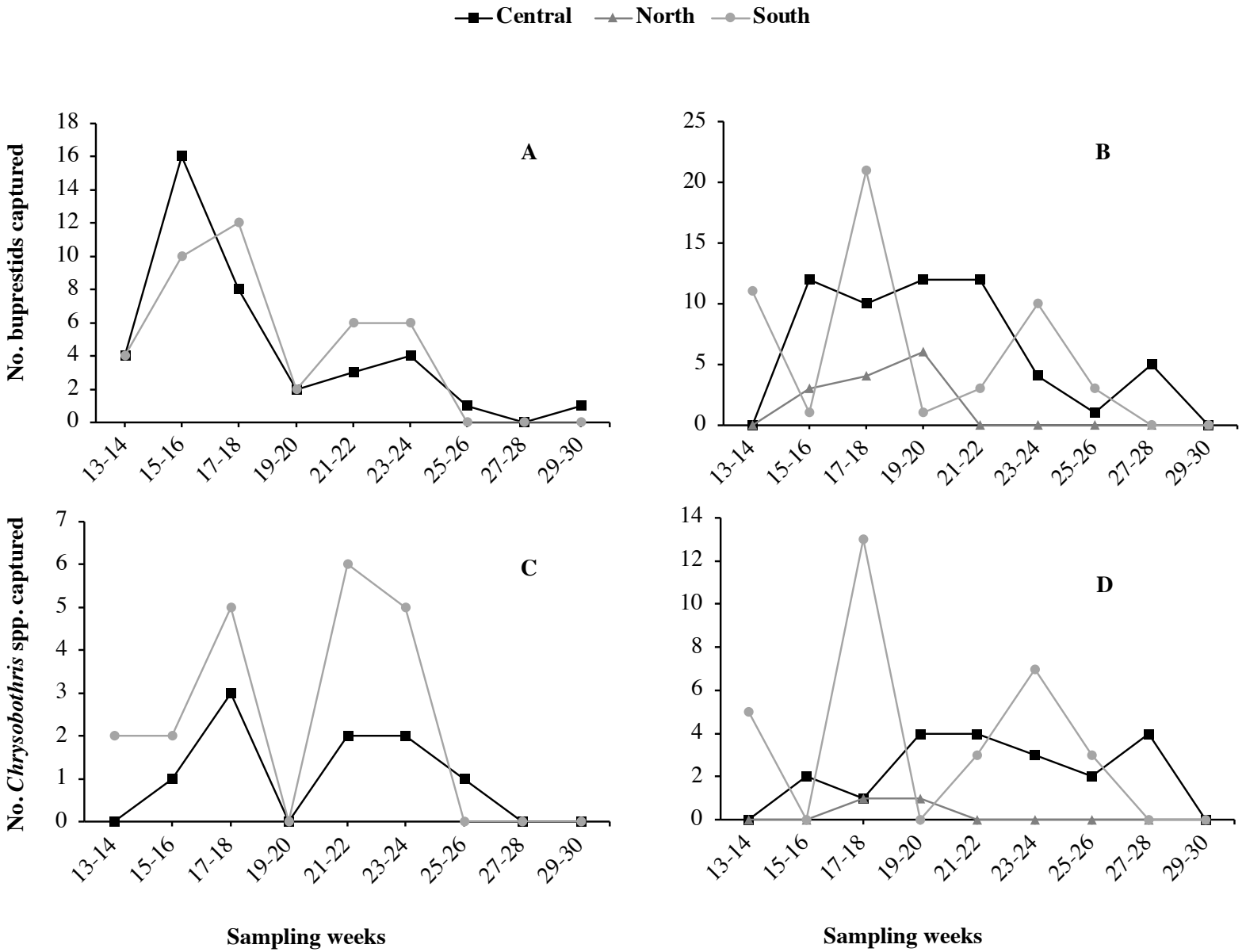

Central
North
South
A
B
C
D
No. buprestids captured
No. Chrysobothris spp. captured
Sampling weeks
Sampling weeks
13-14
15-16
17-18
19-20
21-22
23-24
25-26
27-28
29-30

Figure 2.7. (A, B) The number of buprestids and (C, D) *Chrysobothris* spp. captured by cropping system (ornamentals, pecan, and tree fruit) in (A, C) 2021 and (B, D) 2022. Traps were placed in the nursery or orchard at 11 or 12 weeks each year, with the evaluation for buprestid captures beginning at 13 or 14 weeks. Week 13 corresponds with the middle of March, whereas week 30 corresponds with the end of July.

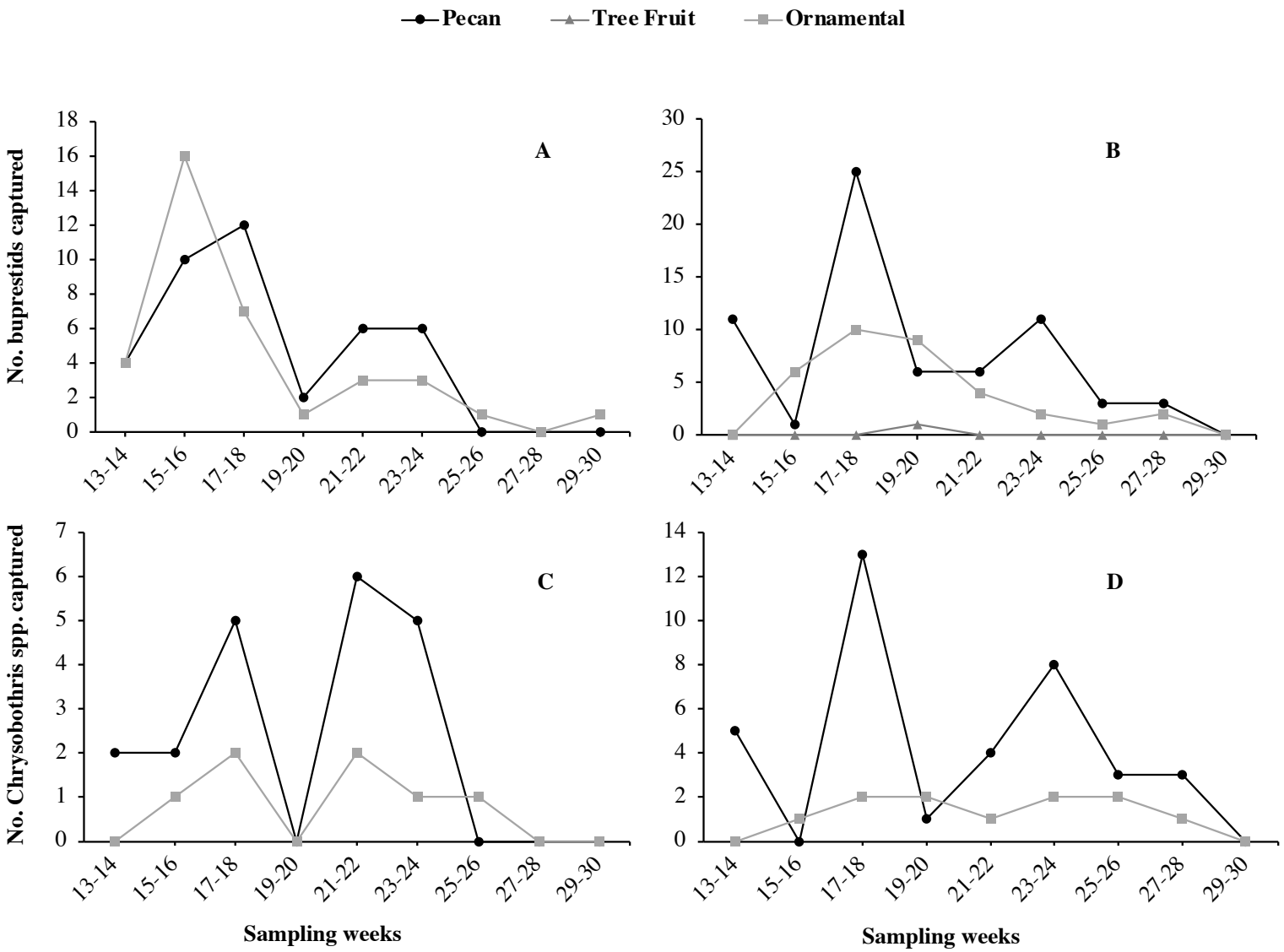
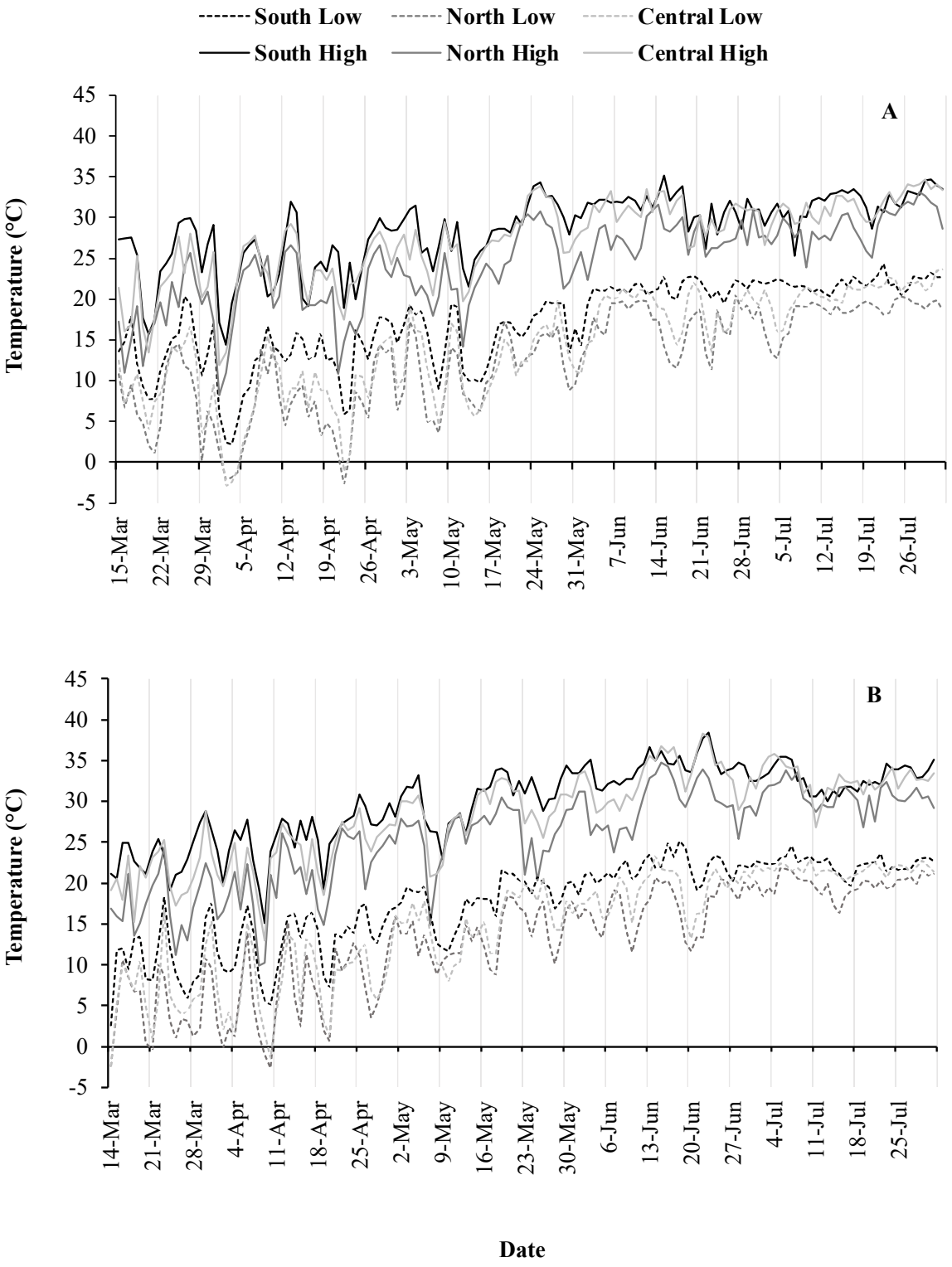

Pecan
Tree Fruit
Ornamental
A
B
C
D
No. buprestids captured
No. Chrysobothris spp. captured
Sampling weeks
Sampling weeks
13-14
15-16
17-18
19-20
21-22
23-24
25-26
27-28
29-30

Figure 2.8. Daily minimum (dotted line) and maximum (solid line) temperature in (A) 2021 and (B) 2022. Temperature data were obtained from three weather stations (University of Georgia weather network) located in the middle of each zone. The Ellijay, Eatonton, and stations were selected for the North, Central, and South zones, respectively. Data is presented for the duration of the trapping period.


South Low
North Low
Central Low
South High
North High
Central High
A
B
Temperature (°C)
45
40
35
30
25
20
15
10
5
0
-5
15-Mar
22-Mar
29-Mar
5-Apr
12-Apr
19-Apr
26-Apr
3-May
10-May
17-May
24-May
31-May
7-Jun
14-Jun
21-Jun
28-Jun
5-Jul
12-Jul
19-Jul
26-Jul
14-Mar
21-Mar
28-Mar
4-Apr
11-Apr
18-Apr
25-Apr
2-May
9-May
16-May
23-May
30-May
6-Jun
13-Jun
20-Jun
27-Jun
4-Jul
11-Jul
18-Jul
25-Jul
Date

CHAPTER 3

EXPLORING RISK FACTORS FOR INSECT BORER ATTACK IN GEORGIA'S URBAN LANDSCAPES[1]

[1] Williamson, Z.V., B.R. Blaauw, and S.V. Joseph. To be submitted to *Journal of Urban Ecology*

Abstract

Urban trees are at risk of stress due to heat island effects and the increased proportion of impervious areas surrounding them. Among pests of trees, insect borers such as ambrosia beetles (Coleoptera: Curculionidae) and flatheaded borers (Coleoptera: Buprestidae) are some of the most devastating, frequently colonizing stressed trees. The objective of the study sought to explore the effect of biotic and abiotic risk factors on borer attacks on trees in 50 urban sites in Atlanta, Georgia, USA, and Augusta, Georgia, USA, in the summer of 2021 and 2022. Specific factors explored include overall tree health, the increase of average high and low temperatures of sites compared to surrounding areas, tree species, and the percentage of the impervious surface surrounding trees. Trees in areas with increased percent pervious area, increased low temperatures, and trees of the species *Acer rubrum* L and *Prunus* × *yedoensis* Matsum were all found to experience higher rates of borer attack, while *Ulmus parvifolia* Jacq. was found less susceptible. Ambrosia beetles were less prone to attack healthy trees. Damage from borers, in general, was also analyzed, exploring trends for both ambrosia beetles and flatheaded borers overall. Healthy trees were less likely to be damaged; however, trees with increased impervious cover around them and increased daily high and low temperatures were more likely to be attacked.

Keywords: flatheaded borers, ambrosia beetles, urban ecology, heat island, impervious area

Introduction

Urban trees are an important part of our daily lives and landscapes. Trees can help improve the human perception of urban areas, providing visual and sensory benefits (Smardon, 1988), even increasing property value (Pandit et al. 2013). In addition, urban trees can provide vital ecosystem services, such as carbon sequestration and air filtration, cooling through increased shade, reducing flooding resulting from storms, and providing habitat for urban animals (Roy et al. 2012). As such, protecting urban trees is crucial and thus, understanding the problems that these trees may encounter is essential.

Urban trees often experience stress at much higher rates than trees in surrounding natural areas, such as forests (Cregg et al. 2001, Dale et al. 2016). Potential stresses include exposure to environmental pollutants and increased soil salt content due to de-icing procedures (Cregg and Dix 2001). Reduced pervious surfaces and an increased proportion of impervious surfaces, such as sidewalks, parking lots, and roads, are common in urban areas (Dutta et al. 2021). These impervious surfaces reduce water infiltration and often have firmly packed and compacted soil beneath them, reducing the rootable soil volume of trees (Jim 2017). In addition, heat island effects can result in increased temperatures in the area due to the increased absorption of solar radiation (Dale et al. 2016, Just et al. 2018). Because of these stresses, urban trees are often at higher risk of insect attack than trees in other more suitable areas like forests (Dale and Frank 2014, Dale et al. 2016).

Planting trees in unfavorable urban sites, such as parking lots, right of ways, or parks, may predispose trees to attack by insects or pathogens due to increased plant stress (Poland and McCullough 2006). It has previously been shown that urban trees can experience increased population numbers of gloomy scale, *Melanaspis tenebricosa* Comstock (Hemiptera:

Diaspididae) due to increased impervious surface cover (Dale and Frank 2014, Dale et al. 2016). Tree stress has also been associated with attack from many wood boring insects. However, the impact of urban stress factors on trees with borer pests, such as flatheaded borers (Coleoptera: Buprestidae), ambrosia beetles (Coleoptera: Curculionidae), and longhorned beetles (Coleoptera: Cerambycidae) is still not understood completely.

Flatheaded borers are widespread, impacting many host plants, such as maples, *Acer* spp., oaks, *Quercus* spp., elms, *Ulmus* spp., redbuds, *Cercis* spp., and willows, *Salix* spp. (MacRae 1991, Hansen et al. 2012). The adults of flatheaded borers are bullet-shaped, and the larvae have a wide, flattened thoracic segment behind the head, and thus, they are referred to as "flatheaded borers" (Frank et al. 2013). These borers cause damage primarily through the tunneling of larvae, leading to the formation of cankers, with eventual girdling of small trees over the years (Oliver et al. 2010). The exit holes left by flatheaded borers have a characteristic "D" shape. Similarly, ambrosia beetles, primarily *Xylosandrus crassiusculus* (Motschulsky) and *Xylosandrus germanus* (Blandford) are pests of many tree species (Ranger et al. 2016). The females attack the tree trunk, bore through vascular bundles, and construct galleries in the heartwood. They introduce a fungal symbiont that they carry in their mycangia, which they grow inside the galleries. The developing larval stages and adults of the ambrosia beetles feed only on the fungus (Reding et al. 2010). Entry holes left by ambrosia beetles are about 1 mm in diameter, and tubes of sawdust and frass referred to as toothpicks may emerge from these holes (Ranger et al. 2016). Longhorned beetles are another pest that can potentially attack trees in urban landscapes. Longhorned beetle groups, such as prionids and *Monochamus* species (MacRae 1993), are common in Georgia, USA. The invasive Asian longhorned beetle also poses a constant threat to trees (Sjöman and Östberg 2019), which, although not currently reported from Georgia, has been

found in the neighboring state of South Carolina. One of the symptoms of larval boring in the tree trunk is the splitting of the tree bark. The exit holes of the adult longhorned beetle are large and round-shaped, resulting from adult emergence (Pedlar et al. 2020, Coyle et al. 2021).

As developers and planners of urban locales continue to emphasize and implement urban trees through areas such as green spaces, sustainable management of insects, such as trunk boring beetles, will become increasingly necessary (Lowe et al. 2019). Despite the direct impact of these pests, little work has been conducted to explore risk factors that enhance borer attacks in urban settings, especially in Georgia, USA. Because of this, understanding the various factors that may place trees at higher risk of borer attacks is essential. By exploring ways to prevent or mitigate attacks in the first place, the overall longevity of trees can be improved, and economic loss associated with the death of trees as a result of insect borers can be reduced. As such, the objectives were to determine 1) the proportion of tree densities in the urban area damaged due to borer activity and 2) the influence of biotic and abiotic characteristics contributing to borer infestations in the urban landscapes.

Materials and Methods

Study sites. A study was conducted in urban landscapes of Atlanta, Georgia, and Augusta, Georgia, in 2021 and 2022. In 2021, 30 urban sites and 887 trees were selected for sampling, whereas in 2022, 20 sites and 474 trees were utilized (Fig. 3.1AandB, Table 3.1). Most of the selected sites were established with trees planted within the last 15 years; however, five sites in 2021 and three sites in 2022 predating that period were included in the study. The types of sites included in the study ranged from subsections of large shopping center areas to smaller green space sites, such as public parks and their associated parking lots. Each site was visited once in

the span of early June to early August. Sites were selected using Google Maps Pro and the time progression feature to view areas of development. Locations of developmental interest were examined more closely, and sites were selected from those areas. More details about selected sites are provided in Table 3.1.

Biotic factors. Both biotic and abiotic factors were measured in the study with the response variables of flatheaded borer, longhorned beetle, and ambrosia beetle damage. Borer damage was quantified by counting visible entry or exit holes on the trunk of the tree at a height of 1.5m and below. Only single-trunked deciduous trees were evaluated. As previously stated, flatheaded borer damage was indicated by the presence of "D" shaped exit holes, small "pinholes" indicated ambrosia beetle damage, and longhorned beetle damage was indicated by large round holes (Oliver et al. 2010, Ranger et al. 2016, Coyle et al. 2021).

Overall tree health was assessed and assigned a value ranging from 1-4, similar to that used in Just et al. (2018). Tree health was rated as dead, poor, fair, or good, with values of 1-4 being assigned, respectively. Trees in good condition had minimal damage (cankers, scrapes, self-girdling, leaf scorch, etc.), branch breakage, or canopy dieback. Trees that were in fair condition had few numbers of dead branches or canopy dieback but presented less damage than poor trees, which may have had multiple dead branches, damaged central leaders, severe injuries, and other easily observable issues. Trees were considered dead when no leaves were present, and twigs were brittle. The trees examined in the study were identified to species, and the diameter of the trunk was taken at breast height using a Vernier caliper, or the point of the first branch, depending on which of the two is the shortest distance from the ground.

The following factors were evaluated, and responses were recorded as a "yes or no" system: Tree dieback, central leader damage, other nonborer-related insect damage, irrigation status, canker, resin soaking, stunting, scraping, or scratching on the tree, water sprouts, root circling, mistletoe, sunscald, lichen, and witch's brooming. Furthermore, the presence of scale insect was evaluated and a score ranging from 0-10 was assigned, where 0, ≤ 10%; 1, 11-20%; 2, 21-30%; 3, 31-40%; 4, 41-50%; 5, 51-60%; 6, 61-70%; 7, 71-80%; 8, 81-90%; 9, ≥91%; 10 = 100% scale cover on the first branch to arise from the trunk. Any lesions or lesions with gummosis were counted.

Abiotic factors. To determine the relationship of abiotic factors, such as the percentage of impervious surface and air temperature, on the incidence of borer damage, these factors were recorded from all the sites. The percentage of the impervious surface was estimated using the "Pace-to-Plant" technique described by Dale et al. (2016). These measurements were conducted for every tree at a given site. Starting from the tree, 25 steps were taken in four directions spaced 90 degrees apart, essentially generating an "X" pattern. The "starting" direction was determined at a 45-degree angle to the direction with the most pervious cover (Figure 3.2). The number of steps on a pervious surface and an impervious surface were recorded. The estimated percentage of the impervious area surrounding the tree was calculated by dividing the number of steps on the impervious surface by the total number of steps taken.

Furthermore, temperature loggers were deployed to compare daily high and low temperatures of specific sites to the average high and low in the area. Temperature loggers were placed (HOBO, Bourne, MA; Part# UA-002-08) at 2.1 m from the tree crown to prevent public interference with the devices. The temperature loggers were protected by placing them into a

clear plastic cup. Logger site selection was primarily based on the granting of permission from site owners or managers for the placement of a logger. In 2021, 13 loggers were deployed on 1 July and were recovered from the field sites on 3 August. In 2022, 12 loggers were placed in sites and deployed on 1 July, and retrieved on 3 August. Temperatures were logged at hourly intervals for the entire exposure time, and the daily minimum and maximum temperature data were obtained from the loggers using HOBOware software (Onset Computer Corporation, Bourne, MA). Local high and low temperatures were obtained from the nearest UGA Weather Network weather station for each site, and the difference between the daily high and low temperature of the logger and the daily high and low temperature of the weather station was determined. The daily differences between the loggers and stations were then averaged to create an average deviation from the local high and average deviation from the local low temperature for each site where loggers were placed. Due to the study being conducted in public spaces, some data were lost due to tampering or removal of branches by landscape professionals.

Statistical analyses. All statistical analyses were conducted utilizing R software (R Core Team 2021). The data obtained were not normally distributed when the response variables of flatheaded borer damage and ambrosia beetle damage were tested. In addition, because many trees experienced no attack at all, with the potential of excessive zeroes, a generalized linear model following a zero-inflated negative binomial distribution was considered, similar to that used in Minami (2007) and Joseph et al. (2014). As discussed in Minami 2007, zero-inflated models are divided into two distinct states: an imperfect state, where, in the case of this study, incidences of attack are possible, and a perfect state, where no incidences of attack are expected. As further discussed in that manuscript, zero-inflated models, specifically zero-inflated negative

binomial models, are well suited for exploring relatively infrequently encountered species that tend to aggregate when present for not well-known reasons. The perfect phase of the model, or zero-inflation model, follows the binomial distribution, while the imperfect phase, or count model, follows the negative binomial distribution. The zero-inflation model can be interpreted as a positive coefficient reflecting an increased likelihood of being in the "perfect" or non-attacked group. The alternative is the "possible attack" group, where the attack is more likely. The count model displays what factors contribute to the severity of borer attack or holes when it does occur to a given tree (Sheu et al. 2004).

To conduct the analysis, the "pscl" package was implemented through the use of the "zeroinfl()" function to construct the zero-inflated negative binomial models (Zeileis et al. 2008; Jackman, 2020). Candidate models initially included all abiotic factors, tree caliper, and health rating as stand-alone variables. Models were constructed and selected for overall suitability based on backward stepwise regression, with comparisons of Akaike information criterion (AIC) scores and R-squared values of all candidate models. The Vuong test determined whether the zero-inflated model provided the best fit, with the zero-inflated negative binomial model proving to be significantly better than the negative binomial model for both borer damage types (Perumean-Chaney et al. 2013). The susceptibility of tree species was analyzed independently using a generalized linear model following the negative binomial distribution. This decision was made due to the wide range of tree species encountered. The baseline level utilized by the model constructed used trident maple, *Acer buergerianum* Miq.

All processes previously described were repeated with each insect grouping, e.g., flatheaded borer damage or ambrosia beetle damage serving as the response variable, as well as with ambrosia beetles and flatheaded borer damage types combined for "borers in general."

Longhorned beetles were excluded from all analyses due to limited presence, with only two trees experiencing longhorned beetle attacks in the study. Graphics were created utilizing the "ggplot2" package of R (Wickham 2016).

Data from the 2021 and 2022 survey seasons were combined due to insignificant year effect when used as the sole parameter in a zero-inflated negative binomial model of all damage types evaluated.

Based on the AIC scores and R-squared values of candidate models, the selected zero-inflated negative binomial model for incidence of flatheaded borer damage included an analysis of the following factors: Tree health rating, percent pervious area, mean deviation of maximum temperature, and mean deviation of minimum temperature. The selected zero-inflated negative binomial model for the incidence of ambrosia beetle damage included an analysis of tree health rating, percent pervious area, mean deviation of maximum temperature, and mean deviation of minimum temperature. The selected combined flatheaded borer damage and ambrosia beetle damage incidence zero-inflated negative binomial model included testing for the following factors: tree health rating, percent previous area, mean deviation of maximum temperature, and mean deviation of minimum temperature.

Results

We surveyed 1351 trees in the study. Of 1351 trees, 8.8% of trees experienced flatheaded borer attacks, with an overall total of 1001 exit holes being counted. Regarding ambrosia beetles, 2.7% of trees experienced ambrosia beetle attacks, with a total of 438 holes recorded. Descriptive statistics, such as estimates, standard error, z-values, and p-values for abiotic and biotic environmental factors affecting the incidence of borer damage to urban trees, are presented

in Table 3.2 and figures 3.3-3.5. Finally, the effect of tree species on borer damage incidence is displayed in Table 3.3 and Figure 3.6.

Among tree health ratings, incidences of ambrosia beetle attacks were less likely to be encountered in trees that were rated as "good" or "fair" in health (Table 3.2, Figure 3.3B). This was not true, however, for flatheaded borers (Table 3.2, Figure 3.3A), where no significant difference in the count model between tree health ratings was found. Trees rated as "good" or fair" in health had lower counts of borer attack incidences in general. In terms of the zero-inflation portion of the flatheaded borer-only model, trees with a health rating of "good" were more likely to be in the perfect or "no attack" group. Furthermore, trees in "poor," "fair," or "good" health were more likely to be in the "no attack" group than "dead" trees for overall borers (Table 3.2, Figure 3.3C).

More flatheaded borer exit holes can be expected when the low temperatures of an area are increased beyond that of the surrounding area (Table 3.2, Figure 3.5A). While no temperature-related parameter had a significant effect on ambrosia beetle damage counts, trees in areas where the high temperature of the area was increased were more likely to be in the "no attack" group, meaning there is likely some latent variable playing a role in driving attack that was not explored in this study (Table 3.2, Figure 3.5B). In terms of borers in general, the increase of both high and low temperatures in an area contributed to increased borer attack (Table 3.2, Figure 3.5C).

In the study, it was observed that flatheaded borers, as well as borers in general, displayed a trend of increased damage with increased impervious cover. In contrast, ambrosia beetles did not display such a trend (Table 3.2, Figure 3.5A-B). However, while the count model showed that increased impervious cover led to higher amounts of damage from flatheaded borers

as well as borers in general, the zero-inflation portion of the models utilized demonstrated that with increasing pervious cover, the less likely trees are to be in the "no attack" group from flatheaded borers and borers in general (Table 3.2, Figures.5A, and3.5C).

Among tree species, red maple, *Acer rubrum* L., white ash, *Fraxinus americana* L., Yoshino cherry, *Prunus* × *yedoensis* Matsum, white oak, *Quercus alba* L., willow oak, *Quercus phellos* L., and Nuttall oak, *Quercus texana* Buckley experienced more flatheaded borer attacks, with 30.8% *Acer rubrum*, 46.2% of *Fraxinus americana*, 19.4% of *Prunus* × *yedoensis*, 53.8% of *Quercus alba*, 4.68% of *Quercus phellos*, and 5.2% of *Quercus texana* experiencing flatheaded borer attack (Table 3.3, Figure 3.6A). While any one tree species was not more prone to increased ambrosia beetle attack (Table 3.3, Figure 3.6B), *Acer rubrum* and *Prunus* × *yedoensis* experienced more incidences of borer attack in general, with 31.7% and 40.5% of those trees experiencing attack from borers in general, respectively. In comparison, lacebark elm, *Ulmus parvifolia* Jacq. experienced fewer incidences of borer attack, with only 1.1% of those trees displaying symptoms of attack (Table 3.3, Figure 3.6C).

Discussion

Urban sites were surveyed to determine the association of biotic and abiotic factors on insect borer damage on trees. Factors such as percentage pervious area, temperature effects, tree species, and tree health were associated with flatheaded borer and ambrosia beetle attacks. The severity of attacks from flatheaded borers and borers, in general, increased with a higher proportion of impervious surface surrounding a tree. At the same time as tree cover in urban areas of the USA is declining, overall impervious cover is increasing (Nowak and Greenfield 2012). This could increase the exposure of urban trees to stress factors, such as intermittent

flooding or drought events (Dale and Frank 2017, Savi et al. 2015). In urban areas, increased soil compaction may also occur during the construction and development of areas, making water and nutrient uptake difficult for trees (Day and Bassuk 1994). As stressed trees are known to attract flatheaded borers, the more severe attack can be clearly associated with an increased impervious area (Ranger et al. 2012, Frank et al. 2013, Dale and Frank 2017). Although there was a trend of increasing impervious area being linked to more severe flatheaded borer and overall borer attacks, trees in areas where pervious area was higher were more likely to be attacked as per the zero-inflated portion of the model. The exact reasons for this result are unclear, but one possibility could be increased levels of flatheaded borers in surrounding forested areas (Dawadi et al. 2019). However, this forest cover was not quantified in the current study because of the unavailability of satellite data as well as time and access-related limitations. Also, the stressed urban trees may emit stress signals, such as ethanol or a blend of volatiles and concentrations of volatile blends, regardless of pervious or impervious surroundings, which may attract more borers from the forested areas.

With the continuous growth of cities, increasing amounts of urban sprawl, and growing concern for heat island effects, the correlation between insect borer attacks and increased temperatures of the sites surveyed is troubling (Amir and Sodoudi 2019, Balany et al. 2020, Rubiera-Morollón and Garrido-Yserte 2020). Results show that flatheaded borer attacks as well as the severity of the attack increased with the rise of low temperatures. In contrast, ambrosia beetle attacks became more likely with an increase of high temperatures beyond that of surrounding areas as per the zero-inflation model. Previously, *Agrilus planipennis* Fairmaire, an invasive flatheaded borer destroying ash trees in the USA, has been shown to have expedited larval development rates in warmer climates in China (Zhao et al. 2005, Wei et al. 2007).

Specifically, the development of *A. planipennis* in China was driven by the number of frost-free days (Wei et al. 2007). These past studies and results from the current study suggest that flatheaded borer damage in urban areas could be at least partially influenced by increases in low temperatures in urban areas (Nuruzzaman et al. 2015). As entry holes of flatheaded borers often occur through wounds and are cryptic, the exit holes utilized in the study provide clear evidence of larval development and subsequent adult emergence (Burke 1910).

Trees in urban areas with high temperatures were more likely to be attacked by ambrosia beetles based on the zero-inflation model but were not likely to experience more severe attacks as temperatures increased based on the count model. Previously, Bellahirech et al. (2019) showed a correlation between increasing average temperatures and increased colonization of ambrosia beetles in cork oak, *Quercus suber* L. Nevertheless, increase in temperatures did not lead to higher emergence of Kuroshio shot hole borer, *Euwallacea kuroshio* Gomez and Hulcr, where emergence eventually ceased as temperatures increased (Dodge and Stouthamer 2021). Thus, beetle reproduction and the emergence of ambrosia beetles in urban areas could be not just related to high temperatures alone. Other unknown factors might be contributing to attacks on the urban trees, such as ambrosia beetle communities in the urban landscape. However, ambrosia beetle species attacking urban trees were not identified in the current study. Furthermore, some species have individual exit holes, while others exit through the parental entrance hole, making it difficult to distinguish foundress colonization from the emergence of progeny (Maner et al. 2013). Perhaps, the incidence of ambrosia beetle attacks on urban trees could be driven both by varied emission rates of stress signals from urban trees as well as flight events of ambrosia beetles originating from wooded locations into urban areas. Although flight distance varies between ambrosia beetle species, ambrosia beetles have been found to move up to 300 m away

from their source population, indicating that most beetles likely fly from natural wooded areas into urban areas (Hanula et al. 2016, Seo et al. 2017). Both these factors should coincide for attacks and colonization on urban trees; however, potentially limited reproduction in these areas may limit attack severity.

Tree health was shown to play a role in the attack severity and occurrence of urban trees by ambrosia beetles and borers, as well as in the likelihood of attack by flatheaded borers. As exotic ambrosia beetles are known to colonize stressed or freshly dead trees, this is consistent with other studies (Harrington et al. 2014, Ranger et al. 2015, O'Donnell et al. 2016, Gugliuzzo et al. 2021). However, it is somewhat counterintuitive that flatheaded borers displayed no differentiation in attack severity in the count model portion despite "healthy" trees being less likely to be attacked in the zero-inflation portion. As buprestids are known to attack stressed trees more severely (Tluczek et al. 2011), this may be a byproduct of one of the limitations of the study. Stressed trees that have experienced heavy attacks may have been removed from the landscape, omitting them from sampling efforts (Seagraves et al. 2013).

Both flatheaded borers and ambrosia beetles colonize a variety of host trees (Reding et al. 2010, Dawadi et al. 2019). A previous study found that trees native to the area experienced more attacks from spring cankerworm, *Paleacrita vernata* Peck (Lepidoptera: Geometridae), than surrounding exotic tree species (Frank et al. 2014). The current study did not focus on the attack of native versus exotic trees, but certain trees were found differentially susceptible to borer attack in urban areas. It is possible that additional tree species could be susceptible to flatheaded borer attacks, and not all areas will have attacks limited to flatheaded borers. Based on the common trees observed in urban locations, *Acer rubrum* and *Prunus* × *yedoensis* were widely susceptible to borers in urban landscapes. *Ulmus parvifolia* trees were not prone to borer attacks.

As an understory tree, *A. rubrum* grows quickly in a landscape (Collins 1961, Dawadi et al. 2019) and thrives in all environmental conditions (Warren et al. 2004). However, the quick growth of *A. rubrum* makes it susceptible to physiological defects related to internal structural issues and mechanical injury, leading to large amounts of dieback. These issues ultimately weaken the tree and make it more susceptible to attack from insect borers (Walters and Yawney 1990). *A. rubrum* accounts for 10% of all deciduous shade trees sold and has an estimated value of ~$11 million USD in the USA (Census of Agriculture 2017). Thus, their high susceptibility to borers can have major economic implications. The second most susceptible tree, *Prunus* × *yedoensis,* has previously been found susceptible to other pests such as Japanese beetles, *Popilia japonica* Newman (Coleoptera: Scarabaeidae) (Held 2004). They are poorly suited for urban areas because of intolerance to desiccation and increased risk of frost dieback-related stress. The stressed trees then release volatiles which makes them an attractive host to trunk-boring beetles (Snyder 1975, Dunn et al. 1986, Bates and Niemiera 1993; Ranger et al. 2010;). *Prunus* × *yedoensis* is regarded as a high-cost specimen tree, and any insect attack diminishes aesthetic value (Held 2004, Seagraves et al. 2013).

In contrast to *Prunus* × *yedoensis* and *A. rubrum*, *U. parvifolia* is resistant to borer attack. With its common namesake (lacebark elm) ornamental bark and slow growth rate, *U. parvifolia* has been popularized as a street tree hardy for the southern USA (Warren 2000). Other instances of insect resistance has been noticed in field studies of elm trees before, with *U. parvifolia* being recognized as one of the most pest and disease-resistant trees in the *Ulmus* (elm) family. *U. parvifolia* has shown higher resistance to *P. japonica*, various scale insect species such as European fruit lecanium, *Parthenolecanium corni* (Bouché) (Hemiptera: Coccidae), and European elm scale, *Eriococcus spurius* (Modeer) (Hemiptera: Coccidae), (Potter and Redmond

2013) as well as to Dutch elm disease (Potter and Redmond, 2013). They are a good fit for street trees and neighborhoods where uniformity is desired (Warren, 2000). In addition, *U. parvifolia* tolerates frequent droughts and is suited for urban landscapes (Bartens et al. 2010).As young trees have the potential to be killed in one season after being attacked by flatheaded borers, this is a troubling prospect both ecologically and economically, with costs for removing and replacing dead or damaged trees and the associated loss of ecosystem services impacting stakeholders (Asadian et al. 2009, Oliver et al. 2010, Park et al. 2010, Armson et al. 2012, Horvàthovà 2021). Therefore, urban planners should consider planting tree species that are less prone to borer attack to reduce the cost and burden on the customer, even years after planting.

Although efforts were made, obtaining site-specific management information was difficult, and inputs regarding the prevention of insect attacks and tree care likely differed between sites. Although trees were identified to species, we could not identify specific cultivars, with cultivars of trees having potential variance in susceptibility to insect borers (Held 2004, Potter and Redmond 2013). In addition, the survey was limited to trees that remained in the landscape. As cankers and declines in tree health resulting from insect borer colonization are detrimental to the overall appearance of the landscape, afflicted trees may have been removed, omitting them from the study (Seagraves et al. 2013).

Trees in urban landscapes and the people that manage them face unique challenges, with concerns from the public and limitations on intervention methods being called into question (Braman et al. 1998, Mullaney et al. 2015). Despite this, urban trees are essential in mitigating some of the environmental malfeasances associated with urban areas (Asadian et al. 2009, Oliver et al. 2010, Park et al. 2010, Armson et al. 2012, Lüttge and Buckeridge 2020, Horvàthovà 2021). As previously mentioned, some limitations of this study may impact the applicability of

this research to specific locations. However, recommendations may be broadly applied to other locales (Grant et al. 2007). Through implementing a higher percent pervious area around trees when possible, as well as utilizing borer-resistant tree species that are suited for an area, damage and subsequent loss of urban trees can be avoided to the benefit of both landscape managers, arborists, and landowners as well as those who benefit from the ecological services provided by those trees (Roy et al. 2012;, Pataki et al. 2021). Furthermore, this research has wide-reaching implications regarding the impact of heat island effects and urbanization on the health of urban trees and forests. Future research warrants increasing our understanding of how temperature drives colonization and emergence of ambrosia beetles as well as exploring both tree species and cultivar-related differences in insect borer vulnerability.

Acknowledgments

We thank Dr. Uttam Bhattarai for statistical advice and Logan Ibanez, Matt Mitcham, Chris Hardin, and Anna Agi for assistance in field survey work. This research was funded by the USDA Specialty Crop Research Initiative under award # 2020-51181-32199.

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Table 3.1. Details of location and characteristics of selected sites in urban areas in Atlanta and Augusta surveyed from July to August in 2021 and 2022.

County	Site Type	Trees	Site Age (Years)	Site Area (m^2)	Surrounding Areas	Host Trees in Site
2021						
Clayton	Parking Lot	32	19	21823	Buildings, Road	*P. chinensis, Z. serrata, A. rubrum*
Clayton	Parking Lot	24	19	6252	Buildings, Open Area, Road	*U. parvifolia*
Cobb	Parking Lot	25	9	7866	Buildings, Road	*U. parvifolia, A. rubrum*
Columbia	Greenspace	17	22	NA	Buildings, Forest, Open Area, Road	*P. calleryana, Tilia cordata*
Columbia	Parking Lot	56	11	5247	Forest, Road	*Q. texana, Q. lyrata, A. rubrum, A. buergerianum, Q. phellos*
Columbia	Parking Lot	48	16	33591	Buildings, Forest, Road	*Q. rubra, Q. texana, A. rubrum, Q. phellos*
Columbia	Parking Lot	34	14	17414	Buildings, Forest, Road	*Q. texana, A. rubrum, Q. phellos*
Columbia	Parking Lot	32	3	9979	Buildings, Road	*U. parvifolia, Q. texana, A. buergerianum*
Columbia	Parking Lot	26	4	3646	Buildings, Forest, Road	*O. virginiana, A. saccharum, F. americana*
Columbia	Parking Lot	25	6	11298	Buildings, Road	*C. caroliniana, P. chinensis, Q. lyrata, Q. rubra*
Columbia	Parking Lot	20	14	3641	Forest, Open Area, Road	*A. rubrum, A. buergerianum, Q. alba*
Columbia	Parking Lot	20	15	13937	Buildings, Forest, Road	*U. parvifolia, A. rubrum, Q. phellos*
Columbia	Parking Lot	14	11	5126	Buildings, Road	*U. parvifolia, A. rubrum, Q. phellos*
Columbia	Parking Lot	13	7	6691	Buildings, Forest, Road	*P. chinensis, A. buergerianum, S. babylonica*
Columbia	Parking Lot	12	12	4325	Buildings, Forest, Road	*A. rubrum*
Columbia	Parking Lot	8	11	3815	Buildings, Forest, Open Area, Road	*Q. texana, A. buergerianum, Q. alba*

Coweta	Parking Lot	69	10	17894	Buildings, Road	*C. caroliniana, P. calleryana, P. chinensis, U. parvifolia, A. rubrum, P. x yedoensis*
Coweta	Parking Lot	42	6	14058	Buildings, Road	*G. biloba, U. parvifolia, A. rubrum*
Fayette	Greenspace	20	3	NA	Buildings, Road	*U. parvifolia, A. buergerianum*
Fulton	Parking Lot	51	5	14997	Buildings, Road	*U. parvifolia, Q. texana*
Fulton	Parking Lot	33	5	5627	Buildings, Road	*F. grandifolia, U. parvifolia, Q. palustris, A. buergerianum*
Fulton	Parking Lot	24	22	7492	Buildings, Forest, Open Area, Road	*A. ginnala, U. parvifolia, Q. texana, A. rubrum*
Gwinnett	Parking Lot	41	8	19748	Buildings, Road	*P. chinensis, Q. phellos*
Gwinnett	Parking Lot	30	4	6943	Buildings, Road	*U. parvifolia, Q. rubra, Q. palustris, Q. coccinea, A. buergerianum*
Henry	Parking Lot	33	6	13778	Buildings, Road	*G. biloba, U. parvifolia, Q. texana, A. rubrum*
Henry	Parking Lot	31	6	8952	Buildings, Forest, Road	*U. americana, Q. palustris, A. rubrum, A. saccharinum, Q. phellos*
Richmond	Parking Lot	38	10	30956	Buildings, Open Area, Road	*F. grandifolia, P. chinensis, Q. texana*
Richmond	Parking Lot	23	14	6287	Buildings, Road	*F. grandifolia, U. americana*
Rockdale	Parking Lot	14	9	10205	Buildings, Road	*Q. palustris, A. rubrum, Q. phellos*
Spalding	Parking Lot	22	11	7833	Buildings, Open Area, Road	*P. x yedoensis*
2022						
Cherokee	Green Space	16	7	NA	Forest, Open Area, Road	*Nyssa sylvatica, G. biloba, B. nigra, A. saccharum*
Cherokee	Parking Lot	39	7	20051	Buildings, Forest, Open Area, Road	*P. chinensis, Q. shumardii*
Cherokee	Parking Lot	16	8	5681	Buildings, Road	*A. saccharum*
Cherokee	Parking Lot	11	15	10982	Buildings, Road	*U. parvifolia, A. saccharum*
Cobb	Parking Lot	84	15	36527	Buildings, Forest, Road	*Z. serrata, Q. palustris, A. rubrum, B. nigra, Q. shumardii, Q. phellos*

Cobb	Parking Lot	30	6	16479	Buildings, Road	*U. parvifolia, Q. shumardii*
Cobb	Parking Lot	15	11	4843	Buildings, Road	*A. rubrum, L. styraciflua, A. buergerianum, Q. phellos*
Cobb	Parking Lot	12	11	9669	Buildings, Road	*Z. serrata, U. parvifolia, Q. phellos, P. x yedoensis*
Coweta	Parking Lot	20	17	14210	Buildings, Road	*U. parvifolia, A. rubrum*
DeKalb	Park/ Green Space	15	11	NA	Buildings, Forest, Open Area, Road	*Q. palustris, A. rubrum, Q. shumardii, A. saccharum*
Douglas	Parking Lot	17	8	16695	Buildings, Forest, Road	*P. chinensis, A. buergerianum*
Fayette	Parking Lot	19	13	13749	Buildings, Forest, Road	*A. ginnala, U. parvifolia, A. rubrum, Q. phellos*
Fayette	Parking Lot	18	16	21815	Buildings, Forest, Road	*A. rubrum, Q. shumardii*
Fayette	Parking Lot	12	6	8990	Buildings, Road	*U. parvifolia*
Fayette	Parking Lot	10	6	1520	Buildings, Forest, Road	*Q. texana, A. rubrum, P. x yedoensis*
Forsyth	Parking Lot	79	7	32255	Buildings, Forest, Open Area, Road	*Q. texana, Q. palustris, A. ruvrum, Q. shumardii, Q. saccharinum, A. buergerianium, Q. phellos*
Fulton	Parking Lot	20	4	11541	Buildings, Road	*P. chinensis, Q. phellos*
Gwinnett	Parking Lot	18	16	10630	Buildings, Forest, Road	*Q. texana, Q. palustris, A. rubrum, Q. shumardii*
Gwinnett	Parking Lot	11	7	4297	Buildings, Road	*U. parvifolia, A. rubrum*
Henry	Parking Lot	12	12	10642	Buildings, Road	*A. rubrum, Q. shumardii, P. x yedoensis*

Table 3.2. Coefficients of parameters by borer species for the count model portion and the zero inflation portion of the zero inflated negative binomial models for sites visited in 2021 (n = 30) and 2022 (n = 20).

Factor	Count Model Portion					Zero Inflated Portion				
	Estimate	**SE**	**Z**	***P***	**Effects**	**Estimate**	**SE**	**Z**	***P***	**Effects**
Flatheaded borer										
Intercept	1.0277	1.060	0.969	0.332		2.681	1.262	2.125	0.034	
Tree health rating of 2	0.207	0.747	0.277	0.782		-1.422	1.096	-1.298	0.194	
Tree health rating of 3	-0.625	0.732	-0.854	0.393		0.891	0.998	0.892	0.372	
Tree health rating of 4	-0.120	0.911	-0.132	0.895		4.140	1.135	3.647	**< 0.001**	***
Percent pervious area	-0.029	0.010	-2.942	**0.003**	**	-0.057	0.016	-3.658	**< 0.001**	***
Mean deviation of max temperature	0.210	0.141	1.494	0.135		-0.098	0.142	-0.693	0.488	
Mean deviation of min temperature	0.468	0.195	2.408	**0.016**	*	-0.376	0.249	-1.509	0.131	
Ambrosia beetle										
Intercept	1.261	0.739	1.707	0.088		-11.707	388.083	-0.030	0.976	
Tree Health Rating of 2	-0.958	0.669	-1.432	0.152		2.536	1.052	2.410	**0.016**	*
Tree Health Rating of 3	-1.241	0.477	-2.603	**0.009**	**	3.722	0.927	4.014	**< 0.001**	***
Tree Health Rating of 4	-2.490	0.684	-3.640	**< 0.001**	**	4.599	1.021	4.506	**< 0.001**	***
Percent Pervious Area	-0.007	0.032	-0.228	0.820		0.000	0.018	-0.017	0.987	
Mean Deviation of Max Temperature	0.528	0.295	1.788	0.074		-0.438	0.207	-2.119	**0.034**	*
Mean Deviation of Min Temperature	-0.139	0.491	-0.283	0.778		0.759	0.537	1.414	0.157	
Borers Overall										
Intercept	0.860	1.481	0.581	0.561		0.712	1.452	0.491	0.624	
Tree Health Rating of 2	-0.266	0.455	-0.583	0.560		12.785	388.082	0.033	0.974	
Tree Health Rating of 3	-1.203	0.426	-2.822	**0.005**	**	15.033	388.082	0.039	0.969	
Tree Health Rating of 4	-1.369	0.613	-2.233	**0.026**	*	17.541	388.082	0.045	0.964	
Percent Pervious Area	-0.031	0.009	-3.643	**< 0.001**	***	-0.052	0.015	-3.573	**< 0.001**	***
Mean Deviation of Max Temperature	0.358	0.121	2.960	**0.003**	**	-0.143	0.129	-1.108	0.268	
Mean Deviation of Min Temperature	0.379	0.167	2.272	**0.023**	*	-0.230	0.233	-0.985	0.325	

Black significance indicators denote a positive effect. Grey denotes a negative effect.

Table 3.3. Coefficients of parameters by tree species for the generalized linear model for borer attack for sites visited in 2021 (n = 30) and 2022 (n = 20)

Tree Species	n	*Flatheaded Borer*				*Ambrosia beetle*				*Borers Overall*			
		Est.	**SE**	**Z**	***P***	**Est.**	**SE**	**Z**	***P***	**Est.**	**SE**	**Z**	***P***
Intercept		-2.674	0.691	-3.871	**< 0.001**	-1.227	1.075	-1.142	0.254	-1.542	0.391	-3.948	**< 0.001**
A. ginnala	14	1.134	1.322	0.858	0.391	0.785	2.406	0.326	0.744	0.862	1.209	0.713	0.476
A. rubrum	240	3.759	0.730	5.151	**< 0.001**	0.667	1.193	0.559	0.576	2.277	0.608	3.743	**< 0.001**
A. saccharinum	12	-19.628	12195.706	-0.002	0.999	-21.075	12195.707	-0.002	0.999	-20.290	7397.000	-0.003	0.998
A. saccharum	32	-19.628	7468.314	-0.003	0.998	-21.075	7468.315	-0.003	0.998	-20.290	4530.000	-0.004	0.996
B. nigra	9	1.863	1.478	1.260	0.208	-21.075	14082.389	-0.001	0.999	0.205	1.491	0.137	0.891
C. caroliniana	11	-19.628	12738.000	-0.002	0.999	-21.075	12738.000	-0.002	0.999	-20.290	7726.000	-0.003	0.998
F. grandifolia	26	1.719	1.040	1.653	0.098	-21.075	8285.351	-0.003	0.998	0.060	0.992	0.061	0.951
F. americana	13	3.575	1.232	2.902	**0.004**	-0.645	2.555	-0.252	0.801	1.977	1.221	1.620	0.105
G. biloba	18	-0.216	1.485	-0.146	0.884	-1.663	2.382	-0.698	0.485	-1.181	1.281	-0.923	0.356
L. styraciflua	1	-19.628	42247.166	0.000	1.000	-21.075	42247.167	0.000	1.000	-20.290	25620.000	-0.001	0.999
N. sylvatica	10	1.470	1.457	1.009	0.313	-21.075	13359.727	-0.002	0.999	-0.188	1.463	-0.129	0.898
O. virginiana	9	-19.628	14082.389	-0.001	0.999	2.121	2.870	0.739	0.460	1.910	1.421	1.344	0.179
P. chinensis	103	-19.628	4162.737	-0.005	0.996	0.330	1.338	0.247	0.805	0.119	0.690	0.172	0.863
P. x yedoensis	37	1.981	0.935	2.118	**0.034**	2.580	1.681	1.535	0.125	2.490	0.841	2.961	**0.003**
P. calleryana	20	-19.628	9446.754	-0.002	0.998	-21.075	9446.754	-0.002	0.998	-20.290	5730.000	-0.004	0.997
Q. alba	13	4.060	1.227	3.309	**0.001**	0.454	2.489	0.182	0.855	2.511	1.216	2.066	0.039

Q. coccinea	2	-19.628	29873.258	-0.001	0.999	-21.075	29873.258	-0.001	0.999	-20.290	18120.000	-0.001	0.999
Q. lyrata	11	-19.628	12738.000	-0.002	0.999	-21.075	12738.000	-0.002	0.999	-20.290	7726.000	-0.003	0.998
Q. palustris	34	-19.628	7245.329	-0.003	0.998	-21.075	7245.329	-0.003	0.998	-20.290	4395.000	-0.005	0.996
Q. phellos	128	1.651	0.775	2.130	**0.033**	-21.075	3734.157	-0.006	0.996	-0.007	0.667	-0.011	0.991
Q. rubra	13	1.901	1.286	1.479	0.139	-21.075	11717.256	-0.002	0.999	0.243	1.276	0.190	0.849
Q. shumardii	81	-19.628	4694.130	-0.004	0.997	-21.075	4694.130	-0.004	0.996	-20.290	2847.000	-0.007	0.994
Q. texana	135	2.354	0.764	3.081	**0.002**	-2.069	1.349	-1.533	0.125	0.745	0.654	1.140	0.254
S. babylonica	4	1.288	2.182	0.590	0.555	-21.075	21123.583	-0.001	0.999	-0.370	2.249	-0.165	0.869
T. cordata	1	-19.628	42247.166	0.000	1.000	-21.075	42247.167	0.000	1.000	-20.290	25620.000	-0.001	0.999
U. americana	26	-19.628	8285.351	-0.002	0.998	-2.031	2.142	-0.948	0.343	-2.242	1.372	-1.634	0.102
U. parvifolia	274	-19.628	2552.245	-0.008	0.994	-2.189	1.222	-1.791	0.073	-2.400	0.687	-3.493	**< 0.001**
Z. serrata	12	-19.628	12195.706	-0.002	0.999	1.3814	2.5502	0.542	0.588	1.170	1.276	0.917	0.359
Unknown: Dead	3	4.060	2.225	1.825	0.068	3.387	4.732	0.716	0.474	3.555	2.313	1.537	0.124

Reference level utilized was *Acer buergerianum*, n=58.

Figure 3.1. Sites (n = 40) in the (A) Atlanta, Georgia in 2021 and 2022, and (B) Augusta, Georgia in 2021 selected for the study. Study sites are marked by semitransparent dots. Map from QGis version 3.22.10 adapted by Zia Williamson.

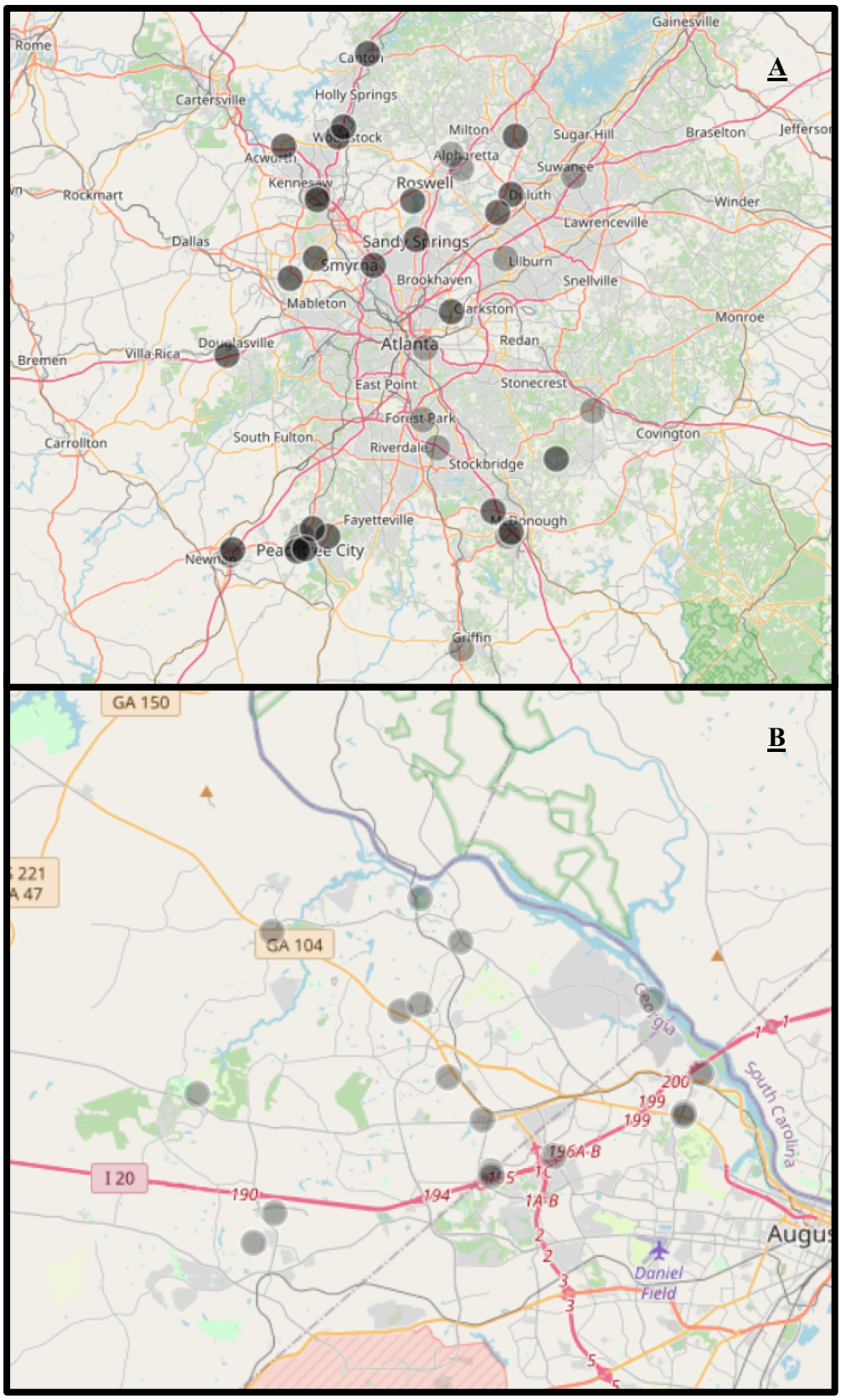

A
Gainesville
Rome
Holly Springs
Cartersville
Milton
Sugar Hill
Braselton
Alpharetta
Suwanee
Acworth
Kennesaw
Roswell
Rockmart
Duluth
Winder
Lawrenceville
Dallas
Sandy Springs
Lilburn
Smyrna
Brookhaven
Snellville
Mableton
Clarkston
Monroe
Douglasville
Atlanta
Redan
Villa Rica
Bremen
East Point
Stonecrest
Forest Park
Carrollton
South Fulton
Riverdale
Covington
Stockbridge
Fayetteville
Griffin
B
GA 150
GA 104
I 20
Georgia
South Carolina
Daniel Field

Figure 3.2. Graphic demonstration of the Pace-to-Plant technique as used in Dale et al. (2016). Four transects, represented by the dashed line, were utilized, originating at the base of the tree being observed. Transects are 90° apart, with the first transect positioned at a 45° angle to the closest impervious edge, such as pavement or sidewalk. Each transect consisted of 25 steps, for a total of 100. The number of steps on the pervious surface, represented by the white portion of the line, out of those 100 were recorded. Aerial image © 2022 Google adapted by Zia Williamson for demonstration purposes.

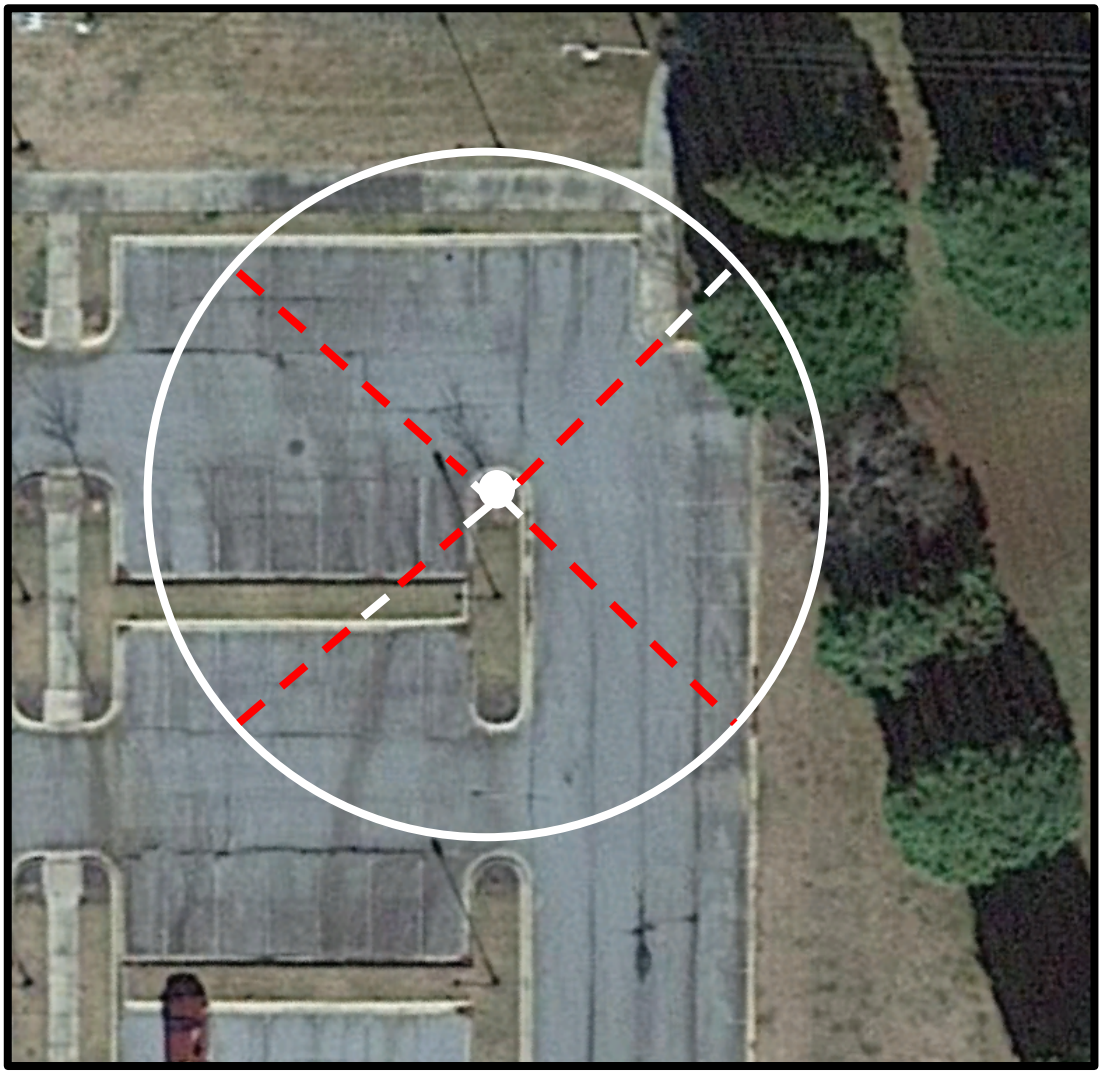


Figure 3.3. Number of entry of exit hole rated using a health scale where 1, dead; 2, poor health; 3, fair health; and 4, good health for (A) flatheaded borer, (B) ambrosia beetle damage, and (C) overall borer damage.

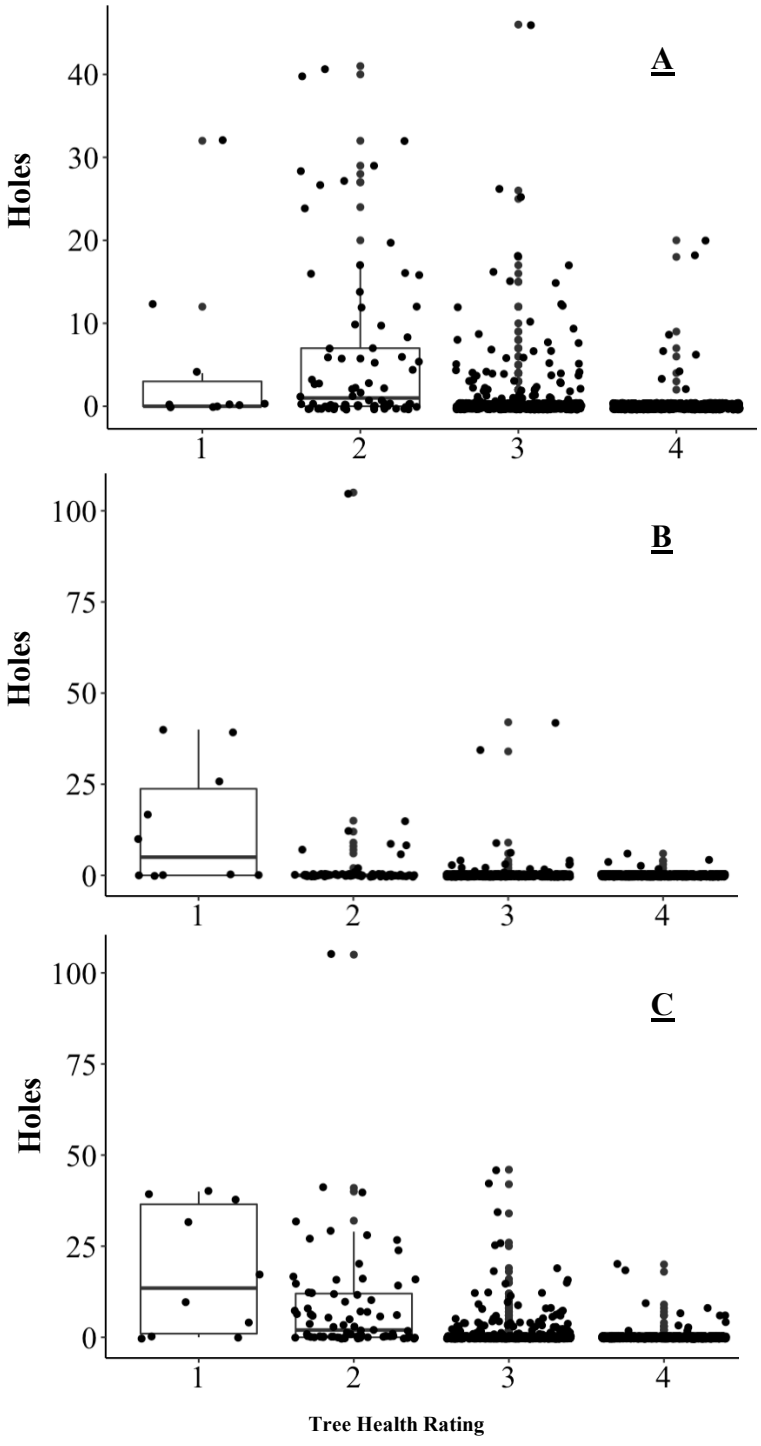

A
B
C
Holes
Holes
Holes
0
10
20
30
40
0
25
50
75
100
0
25
50
75
100
1
2
3
4
Tree Health Rating

Figure 3.4. The numbers of holes of flatheaded borer and ambrosia beetles were recorded on the tree with the average increase of ambient air temperature of each site compared to surrounding areas where (A, C) average high (A, C) and (B, D) low temperatures. Plots including (A, C) and excluding (B, D) trees without borer holes are included. Trendlines are included for parameters determined to be significant in the respective zero-inflated negative binomial model. Temperature loggers were placed in experimental sites from 1 July to 3 August in 2021 and 2022

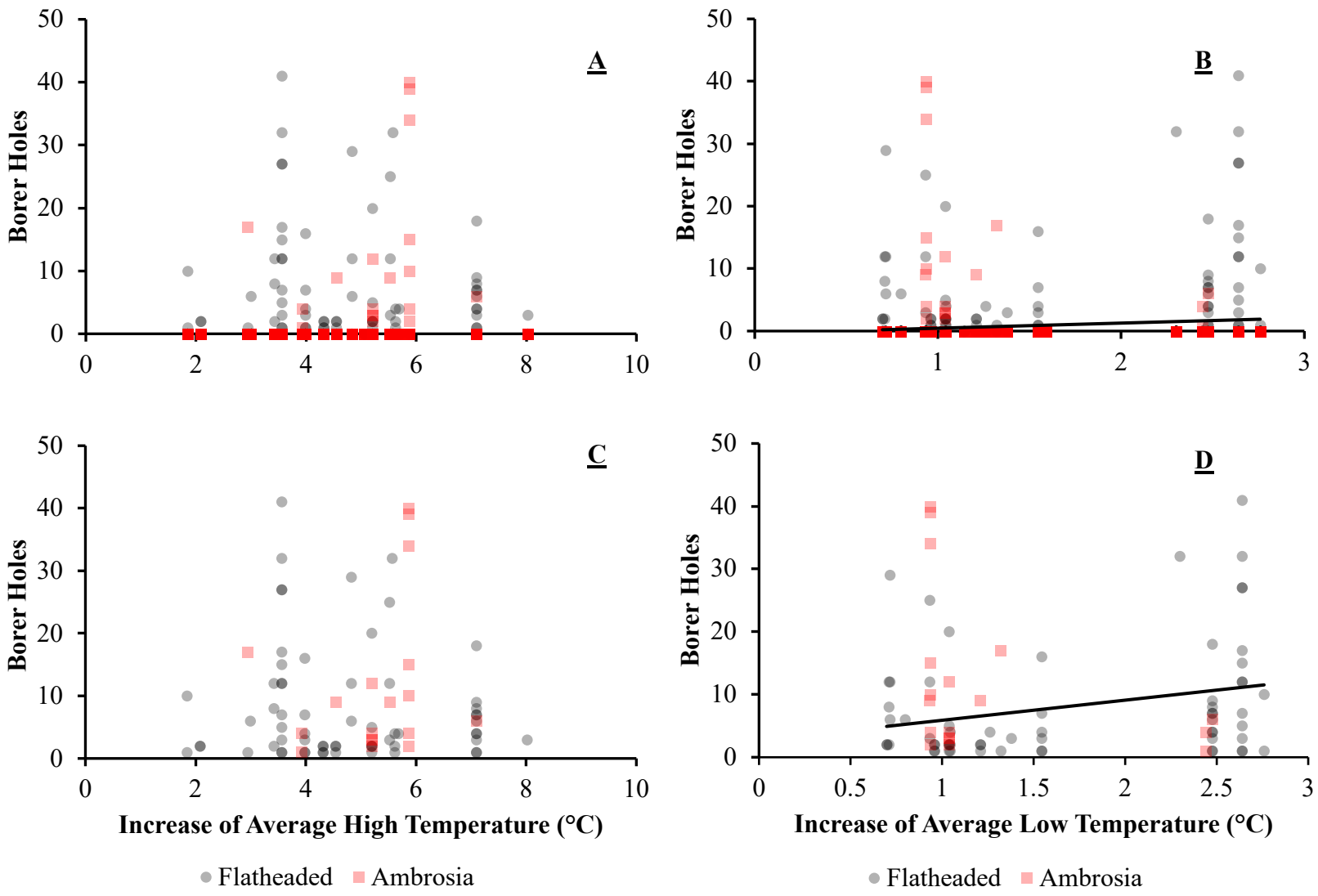

A
B
C
D
Borer Holes
Increase of Average High Temperature (°C)
Increase of Average Low Temperature (°C)
Flatheaded
Ambrosia

Figure 3.5. The numbers of holes of flatheaded borer and ambrosia beetles were recorded on the tree with the percent pervious area around each tree where (A) zero incidences of attack included and (B) excluded. The percent pervious area was determined using the "Pace-to-Plant" technique. Trendlines suggest parameters were significant in the respective zero-inflated negative binomial model.

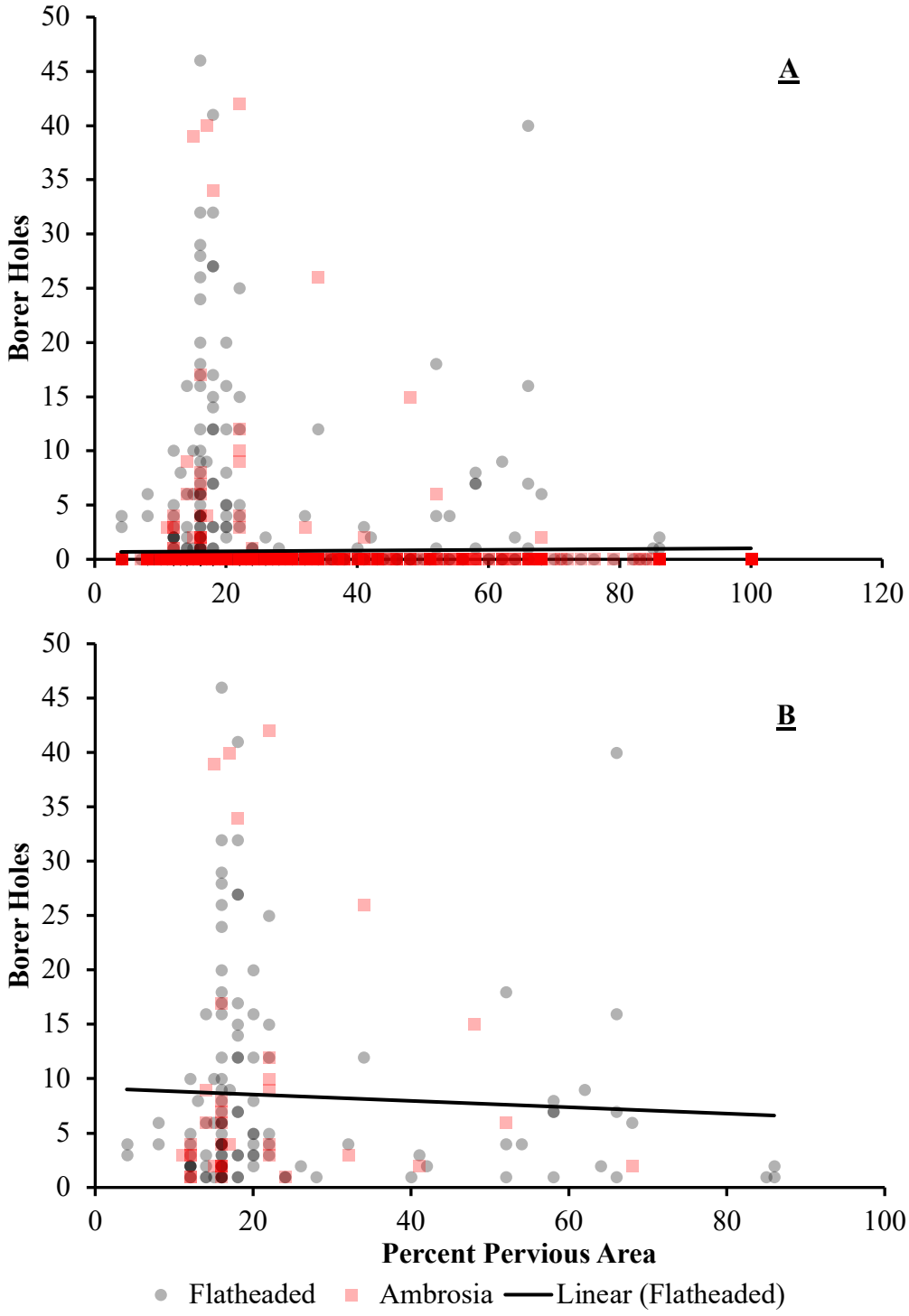
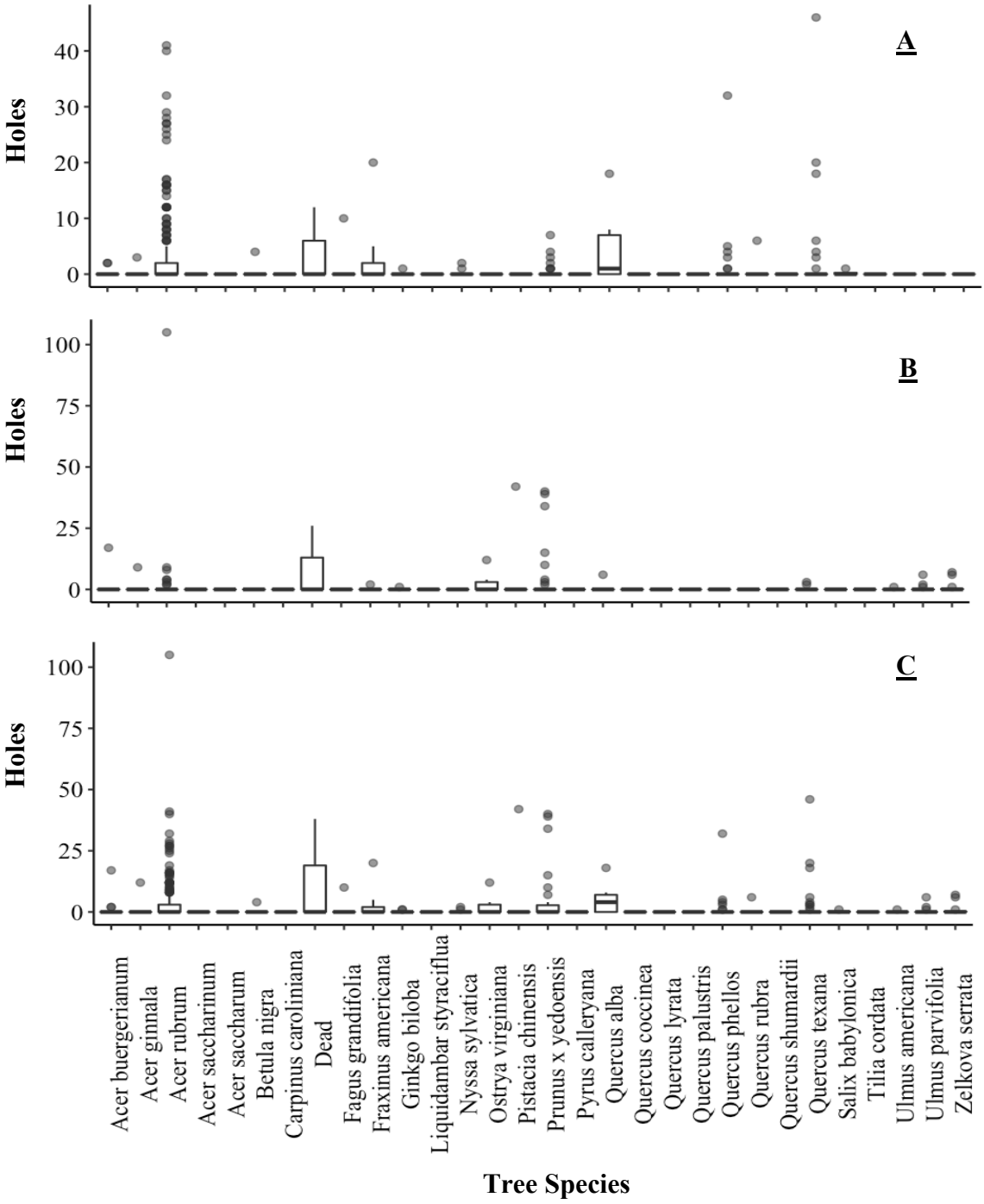

A
B
Borer Holes
Percent Pervious Area
Flatheaded
Ambrosia
Linear (Flatheaded)

Figure 3.6. The numbers of holes of (A) flatheaded borer, (B) ambrosia beetles, and (C) all borers combined by tree species.


A
B
C
Holes
Tree Species
Acer buergerianum
Acer ginnala
Acer rubrum
Acer saccharinum
Acer saccharum
Betula nigra
Carpinus caroliniana
Dead
Fagus grandifolia
Fraxinus americana
Ginkgo biloba
Liquidambar styraciflua
Nyssa sylvatica
Ostrya virginiana
Pistacia chinensis
Prunus x yedoensis
Pyrus calleryana
Quercus alba
Quercus coccinea
Quercus lyrata
Quercus palustris
Quercus phellos
Quercus rubra
Quercus shumardii
Quercus texana
Salix babylonica
Tilia cordata
Ulmus americana
Ulmus parvifolia
Zelkova serrata

CHAPTER 4

EFFECTS OF PERMETHRIN RESIDUES ON AMBROSIA BEETLES IN ORNAMENTAL NURSERIES [1]

[1] Williamson, Z.V., B.R. Blaauw, and S.V. Joseph. To be submitted to *Journal of Insect Science*.

Abstract

Exotic ambrosia beetles (Coleoptera: Curculionidae), such as *Xylosandrus crassiusculus* (Motschulsky), *Xylosandrus germanus* (Blandford), and *Xylosandrus compactus* (Eichoff) are serious pests in southeastern ornamental nurseries (Joseph et al. 2019). Preventative pyrethroid trunk sprays effectively reduce the borer holes in the trees. However, it is unclear how pyrethroids such as permethrin prevent the attack. Thus, the objective was to determine how permethrin-treated bolts interact with invading ambrosia beetles. In 2022, a study was conducted in the nursery on red maple, *Acer rubrum* L., bolts during March and April. The treatments were 1) nonbaited, nontreated bolt, (2) ethanol baited bolt, (3) nonbaited bolt + glue [painted on bolt], (4) ethanol baited bolt + glue, (5) ethanol baited bolt + glue + permethrin, (6) ethanol baited bolt + glue + permethrin + verbenone, and (7) ethanol baited bolt + glue + verbenone. Ambrosia beetles trapped on glue in the pail with soap solution under the bolts, and entry holes on bolts were quantified. Permethrin prevented beetle attacks but did not reduce the densities of ambrosia beetles landing on the treated bolts. Verbenone did not consistently reduce ambrosia beetle landing on the bolts. The numbers of ambrosia beetles in soapy water were not significantly different among treatments. Ambrosia beetles are likely interacting with permethrin as contact repellency, implying that volatiles of fresh permethrin residues may not be necessary for ambrosia beetle management.

Keywords: Ambrosia beetles, *Xylosandrus*, *Xylosandrus crassiusculus*, permethrin, verbenone, field nursery

Introduction

Georgia ornamental nursery production was valued at $444 million USD in 2020 (GFGV 2022). Millions of dollars in losses occur in the Georgia nursery industry as a result of nonsalable plants affected by arthropod pests and pest management expenditures (LeBude et al. 2012). Because of this, it is important to understand the pest management practices administered in ornamental nurseries. Invasive ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are serious pests of ornamental tree nurseries in the southeastern USA (Fulcher et al. 2012, Rabaglia et al. 2006). Among them, *Xylosandrus* species, such as the granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky), the black stem borer, *Xylosandrus germanus* (Blandford), and the black twig borer *Xylosandrus compactus* (Eichoff) are the most damaging pests (Ranger et al. 2016a, Gugliuzzo et al. 2021, Monterrosa et al. 2022).

Xylosandrus species typically have three generations per year in the southeastern USA; however, this is affected by air temperature and other weather-related factors (Frank et al. 2013, Ranger et al. 2016a). Similar to other tribes, Xyleborini ambrosia beetles, *Xylosandrus* spp. larvae develop from eggs laid by foundress females, which bore and inoculate galleries with symbiotic *Ambrosiella* fungus stored in their mycangium (Weber and McPherson 1983, Ranger et al. 2016b). Both adult and immature of *Xylosandrus* spp. feed only on the symbiotic fungi. Eggs are laid singly at the distal regions of the brood chamber, where fungal growth is plentiful (Ranger et al. 2016a). The larvae develop through three instars upon egg hatch before pupating inside the brooding chamber (Ranger et al. 2016a). Adult males are flightless and smaller than adult females. Adult females mate with their brothers and overwinter inside the galleries (Weber and McPherson 1983, Gugliuzzo et al. 2021). Mated females emerge in early spring as temperatures warm up to 18.3 °C for 2-3 d (Reding et al. 2016a, Monterrosa et al. 2022). Other

subsequent emergences occur later in the season when beetles seek new tree hosts (Weber and McPherson 1983, Greco and Wright 2015). Ambrosia beetles use stress signals, especially ethanol, to locate and attack stressed trees (Ranger et al. 2010, 2015). When boring through the bark, ambrosia beetles push out frass and sawdust from the entry holes, which appear as toothpicks; however, these are easily dislodged by wind or rain (Frank et al. 2013).

Preventive insecticide application is critical in managing ambrosia beetle attacks in nurseries because ambrosia beetles are protected from insecticides once they enter the tree bark (Frank and Sadof 2011). Pyrethroids are often used as preventative trunk sprays in early spring before and during peak flights (Mizell et al. 2004, Frank and Sadof 2011, Reding et al. 2013, Ranger et al. 2016b, Frank et al. 2017). Repeated applications of pyrethroids, particularly permethrin or bifenthrin, are recommended between 8 and 17 d intervals (Brown et al. 2020), although a consistent efficacy in preventing ambrosia beetle infestation is still not guaranteed (Ranger et al. 2016b). Other insecticide chemistries have been tested as preventative trunk sprays, but none have provided satisfactory efficacy against *Xylosandrus* spp. to date (Joseph 2022a, 2022b). Currently, preventative applications of pyrethroids, especially bifenthrin and permethrin, are the only insecticide option for ambrosia beetle management.

Although pyrethroids, primarily bifenthrin and permethrin, are widely used for preventing ambrosia beetle attacks, it is unclear how these pyrethroids effectively reduce the *Xylosandrus* spp. from boring into the bark. While studies evaluating modes of repellent behavior of ambrosia beetles to permethrin are limited, the mode of repellency has been explored for many other arthropod pests. For example, permethrin and cypermethrin prevented damage to *Glycine max* (L.) Merr. (soybean) leaves through direct mortality and feeding avoidance of *Epilachna varivestis* Mulsant (Coleoptera: Chrysomelidae), demonstrating contact repellency

(Dobrin and Hammond 1985). Nymphs of *Ixodes scapularis* Say (Ixodida: Ixodidae) elicited contact repellency as most dislodged from the permethrin-treated fabric after exposure (Eisen et al. 2017). Similarly, the probing behavior of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) was reduced after contact with pyrethroid insecticides, likely due to contact repellency (Lowery and Boiteau 1988). Therefore, there is a knowledge gap in understanding the repellency behavior of *Xylosandrus* spp. approaching pyrethroid-treated tree trunks. We hypothesize that pyrethroids, especially permethrin, prevent ambrosia beetle attacks through repellency, although it is unclear if a contact or noncontact mechanism contact induces repellency. The objective was to determine the mechanism that causes reduced attacks on permethrin treated trees. This knowledge will help researchers and growers improve the management of *Xylosandrus* spp. by increasing their understanding of permethrin's effect on ambrosia beetle and corresponding method of ambrosia beetle management. Thus, this study seeks to expand knowledge regarding the successful implementation of preventative pyrethroid sprays in ornamental nurseries, as well as aid in offering better recommendations for growers.

Materials and Methods

Study site. In 2022, a study was conducted at an in-ground tree nursery in Lamar County, Georgia, USA. The site consists of ~35 ha in production, and trees were spaced ~2 m apart. The experiment was employed on the western edge of the nursery. The traps were deployed along the edge of the woodline. The experimental area was surrounded by the trees grown in the nursery and mixed hardwood and pine forest. Common trees grown in the nursery include holly, *Ilex* spp., maple, *Acer* spp., oak, *Quercus* spp., elm, *Ulmus* spp., *Camellia* spp., juniper, *Juniperus* spp., and crape myrtle, *Lagerstroemia* spp. at various ages (from <1 to 5 years). The nursery

trees were under drip irrigation. The nursery tree species adjacent to the woodline were red maple, *Acer rubrum* L., pignut hickory, *Carya glabra* (Mill.) Sweet, American sweetgum, *Liquidamabar styraciflua* L., loblolly pine, *Pinus taeda* L., and post oak, *Quercus stellata* Wangenh. These trees were at least 5 m away from the edge of the woodline. The site was managed following standard production recommendations and commercial pesticide management guidelines. Pesticides were not used for the duration of the experiment near the experimental area.

Bolt trap, insecticide. The bolts were prepared using *Acer rubrum* branches that were obtained from residential yards in Fayette and Henry Counties. Red maple was selected for the experiment because *Xylosandrus* spp. routinely attack this tree species, and it is an important crop in many nurseries in the southeast USA (Frank et al. 2013, COA 2019). The red maple branches were cut into 50 cm long pieces and were temporarily stored in the refrigerator for ~7 d before deployment. These bolts were 5.1-7.3 cm in diameter. Before deployment for the experiment, the bolts were further cut into 25 cm long pieces. A 1.5 × 7 cm (diameter × deep) hole was drilled on the top of each bolt using a handheld drill. Two screws were affixed to the top of the bolts, and a 50 cm long string was then tied to the screws (Fig. 4.1A). The bolts were suspended from a 122 cm metal shepherd's hook. Bolts were hung at ~ 91 cm above the ground.

The insecticide used in the experiment was permethrin (Perm-UP 3.2 EC, 36.8% permethrin; FMC Corporation, Philadelphia, Pennsylvania, USA). The application rate of Perm-UP 3.2 EC was 64 mL per ha. Insecticide solution was prepared with a water volume of 374 L per ha. To do so, 1.6mL of insecticide was mixed with 1000mL of water. The prepared insecticide solution was sprayed on the bolts after suspending them by their hangers from a PVC

pipe for uniform coverage of insecticide until run-off. Insecticide solution was applied using a CO_2-powered single boom handheld sprayer at 206.8 kpa. The nozzle was attached with a TeeJet 8002VS (yellow-colored tip, TeeJet Technologies, Glendale Heights, Illinois, USA).

Experimental design. Experiments were conducted in the spring when the *Xylosandrus* spp. adults were actively flying. The first round of the experiment was conducted from 2 March to 16 March (trial 1) and the experiment was repeated from 11 April to 25 April 2022 (trial 2) in the same nursery. Treatments were: (1) nonbaited, nontreated bolt, (2) ethanol baited bolt, (3) nonbaited bolt + glue, (4) baited bolt + glue, (5) baited bolt + glue + permethrin applied, (6) baited bolt + glue + permethrin + verbenone, and (7) baited bolt + glue + verbenone. The treatments were arranged in a randomized complete block design with five replications. The individual bolt served as the experimental unit. The bolts were deployed 10 m apart along the woodline about 0.5 m inside the nursery from the edge. The bolts were suspended on the shepherd's hooks as described in the previous section.

The nontreated check and the ethanol-baited check bolts were included to ensure the attraction of *Xylosandrus* spp. to the ethanol bait. In some treatments, the glue (Pestik, Phytotronics, Inc. Earth City, Missouri, USA) was applied on the bolt surface to trap the ambrosia beetles landing on the bolts (Fig. 4.1). To ensure glue by itself not attracting the adults of ambrosia beetles, a treatment was added with only glue painted on bolts without ethanol bait. Verbenone is a repellant semiochemical and is used against many ambrosia beetle species (Lindgren et al. 2002; Rivera et al. 2020). Thus, verbenone treatment as a positive control treatment (Verbenone, Synergy Shield Verbenone pouches,97.0% verbenone; Synergy Semiochemical Corporation, Delta British Columbia, Canada) was included. Verbenone pouches

were placed on the shepherd's hooks (5 cm above the bolts) (Fig. 4.1). As described in the previous section, permethrin solution was sprayed on certain treatments to determine the repellent effects.

For treatments with ethanol, 10 mL of 95% ethanol was poured into the hole at the top of the bolt at setup and at the 6 (trial 1) or 7 d (trial 2) of the experiment so that the bolts could remain attractive to the ambrosia beetles (Reding and Ranger 2020). All bolts, even those treatments without ethanol added, had the drilled hole sealed with a 1.25 cm (diameter) cork stopper. For those bolts that received glue, Pestik glue was painted lengthwise, in 2.5 cm wide strips, directly onto the designated bolts using a stiff bristled paintbrush. Thus, there was also 2.5 cm wide of exposed bark (no glue) areas between each glue section (Fig. 4.1). For those permethrin-treated bolts, the glue was applied after the application of permethrin for certain treatments as indicated. The number of glue strips on the bolts varied from three to five strips as the circumference of the maple bolts varied. The coverage area was 1:1 for glue: nonglue surface on bolt surface so that there is sufficient nonglued surface for ambrosia beetles to land. The glue used in the study to capture ambrosia beetles was weather and rain-resistant. Because those ambrosia beetle adults that land on permethrin-treated bolt may become moribund or die, adults could fall. To capture the ambrosia beetle adults falling down from the bolts, a 15.1 L plastic pail with 15 mL of soap (Proctor and Gamble, Kansas City, Kansas, USA) and 250mL of water was prepared as a solution and placed under each bolt (all treatments included; Fig. 4.1). The soap solution was emptied and refilled at every observation date.

Evaluation. Bolts were evaluated at 2, 6, and 14 d for trial 1, and 2, 7, and 14 d in trial 2. The date of the second observation differed between trials because there was a heavy rain forecast on the 7 d for trial 1; thus, data was gathered at 6 d (Fig. 4.2).

At each evaluation date, the cumulative number of ambrosia beetle entry holes on the bolts was quantified. The entry holes were circled using a wax pencil to avoid double counting. All the ambrosia beetles stuck on the glue were counted at each evaluation, with counts recorded for all three evaluations per trial to achieve cumulative totals. All ambrosia beetles were counted, including non-*Xylosandrus* spp., as identifying them was challenging in the field. At the end of the trial, all bolts were collected and placed in plastic bags, transported to the laboratory, and stored in a freezer at -18 °C until processing. Before processing, bolts were allowed to thaw at 21 °C for 2 h. The bolts were removed from the plastic bags, and the bolt diameter and length were measured. The ambrosia beetles caught on the glue were removed from the bolts by painting Histo-Clear (Electron Microscopy Sciences, Hatfield, Pennsylvania, USA) onto the glue with a paintbrush. After 2 mins, ambrosia beetles loosen enough to be removed using pointed forceps. The removed adults were placed into a vial filled with 10 mL of Histo-Clear. After 15 h in the solvent, adults were rinsed in water and 70% ethanol before storage in a vial with 70% ethanol for identification. Ambrosia beetles were not removed from the bolt to determine beetle species in galleries.

Xylosandrus spp. adults collected in the soapy water under the bolts were screened by pouring it through a mesh bag and recovering the contents at the three evaluation dates. From filtered samples, the ambrosia beetles were sorted and removed using a paintbrush and stored in microcentrifuge tubes filled with 70% ethanol for identification. In a few cases, soap samples of individual bolts were lost due to rain and sustained wind. Those samples were considered

missing data points. Ambrosia beetles stored in ethanol were then identified. As *Xylosandrus* spp. has historically been the most damaging ambrosia beetles in Georgia ornamental nurseries (Monterrosa et al. 2022), *Xylosandrus* spp. were identified to species using Bateman and Hulcr's guide to bark and ambrosia beetles (Ranger et al. 2016, Bateman and Hulcr 2017, Gugliuzzo et al. 2021, Monterrosa et al. 2022) and other ambrosia beetles were labeled "others".

Statistical analyses. All statistical analyses were conducted utilizing R software (R Core Team 2021). Count data for cumulative numbers of entry holes, cumulative numbers of beetles in soap, and the cumulative number of beetles captured in glue were square-root transformed. Repeated measures analysis of variance (ANOVA) of data using the aov() function using time, treatment, and block as factors was conducted. Tukey's honest significant difference (HSD) test was then conducted using the Tukey HSD() function of R for post-hoc comparison of means by time. An additional ANOVA analysis using the aov() function was conducted using time, treatment, and a blocking factor in addition to an interaction term for treatment and time to better understand the data.

Because the effect of time was significant, data were analyzed individually by day using two-way ANOVA utilizing the aov() function of R with the primary response factor of cumulative holes, glue captures, or soap captures for that data as well as a blocking factor to account for variation between blocks. Tukey's HSD test ($\alpha = 0.05$) was then conducted using the Tukey HSD() function of R for post-hoc comparison of treatment means. Because the study's primary focus is on the repellency of permethrin, separate analyses for the two-way ANOVA of treatment and blocking were conducted with all treatments included as well as only among treatments that included glue, as the nonglue treatments could disproportionately bias the

outcome of adult beetle entry holes. Data were analyzed separately for each of the two trials and their three evaluations due to inherent differences in temperature and rainfall between trials (Fig. 4.5).

Results

Beetle species and treatment effects

Most beetles captured in the glue on the bolts and soapy water under the bolt samples in trials 1 and 2 were in the genus *Xylosandrus,* and primarily, *Xylosandrus crassiusculus* (Fig. 4.2). For trial 1, the treatment, sampling date, and their interaction were significantly different for density of entry holes, beetles captured on glue and trapped in soap solution (Table 4.1). For trial 2, treatment and sampling date were significantly different for the density of entry holes, beetles captured on glue, and trapped in soap solution. However, the interactions between treatment and sampling date were significantly different for the density of entry holes. Still, they were not significantly different for beetles captured on glue and trapped in soap solution (Table 4.1). Thus, one-way ANOVA was performed by treatment and sampling date for each trial to better understand the effects.

Entrance holes

Trial 1. At 2 d, treatment did not significantly affect the number of holes (Fig. 4.3a). At 6 d, the numbers of holes in the overall model were significantly greater for the ethanol treatment than for the nontreated or glue treatments, whereas the ethanol + glue treatments were similar to the ethanol treatment ($F = 16.5$, df = 6, $P < 0.001$; Fig. 4.3b). The glue treatment was similar in the number of entry holes on the permethrin and permethrin + verbenone treatments. The densities

of entry holes were not significantly different among ethanol + glue, permethrin, and verbenone treatments (Fig. 4.3b).

At 14 d, the numbers of holes in the overall model were significantly greater for the ethanol treatment than for the remaining treatments except for ethanol + glue treatment (F = 13.7, df = 6, $P < 0.001$; Fig. 4.3c). The densities of entry holes were similar between the ethanol and ethanol + glue control treatments. The numbers of entry holes for nontreated, glue, permethrin, and permethrin + verbenone treatments were not significantly different from each other. The entry holes in the verbenone treatment were significantly greater than in the glue and nontreated treatments (Fig. 4.3c).

For the reduced model (where nontreated + nonbaited [none] and ethanol were dropped), at 2 d, treatment did not have a significant effect on the number of entry holes (Fig. 4.3d). At 6 d, the numbers of entry holes were significantly lower for the glue than for the ethanol + glue and verbenone treatment (F = 8.7, df = 4, $P < 0.001$; Fig. 4.3e). The glue + ethanol treatment did not significantly differ in densities of entry holes for the permethrin and verbenone treatments. There were no significant differences in the number of entry holes among permethrin, permethrin + verbenone, and verbenone treatments. At 14 d, the numbers of entry holes were significantly lower for the glue treatment than for the ethanol + glue and the verbenone treatments. In contrast, the permethrin and permethrin + verbenone treatments did not significantly vary from any of the remaining treatments other than the ethanol + glue (F = 6.3, df = 4, $P < 0.001$; Fig. 4.3f).

Trial 2. For the overall model, the effect of treatment on numbers of holes were not significantly different (F = 1, df = 6, P = 0.448; Fig. 4.4a). At 7 d, significantly more numbers of holes were

found for ethanol and ethanol + glue treatments than for the nontreated, glue, permethrin, and permethrin + verbenone treatments (F = 11.0, df = 6, P < 0.001; Fig. 4.4b). The numbers of entry holes were similar for the ethanol, ethanol + glue control, and verbenone treatments. There were no significant differences between the verbenone treatment and the permethrin + verbenone, permethrin, glue, and nontreated treatments. At 14 d, the nontreated, glue, permethrin, and permethrin + verbenone treatments had significantly lower numbers of entry holes than for the ethanol and ethanol + glue treatments (F = 8.1, df = 6, P < 0.001; Fig. 4.4c). The numbers of entry holes for the verbenone treatment were not significantly different from any control or remaining treatments.

For the reduced model, the effect of treatment on the number of holeswas not significantly different at 2 d (No holes in any reduced treatments, Fig. 4.4d). At 7 d, the ethanol + glue treatment had significantly more numbers of holes than for the glue, permethrin, and permethrin + verbenone treatments (F = 11.7, df = 4, P < 0.001; Fig. 4.4d). The verbenone treatment was not significantly different from any control treatments. At 14 d, the ethanol + glue as well as the verbenone treatment, had significantly more numbers of holes than the glue (F = 10.1, df = 4, P < 0.001; Fig. 4.4f). The permethrin and permethrin + verbenone treatments were similar in densities of entry holes to the nontreated treatment.

Ambrosia beetle captures in glue

Trial 1. Because glue was only present on five out of seven treatments in the overall model, results from the overall model and reduced model are identical. At 2 d, the numbers of ambrosia beetle adults captured in glue were significantly lower for the glue treatment than for the remaining treatments (F = 14.9, df = 4, P < 0.001; Fig. 4.3a or d). At 6 d, the glue captured

lower densities of adults in the glue than any of the remaining treatments (F = 38.7, df = 4, P < 0.001; Fig. 4.3b or e). The permethrin and permethrin + verbenone treatments had similar numbers of adult captures, which were significantly greater than the verbenone treatment. The permethrin + verbenone treatment had significantly more numbers of adult captures than the verbenone treatment, glue + ethanol, and glue treatments. The numbers of adults captured for the permethrin and verbenone treatments were similar to the ethanol + glue treatment. At 14 d, the glue treatment captured lower densities of adults than the remaining treatments, with the verbenone treatment having the second lowest captures (F = 60.9, df = 4, P < 0.001; Figs. 4.3c or e, f). The ethanol + glue control treatment had significantly fewer numbers of adult captures than the permethrin + verbenone treatment; however, the permethrin treatment was similar to both the ethanol + glue and the permethrin + verbenone treatments.

Trial 2. The numbers of ambrosia beetles captured in the glue were significantly lower for the glue treatment than for the remaining treatments at 2 (F = 17.8, df = 4, P < 0.001; Fig. 4.4a or d), 7 (F = 30.6, df = 4, P < 0.001; Fig. 4.4b or e) and 14 d (F = 30.8, df = 4, P < 0.001; Fig. 4.4c or f). At 7 d, the numbers of adult captures were significantly greater for the permethrin treatment than for the permethrin + verbenone treatment, whereas the ethanol + glue and verbenone treatments were similar to the permethrin and permethrin + verbenone treatments. At 14 d, the permethrin treatment had significantly more adult captures than the verbenone and verbenone + permethrin treatments. There were no significant differences in adults captured in the glue between the ethanol + glue treatments and the remaining treatments.

Ambrosia beetle captures in soap solution

Trial 1. At 2 d in the overall model, captures of adult ambrosia beetle in soap solution for the nontreated, glue, permethrin, and permethrin + verbenone treatments were significantly lower than for the ethanol treatment (F = 8.4, df = 6, P < 0.001; Fig. 4.3a). Adult captures were not significantly different among the ethanol, ethanol + glue, and verbenone treatments. Differences between the ethanol + glue and permethrin, verbenone, and permethrin + verbenone treatments were not significant. At 6 d (F = 17.2, df = 6, P < 0.001; Fig. 4.3b) and 14 d (F =17.7, df = 6, P < 0.001; Fig. 4.3c) in the overall model, adult captures in soap were significantly lower for the nontreated treatment and glue than for the remaining treatments. In the reduced model, the number of adult ambrosia beetles captured in soap solution was significantly lower for the glue treatment than for the remaining treatments at 2d (F =3.9, df = 4, P = 0.025; Fig. 4.3d), 6 (F = 10.6, df = 4, P < 0.001; Fig.4.3e), and 14 d (F=10.8, df = 4, P < 0.001; Fig. 4.3f)

Trial 2. For the overall model, the adult captures in soap solution were significantly lower for the nontreated and glue treatments than for the remaining treatments at 2 d (F=13.9, df = 6, P < 0.001; Fig. 4.5a), 7 d (F=18.0, df = 6, P < 0.001; Fig. 4.5b), and 14 d (F=18.6, df = 6, P < 0.001; Fig. 4.5c). For the reduced model, captures of ambrosia beetles were significantly lower for the glue treatment than for the remaining treatments at 2 (F =11.8, df = 4, P < 0.001; Fig. 4.4d), 7 (F =13.4, df = 4, P < 0.001; Fig. 4.4e), and 14 d (F =13.7, df = 4, P < 0.001; Fig. 4.4f).

Sampling date

Trial 1. The numbers of entry holes for the 6 d and 14 d evaluation dates were significantly greater than for the 2 d evaluation (F = 33.6, df = 2, P < 0.001). The numbers of ambrosia beetles

on the glue were significantly greater for the 6 and 14 d evaluation dates than for the 2 d evaluation ($F = 39.2$, df = 2, $P < 0.001$). The numbers of ambrosia beetles in the soapy water solution were significantly greater for the 6 and 14 d evaluations than for the 2 d evaluation ($F = 37.9$, df = 2, $P < 0.001$).

Trial 2. The numbers of entry holes for the 7 d and 14 d evaluation dates were significantly greater than for the 2 d evaluation ($F = 17.9$, df = 2, $P < 0.001$). The densities of ambrosia beetle on glue captures for the 14 d evaluation were significantly greater than for the 2 and 7 d evaluations ($F = 17.1$, df = 2, $P < 0.001$). The numbers of ambrosia beetles in the soapy water solution were significantly greater for the 7 and 14 d evaluations than for the 2 d evaluation ($F = 26.2$, df = 2, $P < 0.001$).

Discussion

Xylosandrus crassiusculus was the most commonly captured species of ambrosia beetle in the current study, which is consistent with previous studies where more than half of all ambrosia beetle captures were *X. crassiusculus* (Ranger et al. 2016b, Gugliuzzo et al. 2021, Monterrosa et al. 2022). Results show that the densities of ambrosia beetle captured on glue painted on bolts treated with and without permethrin were not different, or in some cases, greater on bolts treated with permethrin than without permethrin. This suggests that ambrosia beetles are not repelled after sensing the volatiles of permethrin from the treated bolts through noncontact repellency. However, the densities of entry holes were lower on permethrin-treated bolts than on non-permethrin-treated bolts. This indicates that the underlying mechanism could be either contact repellency or intoxication after ambrosia beetles come in contact with permethrin-treated

bolts. In a contact repellency scenario, those ambrosia beetle adults who land on the permethrin-treated bolts might have flown off after sensing permethrin residues instead of boring entrance holes. The other possibility is that they were intoxicated and became moribund or dead after contact with permethrin residues. Because the densities of ambrosia beetles collected in the pail with soap solution were not different with and without permethrin treatment, it implies that adult beetles getting intoxicated or killed after contact with permethrin residues is not the driving mechanism. Thus, the ambrosia beetles are more likely to leave the permethrin-treated bolt surface as a form of contact repellency, although some adults might have been knocked down post-permethrin exposure as moribund or dead. This is consistent with previous studies where contact repellency was the leading mechanism in the prevention of damage from pests such as *E. varivestis, M. persicae*, and *Stephanitis pyrioides* Scott (Hemiptera: Tingidae) (Dobrin and Hammond 1985, Lowery and Boiteau 1988, Joseph 2020). Other studies exploring the effects of permethrin on *Aedes aegypti* Linnaeus (Diptera: Culicidae) found that densities of mosquito bites were reduced when permethrin-treated clothing was used (Orsborne et al. 2016, Bowman et al. 2018). Furthermore, *Apis mellifera* Linnaeus (Hymenoptera: Apidae) avoided foraging on feeders treated with permethrin versus nontreated feeders (Rieth and Levin 1988). In these studies, all organisms came in contact with permethrin-treated surfaces and then subsequently attempted to leave the permethrin-treated area, even among drastically different systems and insect groups.

In the current study, more ambrosia beetles were captured on the glue with permethrin-treated bolts compared to non-permethrin-treated bolts. The exact reasons for this behavior are unclear, but one possible explanation could be that pyrethroids are known to cause excitatory behavior when insects come in contact with residues. As a sodium channel modulator,

pyrethroids cause the sodium channels involved in the generation of action potentials along nerve axons to remain open (IRAC 2022). A member of the pyrethroid family, permethrin kills by making the nervous system hypersensitive to stimuli (Cox 1999). This hypersensitivity of the nervous system causes excitation and irritation behaviors. For example, there was a visible excitatory reaction when *Ixodes ricinus* (Linnaeus) (Ixodida: Ixodidae) crawled on permethrin-impregnated fabric (Faulde et al. 2008). Mosquitoes exposed to permethrin displayed the characteristic "hot feet" sign of contact irritancy and reduced blood-feeding rates (Orsborne et al. 2016). Numerous other studies confirm this phenomenon, with organisms exhibiting behavioral avoidance of pyrethroids, including permethrin (Meyer et al. 2006; Yan et al. 2011, Boonyuan et al. 2016, Joseph 2020). Because of this, it is possible that ambrosia beetles landed on the surface of the bolt and upon exposure to permethrin, may have elicited excitatory, erratic movement as more numbers of ambrosia beetles were caught on the glue coated on ethanol baited bolts.

The standalone deployment of a verbenone dispenser did not conclusively reduce ambrosia beetle attacks on the ethanol-baited bolts, although ambrosia beetle captures were lower than ethanol-baited bolts in some cases. Verbenone is a known repellent as it repels bark beetles, serving as an anti-aggregation pheromone for species such as *Dendroctonus ponderosae* Hopkins*, Dendroctonus frontalis* Zimmerman, and *Ips typographus* L. (Pitman and Vité 1969, Pitman et al. 1969, Lindgren and Miller 2002). This compound is produced by fungal symbionts of various bark beetle species, such as *Ips typographus* and *D. frontalis* which oxidize trans-verbenol to verbenone (Brand et al. 1976, Leufvén et al. 1984). This chemical functions by interrupting the attraction of beetles to conspecific pheromones and host volatiles, such as ethanol (Ranger et al. 2021). Because of this, its use in management programs is often explored.

Verbenone has been evaluated for ambrosia beetles in ornamental nurseries with variable efficacy in reducing ambrosia beetle attacks. Verbenone reduced sticky trap captures of the redbay ambrosia beetle, *Xyleborus glabratus* Eichoff (Hughes et al. 2017). Similarly, the verbenone dispenser reduced attacks on trap trees, but attacks did occur to all trees. In that study, ethanol-baited traps captured primarily, *Xylosandrus germanus* (Ranger et al. 2013). In another study, *X. germanus* trap captures were lower in verbenone-treated funnel traps, although verbenone did not reduce attacks on the tree (Dodds and Miller 2010). Thus, verbenone may deter ambrosia beetles in some cases by preventing landing, but those beetles that do land were able to bore into heartwood and effectively colonize the tree. The tolerance for ambrosia beetle attacks in ornamental production is minimal, and inconsistent efficacy when only using verbenone for the management of ambrosia beetles may affect the aesthetic value and marketability of the trees. The utility of verbenone as a standalone method for managing ambrosia beetles is a risky approach. However, verbenone may serve as a component of integrated pest management programs when combined with other methods of intervention, such as permethrin trunk sprays (Frank et al. 2011, Joseph 2022a, 2022b).

Understanding the method of repellency of permethrin to ambrosia beetles has obvious implications for managing ambrosia beetles in nursery production. The results of the current study showed that permethrin sprays reliably deterred boring and colonization by contact repellency. Many nursery growers have a notion that fresh residues of pyrethroid are required as the volatiles from those freshly applied residues prevented attacks from ambrosia beetles. The results show that multiple applications of pyrethroids at close intervals may be unnecessary, even though the ambrosia beetles could infrequently fly during fluctuating warmer temperatures in early spring. Those ambrosia beetles that land on the tree trunk treated with permethrin may

leave or get intoxicated and not bore into the tree trunk. Moreover, Brown et al. (2020) suggested that permethrin applied at 8 -17 d intervals reduced adult beetle attacks. Thus, concurrent applications of permethrin at closer than 14 d offer no benefit. With concerns for harmful effects on natural enemies and pollinators resulting from permethrin application (Frank and Sadof 2011, Lebude et al. 2012), this information is important for ornamental growers to reduce pyrethroid exposure to nontargets.

The current study also has its share of limitations. During the trials, weather conditions were variable, such as intermittent frost and heavy rain (Fig. 4.5). Secondly, the cumulative glue captures decreased in trial 2, which occurred after heavy rains (Fig. 4.5) as some glue with beetles rinsed off the bolts. Thirdly, species-level identification of ambrosia beetles collected on the glue applied on bolts were only obtained at 14 d when the bolts were removed from the field. Finally, it could not be determined whether beetles were killed after permethrin exposure and fell into the bucket placed below the bolts and trapped in soap solution or if they left the bolts after landing on it.

In summary, permethrin did not stop the ambrosia beetles from landing on the treated bolt but prevented successful colonization. Captures of ambrosia beetles on glue were greater on permethrin-treated than nontreated bolts, possibly because of excitatory movement post-permethrin exposure. This may be a function of exposure to a sodium channel modulator-based insecticide such as permethrin. Future research exploring rate-dependent effects of permethrin deterrence to ambrosia beetles is warranted. Previous studies showed that verbenone did not consistently deter ambrosia beetle from landing on the ethanol-baited bolts. This suggests that a more thorough understanding of the relationship between ethanol emittance doses and verbenone on deterrence of approaching ambrosia beetles may provide valuable information regarding

verbenone as a non-insecticide strategy. The results from the current study expand our understanding of how permethrin interacts with stressed trees emitting ethanol signals and subsequent ambrosia beetle attacks. Thus, it will be valuable information for nursery growers and researchers alike.

Acknowledgments

We thank Chris Hardin and Logan Ibanez for their assistance in field sampling. Mention of insecticide active ingredients in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement. This work is funded by USDA NIFA SCRI #5950821011.

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Table 4.1. Parameters associated with the analysis of variance by treatment, sampling date, and treatment interaction and sampling date of trial 1 and trial 2.

Effects	Holes			Glue captures			Soap captures		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
Trial 1									
Treatment	31.7	6	<0.001	90.5	4	<0.001	42.8	6	<0.001
Time	60.1	2	<0.001	50.0	2	<0.001	43.8	2	<0.001
Treatment × Time	7.0	12	<0.001	3.2	8	0.005	2.2	12	0.021
Trial 2									
Treatment	18.3	6	<0.001	83.3	4	<0.001	54.6	6	<0.001
Time	25.7	2	<0.001	17.8	2	<0.001	25.1	2	<0.001
Treatment × Time	4.3	12	<0.001	1.3	8	0.251	0.7	12	0.764

Figure 4.1. Set up of wooden bolt with glue bands (dotted arrow) painted on the bolt and verbenone pouch (striped arrow) suspended from the shepherd's hook in a field nursery. Permethrin was trunk sprayed before the glue was painted on the bolt. A 18.9 L pail (black arrow) with soap solution was placed below the bolt to collect the dropping ambrosia beetles.

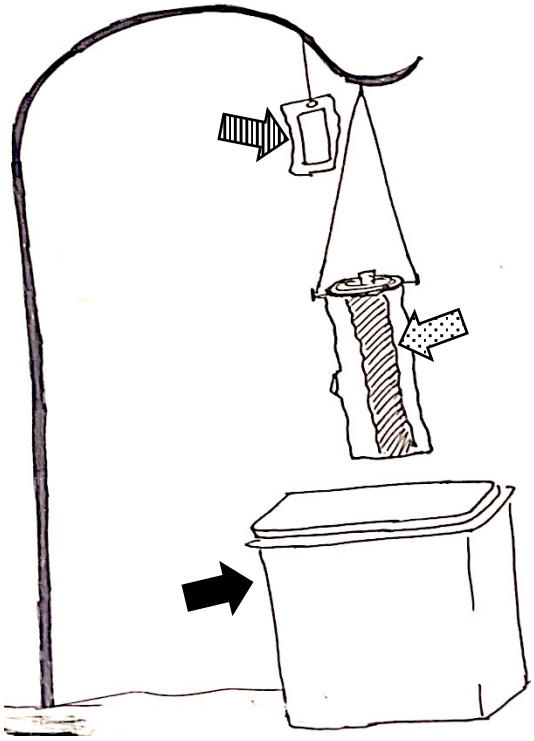


Figure 4.2. Cumulative captures of ambrosia beetles by species in (A, C) soap solution in pails and (B, D) glue painted on bolts for trial 1 (A, B) and trial 2 (C, D). Abbreviations: E, Ethanol; G, Glue; P, Permethrin; and V, Verbenone.

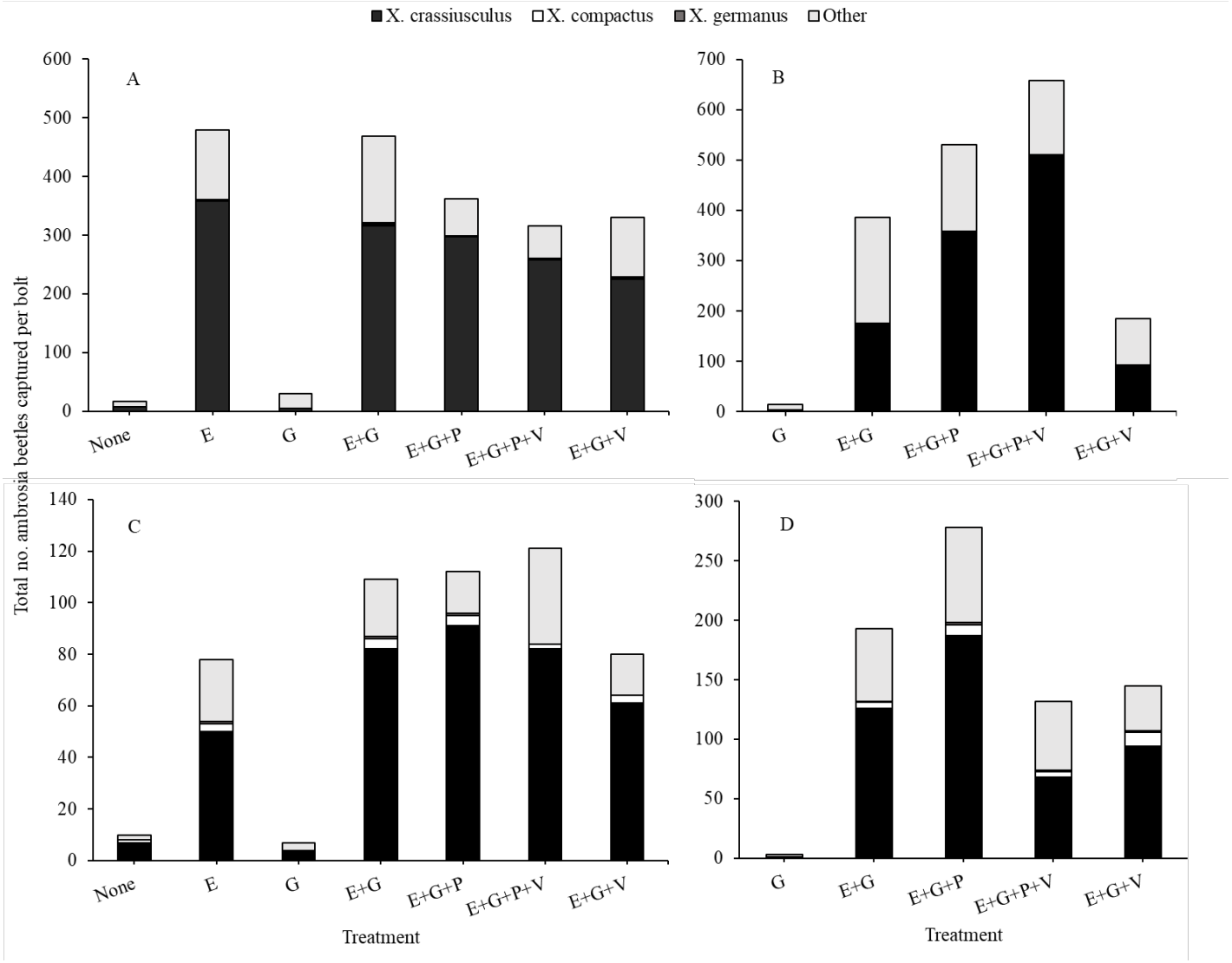

X. crassiusculus
X. compactus
X. germanus
Other
A
B
C
D
Total no. ambrosia beetles captured per bolt
Treatment
None
E
G
E+G
E+G+P
E+G+P+V
E+G+V

Figure 4.3. Means ± (SE) number of ambrosia beetles collected on various treatments in trial 1 where (A, C, E) include all treatments and (B, D, F) include only treatment with glue painted on the bolts. Bars with the same letter types (regular, italics and bold fonts) were compared among treatments and same letters among treatments are not significantly different (Tukey's HSD Test, α = 0.05). Abbreviations: E, Ethanol; G, Glue; P, Permethrin; and V, Verbenone. Because glue was not painted for nontreated (none) and ethanol treatments, letters are not provided on ambrosia beetle captures.

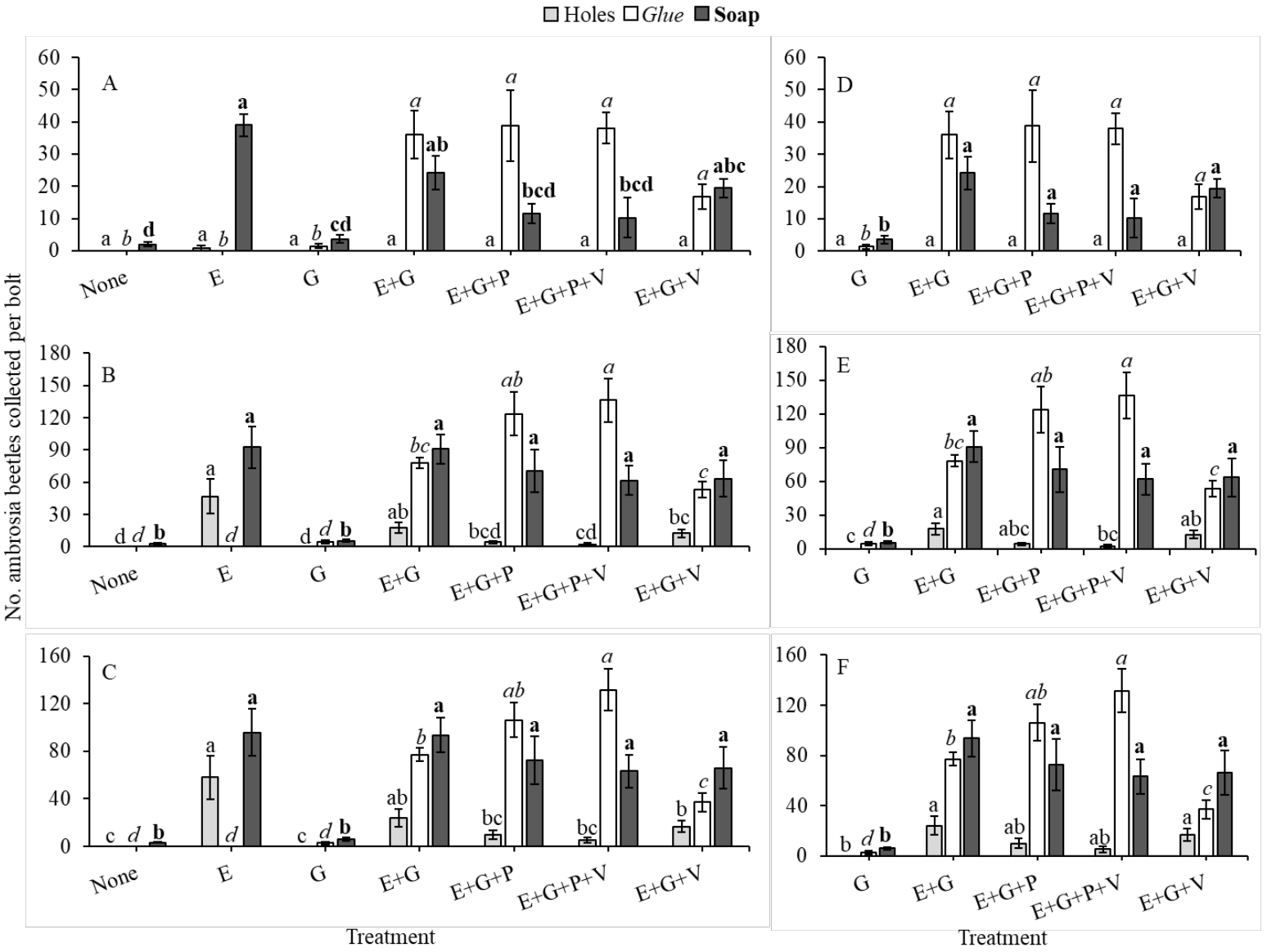

Holes Glue Soap
No. ambrosia beetles collected per bolt
A
B
C
D
E
F
None
E
G
E+G
E+G+P
E+G+P+V
E+G+V
Treatment
Treatment

Figure 4.4. Means ± (SE) number of ambrosia beetles collected on various treatments in trial 2 where (A, C, E) include all treatments and (B, D, F) include only treatment with glue painted on the bolts. Bars with the same letter types (regular, italics, and bold fonts) were compared among treatments, and the same letters among treatments were not significantly different (Tukey's HSD Test, $\alpha = 0.05$). Abbreviations: E, Ethanol; G, Glue; P, Permethrin; and V, Verbenone. Because glue was not painted for nontreated (none) and ethanol treatments, letters are not provided on ambrosia beetle captures.

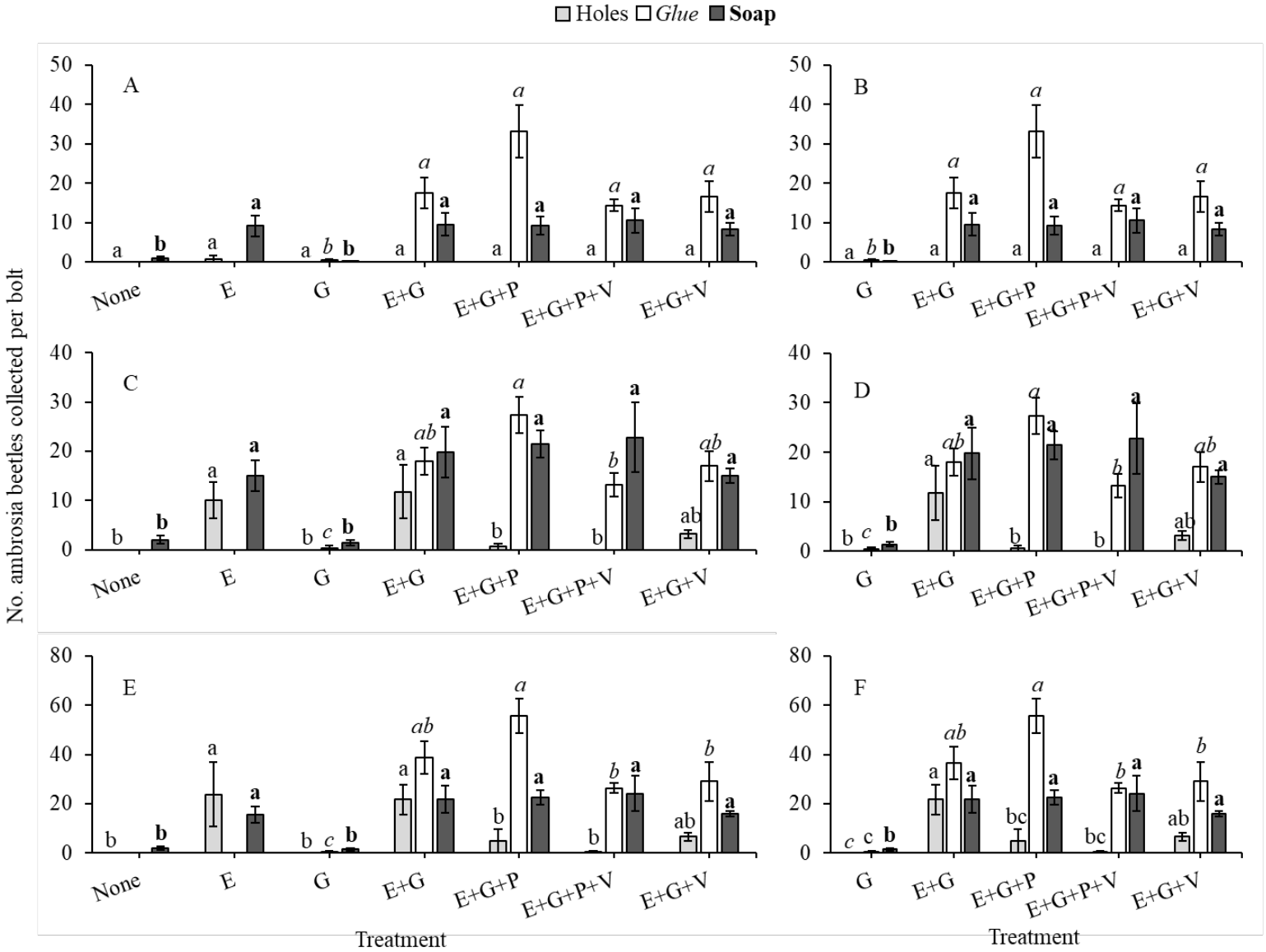
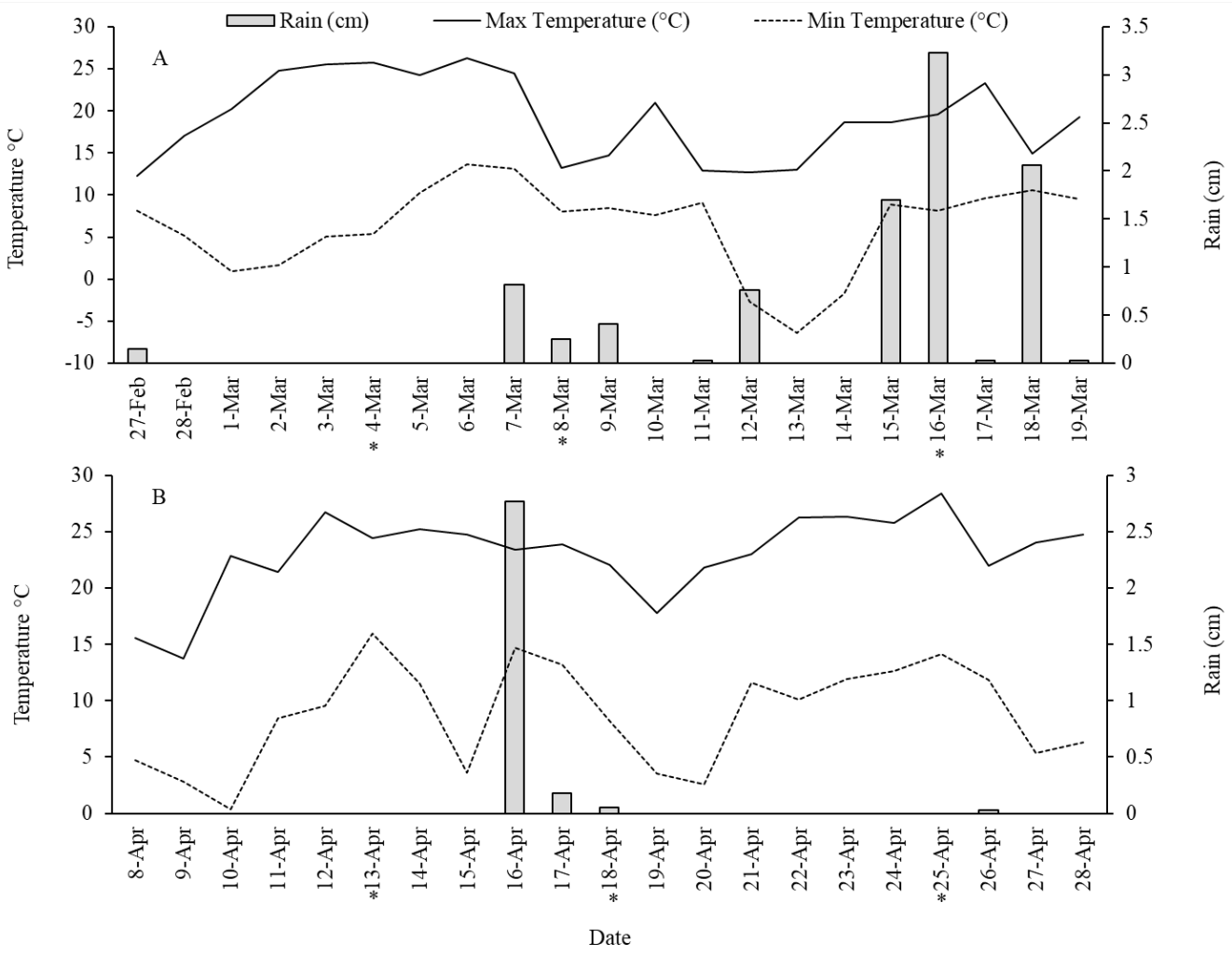

Holes Glue Soap
No. ambrosia beetles collected per bolt
A
B
C
D
E
F
None
E
G
E+G
E+G+P
E+G+P+V
E+G+V
Treatment
Treatment

Figure 4.5. Temperature and rainfall data were obtained from the Williamson weather station part of the University of Georgia Weather network three days before and after the start and end of the trial, respectively, for (A) trial 1 and (B) trial 2. Sampling dates are denoted by asterisk (*).


Rain (cm)
Max Temperature (°C)
Min Temperature (°C)
A
B
Temperature °C
Rain (cm)
Date
27-Feb
28-Feb
1-Mar
2-Mar
3-Mar
* 4-Mar
5-Mar
6-Mar
7-Mar
* 8-Mar
9-Mar
10-Mar
11-Mar
12-Mar
13-Mar
14-Mar
15-Mar
* 16-Mar
17-Mar
18-Mar
19-Mar
8-Apr
9-Apr
10-Apr
11-Apr
12-Apr
*13-Apr
14-Apr
15-Apr
16-Apr
17-Apr
*18-Apr
19-Apr
20-Apr
21-Apr
22-Apr
23-Apr
24-Apr
*25-Apr
26-Apr
27-Apr
28-Apr

CHAPTER 5

CONCLUSION

In March through July 2021 and 2022, a study was conducted to trap buprestids in ornamental nursery, pecan, and tree fruit production sites. In 2021 and 2022, 18, and 17 sites were selected for sampling, respectively. A total of 79, and 119 buprestids were trapped in 2021 and 2022, resepectively. In 2022, more buprestids were collected from ornamental and pecan sites than tree fruit. Flatheaded borers collected were *Acmaeodera, Agrilaxia, Agrilus, Anthaxia, Brachys, Chrysobothris*, and *Ptosima* species*Chrysobothris* spp. captures were identified to nine species. Those species were *Chrysobothris adelpha* (Harold), *Chrysobothris chrysoela* (Iliger), *Chrysobothris cribraria* (Mannerheim), *Chrysobothris femorata* (Olivier), *Chrysobothris quadriimpressa* (Gory and Laporte), *Chrysobothris rugosiceps* (Melsheimer), *Chrysobothris scitula* (Glory), *Chrysobothris sexsignata* (Say), and *Chrysobothris viridiceps* (Melsheimer). Phenological results suggest that adult beetles emerge in early March to mid-June, with some regional variations.

In order to evaluate the impact of urban stressors on insect borer attacks on trees, 50 urban sites were selected in Atlanta, Georgia, USA, and Augusta, Georgia, USA, in the summer of 2021 and 2022. Factors evaluated include overall tree health, the increase of average high and low temperatures of sites compared to surrounding areas, tree species, and the percentage of impervious surface surrounding trees. Trees in areas with increased percent pervious area or increased low temperatures were found to experience higher rates of flatheaded borer attacks. Ambrosia beetles were found to be less prone to attacking healthy trees. Healthy trees were less

likely to be damaged; however, trees with increased impervious surfaces around them and increased daily high and low temperatures were more likely to be attacked. Trees of the species *Acer rubrum* L and *Prunus* × *yedoensis* Matsum were more likely to be attacked, although *Ulmus parvifolia* Jacq. was found less susceptible to attack from borers.

Since the mode of management is unclear, a study was conducted in a field nursery during March and April to determine how permethrin prevented ambrosia beetles damage on the trees. The treatments were: (1) nonbaited, nontreated bolt, (2) ethanol baited bolt, (3) nonbaited bolt + glue [painted on bolt], (4) ethanol baited bolt + glue, (5) ethanol baited bolt + glue + permethrin, (6) ethanol baited bolt + glue + permethrin + verbenone, and (7) ethanol baited bolt + glue + verbenone. Ambrosia beetles trapped in glue, found in the pail with soap solution placed under the bolts, and the number of entry holes on bolts were quantified. *Xylosandrus crassiusculus* Motschulsky was the most commonly captured species. Verbenone did not consistently reduce ambrosia beetle landing on the bolts, indicating that verbenone alone is not sufficient for effective ambrosia beetle management in ornamental nurseries. The numbers of ambrosia beetles in soapy water were not significantly different among treatments. In conclusion, permethrin prevented ambrosia beetle attacks but did not reduce beetles from landing on the permethrin-treated bolts. Also, the densities of ambrosia beetles captured in the pails were not different regardless of exposed to permethrin. Thus, the underlying mechanism for reduced ambrosia beetle attacks on the bolts is likely contact repellency. The information obtained from all three projects will be integrated into managing flaheaded borer and ambrosia beetles in nurseries and landscapes.