ABSTRACT

BOHANNON, GEORGE RYAN. Optimizing Biological Control of Emerald Ash Borer in North Carolina: Host Phenology and Parasitoid Recovery. (Under the direction of Dr. Kelly Oten and Dr. Robert Jetton).

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive beetle that is causing devastating mortality of ash (*Fraxinus* spp.) trees across eastern and central North America. Individual trees can be protected using insecticides, but chemical control is not practical across forested landscapes. Classical biological control using introductions of parasitoid wasps that attack EAB eggs or larvae may provide a sustainable approach to managing EAB. However, establishment of parasitoids in the southern United States has been difficult. The phenology of EAB was studied in the warm climate of central North Carolina to inform appropriate parasitoid release timings that align with the seasonal availability of susceptible EAB life stages in this region. Biweekly EAB life stage assessments were conducted in stands of EAB-infested green ash (*Fraxinus pennsylvanica* Marshall) in Garner, North Carolina over 26 consecutive months (June 2019 through August 2021). Adult trapping was also conducted in these stands in the spring and summer of 2019, 2020, and 2021. Based on these collections, EAB exhibits a univoltine (one-year) life cycle. Parasitoid-susceptible larvae (third and fourth instars) are present from late June through October (~1,100–3,000 degree days base 10ºC) and are mostly absent during the remainder of the year. Parasitoid release timings and the life history of selected parasitoid species should be aligned with this window of host availability to be effective. This characterization of EAB phenology and voltinism will inform EAB management as this forest pest continues to spread in southern North America.

Introduced parasitoid establishment and existing natural enemy communities were also assessed through recovery efforts at new and previous release sites in North Carolina. The larval

parasitoid *Spathius agrili* Yang (Hymenoptera: Braconidae) was released in Garner in the summers of 2019 and 2020. To assess whether *S. agrili* successfully established, recovery of this species was attempted in the summer of 2021 using felled ash trees in emergence cages, yellow pan traps, and sentinel ash bolts containing EAB larvae. *Spathius agrili* was not detected using any recovery method. Establishment was likely hindered by temporal mismatches between release timings and the susceptibility of EAB to parasitism, the limited number of releases related to the COVID-19 pandemic, and acute declines in EAB and living ash trees. In the future, more numerous releases of *S. agrili* with timings that better align with the seasonal availability of parasitoid-susceptible larvae in univoltine EAB populations should increase the potential for *S. agrili* establishment in the Southeast. The recovery efforts in Garner did identify a diverse community of native or naturalized natural enemies, including the confirmed EAB parasitoids *Atanycolus* sp. (Hymenoptera: Braconidae) and *Balcha indica* Mani and Kaul (Hymenoptera: Eupelmidae) and the native *Spathius elegans* Matthews (Hymenoptera: Braconidae). In the late summer of 2021, additional recovery efforts were conducted using sentinel bolts at sites in Granville and Wayne counties where EAB parasitoids had been released between 2013 and 2019. While these efforts also did not detect introduced parasitoids, they provide a foundation for ongoing, more extensive sentinel bolt deployments to assess the establishment and effectiveness of EAB biological control efforts in North Carolina.

Optimizing Biological Control of Emerald Ash Borer in North Carolina: Host Phenology and Parasitoid Recovery

by
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DEDICATION

To my parents, Cynthia and George.

BIOGRAPHY

Ryan Bohannon grew up in Mebane, North Carolina, and he has held a deep love for exploring and learning about the outdoors from the beginning of his life. Ryan arrived at North Carolina State University in August 2016 to pursue his passion for conservation in the College of Natural Resources. During his time as an undergraduate, Ryan completed NC State's fisheries and wildlife summer camp program, conducted field research on American alligators with the North Carolina Wildlife Resources Commission, and worked on the certification and management of a conservation property. Ryan graduated with his Bachelor of Science degree in Fisheries, Wildlife, and Conservation Biology with a minor in Forest Management in May 2020. He returned to NC State later that year to pursue a Master of Science degree in Forestry and Entomology, and as a graduate student he discovered his love for the field of forest health. As a graduate research assistant in the NC State Forest Health program, Ryan has developed his passion for research and extension through his work on a variety of forest entomology issues, including emerald ash borer biological control, lingering ash, and hemlock woolly adelgid.

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The completion of these projects could not have been possible without the dedicated contributions of an incredible team of researchers. Even more importantly to me, my journey as a graduate student has been sustained by the kindness, mentorship, and friendship of many amazing people.

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I am grateful to North Carolina State University and specifically the College of Natural Resources for being a wonderful home for the past six years. I would especially like to thank the students and faculty of the Entomology, Forestry, and Fisheries, Wildlife, and Conservation Biology programs for their friendship and mentorship.

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help navigating the mysteries of graduate school, need advice on machete safety, or just need someone to talk to.

Finally, I am eternally grateful for my parents, Cynthia and George Bohannon. Your unconditional love, support, encouragement, and faith have sustained me throughout my life and inspire in me the strength to pursue my dreams with courage, integrity, and peace.

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CHAPTER 1

The Emerald Ash Borer and its Management, with an Emphasis on Biological Control and Southern North America

INTRODUCTION

Introductions of non-native insects and diseases have caused devastating impacts to the ecological, economic, and cultural values provided by forests around the world. One of the most destructive non-native insects to affect North American forests is the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). The larvae of this species of jewel beetle feed in the vascular tissue of ash (*Fraxinus* spp.) trees, forming tunnels called galleries in the inner bark and outer wood. Emerald ash borer is native to eastern Asia, including China, Korea, and the Russian Far East (Liu et al. 2007, Orlova-Bienkowskaja and Volkovitsh 2018). This insect was accidentally imported to North America, likely on wood packing material, and it has been present on the continent since the 1990s (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006, Siegert et al. 2014). Emerald ash borer was first detected in the area of Detroit, Michigan, United States and Windsor, Ontario, Canada in the summer of 2002 (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006). In the 20 years since this initial infestation, EAB has spread across eastern and central North America. As of spring 2022, this insect has been found in 35 states and the District of Columbia in the United States in addition to five provinces of Canada (Emerald Ash Borer Information Network 2022). Emerald ash borer was first detected in North Carolina in 2013, and it is now found in more than 60 of the state's 100 counties (NCFS 2017, 2021).

Unlike the ash hosts of EAB in its native range in Asia, ash species native to North America are highly susceptible to mortality from EAB. North American ash species do not share a coevolutionary history with the beetle and possess poor resistance against EAB attack (Liu et

al. 2007, Rebek et al. 2008, Villari et al. 2016). Millions of ash trees have been killed by EAB throughout its expanding non-native range, and six North American ash species are now classified as critically endangered or endangered by the International Union for Conservation of Nature's Red List of Threatened Species (Bauer et al. 2014, Herms and McCullough 2014, IUCN 2022). The loss of ash in North America has far-reaching ecological consequences for ecosystem components as diverse as carbon cycling, forest succession and species composition, larval development of amphibians, and the survival of many ash-specialist arthropods (Flower et al. 2013, Stephens et al. 2013, Wagner and Todd 2016). Emerald ash borer impacts in urban areas, where ash species are extensively planted as high-value shade trees, are projected to cost many billions of dollars, and timber industries also suffer losses (Poland and McCullough 2006, Kovacs et al. 2010, Herms and McCullough 2014, McConnell et al. 2019). Ash trees also hold incalculable and irreplaceable cultural and economic values, particularly among Native American and First Nation basket makers in EAB-impacted regions (Herms and McCullough 2014, Bolen 2020).

EMERALD ASH BORER BIOLOGY

Host Plants

Overview

Ash trees, which belong to the family Oleaceae, are the host plants primarily attacked by EAB. In Asia, EAB feed on native ash species such as Manchurian ash (*F. mandshurica* Rupr.) and Korean ash (*F. chinensis* var. *rhynchophylla* Roxb.) as well as introduced North American ash species such as green ash (*F. pennsylvanica* Marshall) and velvet ash (*F. velutina* Torr.) (Liu et al. 2007, Wei et al. 2007, Wang et al. 2010). North America is home to 16 native ash species across the continent, with green ash, white ash (*F. americana* L.), and black ash (*F. nigra*

Marshall) being the most widespread and well studied (Preston Jr. and Braham 2002, MacFarlane and Meyer 2005, Herms and McCullough 2014). All ash species that have been exposed to EAB in North America are susceptible to EAB mortality (Cappaert et al. 2005, Herms and McCullough 2014). Vulnerability to EAB does vary between ash species, with green ash suffering higher mortality compared to white ash (Robinett and McCullough 2019).

Ash trees were once thought to be the only suitable hosts of EAB in its invasive range (Herms and McCollough 2014). However, the white fringetree (*Chionanthus virginicus* L.), another oleaceous species native to North America, has since been confirmed as a viable host in which EAB can successfully complete development in both laboratory and field settings (Cipollini 2015, Cipollini and Rigsby 2015). Different susceptibility of Asian and North American host species may be present in both *Fraxinus* and *Chionanthus* species, as Chinese fringetree (*C. retusus* Lindl. and Paxton) appears to be resistant to EAB (Cipollini and Rigsby 2015). White fringetree appears to support poorer EAB larval performance and experience less severe impacts from EAB attack compared to ash species (Peterson et al. 2020, Peterson and Cipollini 2021). Adult development of EAB is also possible on cultivated olive (*Olea europaea* L.), although this species is considered a poor host (Cipollini et al. 2017, Peterson et al. 2020). Early laboratory tests found that privet (*Ligustrum* spp.), also in the family Oleaceae, could be potentially suitable for EAB larvae, while unrelated species such as shagbark hickory (*Carya ovata* (Mill.) K.Koch), hackberry (*Celtis occidentalis* L.), black walnut (*Juglans nigra* L.), and American elm (*Ulmus americana* L.) did not appear to support proper larval development (McCollough et al. 2004).

Potential Hosts South of the United States

The potential expansion of EAB southward beyond the United States has received little formal study but could have important impacts. Approximately 13 species of ash are found in Mexico, and the native range of tropical ash (*F. uhdei* (Winzig) Lingelsh) extends south to at least Guatemala (Francis 1990, Bonfil 2010). Tropical ash is extensively used in urban forestry and for reforestation in Mexico, and it is also planted in the Andean region, Costa Rica, and Puerto Rico (Francis 1990, Filgueira et al. 2004, Bonfil 2010, Rojas-Rodríguez and Torres-Córdoba 2016). Tropical ash appears to be a highly suitable EAB host plant based on its use in laboratory rearing of EAB larvae (Duan et al. 2013, Duan et al. 2014b, Duan et al. 2021). Due to the presence of these species, it appears likely that EAB would encounter suitable host plants south of the United States. However, expansion into more tropical regions could be limited if EAB are not exposed to a period of cold temperatures necessary for development to adulthood (Duan et al. 2021). Deeper understandings of EAB phenology and climate-related limitations to development and survival are crucial to understanding the potential for further EAB expansion into warmer climates.

Life Cycle

Overview

Emerald ash borer adults lay their eggs in or under crevices and layers of the bark of host trees. Oviposition occurs in mid-May to July in northeastern China, peaks between late June and early July in Michigan, and begins around mid-June in New York, but oviposition can continue into August (Bauer et al. 2004, Liu et al. 2007, Wei et al. 2007, Wang et al. 2010, Jones et al. 2020). A female EAB may lay a maximum of more than 200 eggs (Lyons et al. 2004). Several weeks after oviposition, EAB larvae hatch and bore into the tree (Bauer et al. 2004, Cappaert et

al. 2005, Poland and McCollough 2006, Wang et al. 2010, Jones et al. 2020). New larvae from eggs laid in the same spring are first observed in late May or early June in Tianjin, China (Wei et al. 2007, Wang et al. 2010). Larvae create expanding, serpentine galleries as they move through the vascular system and cambium (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006, Wang et al. 2010, Jones et al. 2020). This feeding damages the phloem and xylem of the host tree, interfering with nutrient and water transport and eventually killing the branch or trunk by girdling (Wang et al. 2010, Herms et al. 2019, Oten 2020). Larvae develop through four stages, or instars (Bauer et al. 2004, Cappaert et al. 2005, Wang et al. 2010, Jones et al. 2020). Larvae can feed into the autumn, with feeding stopping by October or November in the northern United States and Tianjin, China (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006, Wang et al. 2010, Jones et al. 2020).

After feeding is completed, fourth-instar EAB larvae create pupal chambers deeper in the tree in which they overwinter. These cells are located in the outer xylem or bark (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006, Wang et al. 2010, Jones et al. 2020). Excavation of pupal chambers can begin in July in New York and Tianjin (Wang et al. 2010, Jones et al. 2020). Mature larvae bore an exit hole filled with frass leading from the pupal chamber (Wang et al. 2010). A larva in a chamber ceases feeding and initially folds into a “J” shape. J-shaped larvae later condense and straighten into a prepupa form; this occurs in March in Tianjin (Wei et al. 2007, Wang et al. 2010). J-larvae and prepupae are sometimes referred to interchangeably (Jones et al. 2020). Pupation begins in April in Michigan and northeastern China and can extend into June in China (Cappaert et al. 2005, Wei et al. 2007, Wang et al. 2010). Pupation of EAB that overwinter as prepupae begins in early May in New York (Jones et al. 2020).

Development of EAB to adulthood may depend on larvae undergoing obligatory diapause at the end of the growing season. Larvae would then need to experience a period of cool temperatures in fall or winter that terminates their diapause and allows them to complete adult development when warm temperatures return the next year. There is evidence that EAB mostly complete this diapause as J-larvae/prepupae in order to complete adult development, while few adults develop when larvae (instars 1–4) are exposed to the same simulated overwintering conditions (Duan et al. 2021). The length of EAB development from egg to adult can vary between one and two years. A life cycle in which EAB overwinter once as a J-larva/prepupa in a pupal chamber and emerge in the spring is classified as a one-year, or univoltine, life cycle. Emerald ash borer can also exhibit a two-year, or semivoltine, life cycle if larvae do not develop to the J-larva/prepupa stage by the end of their first growing season. In a semivoltine life cycle, EAB spend their first winter as early instar larvae in their existing gallery, begin feeding again in the spring, develop into J-larvae/prepupae during the summer, and complete development after overwintering again (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006, Wei et al. 2007, Bauer et al. 2014, Jones et al. 2020, USDA–APHIS/ARS/FS 2020, Duan et al. 2021).

In both the univoltine and semivoltine life cycles, EAB adults exit their pupal chambers and emerge from their host tree through D-shaped exit holes (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006, Oten 2020). Eclosed adults may remain in pupal chambers for several days to almost two weeks before emerging from the tree (Wei et al. 2007, Wang et al. 2010). Based on studies in Michigan, New York, and the Washington, DC area, emergence of EAB adults begins in May or June at ~400–550 growing degree days base 10°C (DD_{10}) accumulated beginning 1 January (Brown-Rytlewski and Wilson 2004, Poland et al. 2011, Abell et al. 2019, Herms et al. 2019, Jones et al. 2020). Adult emergence begins in early to late May in

northeastern China (Wei et al. 2007, Wang et al. 2010). After emerging, EAB adults begin feeding on ash foliage; damage from EAB defoliation is considered superficial (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006). Mating does not begin until adults have fed for five to seven days, after which adult females will mate with multiple males. Females require at least an additional five to seven days of feeding before oviposition begins (Bauer et al. 2004, Lyons et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006). EAB adults can live for three to six weeks, with an average of about four weeks in laboratory conditions (Lyons et al. 2004, Cappaert et al. 2005, Wang et al. 2010). Activity of EAB adults peaks in about late June to mid-July (800–1,200 DD_{10}) in Michigan and about late May to late June (1,000–1,700 DD_{10}) in the Washington, DC area (Poland et al. 2011, Abell et al. 2019). Adult activity begins and peaks in May and ends in early July in northeastern China (Wei et al. 2007, Wang et al. 2010). Natural spread of EAB adults is poorly understood, but it is generally believed to be limited to several miles per year with the potential for some individuals to disperse farther (Taylor 2010, Siegert et al. 2015, Oten 2020). Emerald ash borer adults have been attracted to traps located at least 300 m away from ash trees (Poland et al. 2011). Human-assisted movement of infested materials is usually considered to have a greater potential for increasing the spread of EAB (Siegert et al. 2015, Oten 2020).

Climate-based Variability

Emerald ash borer activity and development is dependent on temperature. As a result, the length of the EAB life cycle is variable between regions with different climates. Most research on the EAB life cycle in North America has been conducted in northern regions because the insect has radiated from its initial detection in Michigan and Ontario. However, EAB is known to develop faster in the warmer conditions of the southern United States. A shorter, univoltine life

cycle is likely more common in warmer, southern regions relative to colder, northern regions because of the more rapid development of eggs and larvae (Duan et al. 2018, USDA–APHIS/ARS/FS 2020). Faster larval development related to longer or warmer growing seasons would increase the ability of larvae to reach the J-larval/prepupal stage before overwintering, allowing them to complete their development to adulthood after their first winter (Duan et al. 2021). Emerald ash borer exhibits a univoltine life cycle in the Washington, DC area, with more than 90% of EAB reaching the prepupal stage by the end of October (Abell et al. 2019). A univoltine life cycle is also dominant in Tianjin, China, which lies at the southern end of the studied EAB native range in Asia (Wang et al. 2010). Emerald ash borer is also believed to be univoltine in North Carolina, and the United States Department of Agriculture (USDA) predicts that the two-year EAB life cycle is rare throughout the state except in the cooler Appalachian Mountains (Gould et al. 2020, Oten 2020, USDA–APHIS/ARS/FS 2020). The start of adult EAB emergence also occurs earlier further south, varying by a month between southern Indiana and central Michigan (Herms et al. 2019). Control of EAB in the southern United States may be hindered if management decisions are based on phenology and voltinism information from northern regions that does not match EAB development in warmer climates (Duan et al. 2018).

EMERALD ASH BORER MANAGEMENT

Regulatory Control

Efforts to combat EAB in North America are currently focused on management rather than eradication. While eradication was pursued in the first few years after EAB was detected in North America, these efforts were hindered due to ineffective surveying, a lack of control measures, and funding constraints (GOA 2006, Herms and McCullough 2014, Bauer et al. 2015, Duan et al. 2018). Beginning in 2003, the USDA Animal and Plant Health Inspection Service

(APHIS) established a quarantine program that regulated the commercial interstate movement of ash materials and all hardwood firewood from EAB-affected areas. State agencies have also enforced corresponding regulations on movement of materials that could potentially harbor EAB (Federal Register 2003, Federal Register 2020). The North Carolina Department of Agriculture and Consumer Services instituted a statewide EAB quarantine in 2015 (NCDA&CS 2015). In 2020, the land area under EAB quarantine encompassed more than a quarter of the contiguous United States (Federal Register 2020). Despite regulatory efforts, EAB has continued to spread in North America due to the continued movement of infested materials, an inability to detect new infestations quickly and effectively, and natural dispersal (Taylor et al. 2010, Ryall 2015, Federal Register 2020). In December 2020, USDA APHIS made the decision to remove the domestic quarantine regulations for EAB, effective January 2021, on the basis that the regulations have failed to effectively prevent the spread of EAB in the United States. Instead, the agency stated its intention to focus resources on other management options such as biological control and host plant resistance. Despite the removal of the federal quarantine, state-level quarantine regulations will remain in effect based on the decisions of individual states (Federal Register 2020).

Monitoring

Monitoring of EAB is critical to the management of this pest in order to detect and address new and existing infestations. Surveying for EAB can be done visually by looking for signs and symptoms of infestation, including canopy dieback, vertical bark cracks, epicormic branching, woodpecker excavation, and D-shaped EAB exit holes. However, visual surveying is not effective for stands that are only lightly or moderately infested because EAB symptoms are usually only noticeable after trees have become heavily infested (Cappaert et al. 2005, Poland and McCollough 2006, Poland et al. 2011). Emerald ash borer attacks typically begin in the

canopy, which means that exit holes are not visible to surveyors working on the ground (Cappaert et al. 2005). Girdled trap trees have also been used for EAB monitoring, but this method is labor intensive and limited at large scales. The development of traps and attractants for EAB has been important for improving the effectiveness of monitoring (Cappaert et al. 2005, Poland and McCollough 2006, Poland et al. 2011, Ryall 2015). Various trap configurations are used, but most incorporate purple or green sticky panels or green funnels and use olfactory attractants containing chemicals released by ash trees (Poland et al. 2011, Ryall 2015, Tobin et al. 2021). Remote sensing and biosurveillance using the predatory wasp *Cerceris fumipennis* Say (Hymenoptera: Crabronidae) are additional strategies that have been used to monitor EAB (Swink et al. 2014, Ryall 2015, Murfitt et al. 2016).

Silviculture

If an EAB infestation occurs on managed forestland, conducting a salvage harvest of infested ash trees can be beneficial to the land manager. Infested ash trees can retain commercial value if the harvest is conducted early because EAB damage generally does not reach the inner portions of the tree (NCFS 2017, Oten 2020). However, salvage harvests are projected to have a limited ability to mitigate lost timber value (McConnell et al. 2019). Proactively harvesting all of the ash on a property to avoid EAB impacts is not recommended if ash trees are currently healthy (NCFS 2017). Avoiding the premature harvest of healthy ash trees may help the long-term survival of ash species by conserving trees that may possess genetic resistance to EAB.

Chemical Control

Chemical control using systemic insecticides can be highly effective at protecting individual ash trees from EAB mortality. Systemic insecticides are applied to the soil or trunk and then transported throughout the tree by its vascular system. Emamectin benzoate

(Arbormectin™, TREE-äge™) is a trunk-injection insecticide that provides two to three years of protection and is highly effective at killing EAB. Neonicotinoids such as imidacloprid (Merit®, Imicide®, Bayer Advanced™ products, Optrol™, etc.) and dinotefuran (Safari™, Transtect™, Ortho® products, etc.) applied to the soil or using basal trunk sprays are less expensive and more readily available options, but they provide less effective and shorter-term control (Herms et al. 2019, Oten 2020). Due to the high costs, environmental concerns, and logistical constraints of treating trees on a large scale, chemical control of EAB is impractical for long-term control across forested landscapes and is mostly limited to high-value trees in urban areas (Davidson and Rieske 2016, Herms et al. 2019, Oten 2020). However, protecting ash trees with insecticides is cost effective in urban areas compared with the expense of removing and replacing trees infested and killed by EAB (Herms et al. 2019).

Host Plant Resistance

A major focus for managing EAB into the future is the development of ash trees that are genetically resistant or tolerant to EAB attack. Research on this management strategy is centered on ash trees, termed "lingering ash," that have survived and remained healthy in EAB-affected areas (Koch et al. 2015). The survival of some ash trees is likely a result of lower EAB densities or other factors rather than inherent differences in resistance (Koch et al. 2015, Robinett and McCollough 2019). However, researchers are also attempting to identify genetically controlled mechanisms in ash that make trees less attractive for selection by adult EAB or allow trees to successfully defend themselves against feeding by EAB larvae. Foliage feeding by adult EAB, larval mortality due to host defense, and larval development have been shown to differ significantly between potential lingering green ash trees and control trees. These lingering ash trees could be used to breed North American ash trees with heightened resistance to EAB (Koch

et al. 2015). The introduction of EAB-resistance traits from Asian ash species to North American ash species through hybridization of compatible species or genetic engineering has also been proposed (Kelly et al. 2020).

EMERALD ASH BORER BIOLOGICAL CONTROL

Of the EAB control strategies in use, the method currently considered to have the most potential for long-term management of EAB is classical biological control (USDA–APHIS/ARS/FS 2020). This strategy involves the importation, release, and establishment of natural enemies of EAB from its native range in Asia. The most important of these natural enemies are several species of parasitoid wasps that attack either the eggs or larvae of EAB. These species were identified and collected by researchers in China, Korea, and the Russian Far East during field explorations beginning in 2003. Three of the significant EAB biocontrol agents currently in use in North America were collected from China: *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), *Spathius agrili* Yang (Hymenoptera: Braconidae), and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae). The remaining biocontrol agent currently in use, *Spathius galinae* Belokobylskij and Strazanac, was collected from Russia (Bauer et al. 2015, Duan et al. 2018, USDA–APHIS/ARS/FS 2020).

Before potential biocontrol agents could be approved for release in North America, testing was conducted to determine the host specificity of these species and their potential effects on nontarget organisms. The general conclusion of these assessments was that nontarget effects of introduced parasitoids are likely to be low. Effects that could occur are believed to be constrained to other members of the genus *Agrilus* and further limited by parasitoids' attraction to ash odors when seeking hosts (Yang et al. 2008, Bauer et al. 2015, Duan et al. 2018). For example, no-choice tests using the larvae of a variety of Asian and North American insect

species found that *S. agrili* only parasitized *Agrilus* species. Field observations of wood-boring insects in China found that *S. agrili* and *T. planipennisi* did not parasitize any insect species other than EAB. In addition, tests of female *S. agrili* attraction to various plant leaf volatiles indicated that *S. agrili* was only attracted to ash leaves (Yang et al. 2008). Following such assessments, *O. agrili*, *S. agrili*, and *T. planipennisi* were approved for release in 2007 in the United States and in 2013 in Canada (Bauer et al. 2015, Duan et al. 2018). The most recently introduced parasitoid, *S. galinae*, began to be released in the United States after approval in 2015 (Duan et al. 2018). Post-release assessments of *S. agrili* impacts on the nontarget, native bronze birch borer (*Agrilus anxius* Gory) and two-lined chestnut borer (*A. bilineatus* Weber) found successful but limited parasitism of bronze birch borer in a field experiment (Bauer et al. 2014). As the classical biocontrol program for EAB expands, further studies of parasitoid impacts should consider nontarget impacts in forest ecosystems to ensure the integrity of the program.

Egg Parasitoid: *Oobius agrili*

Oobius agrili is unique among the introduced parasitoids in that it attacks the eggs of EAB. This species was described from Jilin in northeastern China (Zhang et al. 2005). *Oobius agrili* reproduces by laying a single egg inside an EAB egg. *Oobius agrili* develops inside of the EAB egg and eventually emerges as an adult, killing the EAB egg in the process (USDA–APHIS/ARS/FS 2020). On green ash in Jilin, *O. agrili* parasitism is well synchronized with the period in July and August when EAB are laying eggs. The parasitism level on EAB eggs during this period was close to 60% (Liu et al. 2007). *Oobius agrili* can reproduce through parthenogenesis, so only female wasps are released; all offspring are also female (USDA–APHIS/ARS/FS 2020).

Overview of Larval Parasitoids

The remaining introduced parasitoids, *S. agrili*, *S. galinae*, and *T. planipennisi*, parasitize EAB larvae. All three larval parasitoids target the third and fourth EAB instars. *Spathius* eggs are laid and larvae develop and feed on the outside of EAB larvae, while those of *T. planipennisi* are laid, develop, and feed inside EAB larvae (USDA–APHIS/ARS/FS 2020). A female *Spathius* also paralyzes the EAB larvae that it oviposits on, stopping the larvae's activity, while EAB larvae parasitized by *T. planipennisi* are not paralyzed and temporarily continue to feed and move (Yang et al. 2005, Wang et al. 2015, USDA–APHIS/ARS/FS 2020). Another difference between the larval parasitoids that is important for EAB biocontrol is that the ovipositor of the two *Spathius* species is longer than that of *T. planipennisi*. This means that *T. planipennisi* is well suited for attacking EAB larvae in ash saplings and small branches, but it is not as effective of a biocontrol agent for larger trees with thick bark. *Spathius* species are better able to access EAB in larger, thick-barked ash trees, so they can play a complementary role with *T. planipennisi* in the biocontrol program for a site (Jones et al. 2020, USDA–APHIS/ARS/FS 2020).

Larval Parasitoid: *Spathius agrili*

The larval parasitoid *S. agrili* was described from Tianjin in northeastern China (Yang et al. 2005). Adult female *S. agrili* seek out EAB larvae by sensing ash bark with their antennae (Yang et al. 2005, Yang et al. 2010). The female wasp accesses an EAB larva in its gallery by using its ovipositor to drill through the bark (Yang et al. 2005, Yang et al. 2010, USDA–APHIS/ARS/FS 2020). The wasp then lays between one and 35 eggs on the larva, of which one to 18 wasps, or about eight on average, will develop to maturity. After they complete their external feeding in about seven to 10 days, the *S. agrili* larvae create silken cocoons in which

they pupate or overwinter as prepupae if necessary (Yang et al. 2005, Yang et al. 2010). *Spathius agrili* may also overwinter as larvae (USDA–APHIS/ARS/FS 2020). After pupating, adult *S. agrili* emerge by chewing their way out of the tree (Yang et al. 2005, Yang et al. 2010). *Spathius agrili* can complete between one and four generations per year, with the first generation of adults emerging in the summer (Yang et al. 2005, Yang et al. 2010, USDA–APHIS/ARS/FS 2020). Emergence commences in late May or June in Tianjin and continues for more than two months (Yang et al. 2010). *Spathius agrili* reared in an open-air insectary in New York emerged at ~750–1,100 DD_{10} (July to August) (Jones et al. 2020). Parasitism levels for *S. agrili* on EAB in China have been frequently recorded at 30% to 50% and as high as >90% in some velvet ash stands in Tianjin (Yang et al. 2005, Yang et al. 2010).

Larval Parasitoid: *Spathius galinae*

Spathius galinae was collected from the Russian Far East, a colder, more northern region of Asia compared to the origin of *S. agrili*. *Spathius galinae* was also described from South Korea (Belokobylskij et al. 2012). The life history of *S. galinae* is similar to that of *S. agrili*, as both are gregarious larval ectoparasitoids. *Spathius galinae* can complete two to three generations per year (Belokobylskij et al. 2012, USDA–APHIS/ARS/FS 2020). Unlike *S. agrili*, adult *S. galinae* emerge in the spring rather than the summer (USDA–APHIS/ARS/FS 2020).

Larval Parasitoid: *Tetrastichus planipennisi*

In addition to the differences previously described, *T. planipennisi* is distinguished from the *Spathius* species in that as many as 130 adult wasps can develop from a single EAB larva. *Tetrastichus planipennisi* overwinter as larvae or prepupae and emerge in the spring as with *S. galinae*, completing multiple generations in a year (USDA–APHIS/ARS/FS 2020). The

parasitism level for *T. planipennisi* on EAB in green ash in Jilin, China has been observed to average over 20% with a maximum of about 40% (Liu et al. 2007).

Regional Suitability of Introduced Larval Parasitoids

Successful development and establishment of EAB parasitoids is dependent on the availability of EAB larval stages that are susceptible to parasitism. The availability of third- and fourth-instar EAB larvae throughout the year varies by region based on climatic conditions that speed or slow larval development and result in a univoltine or semivoltine life cycle (Liu et al. 2007, Wang et al. 2007, Jones et al. 2020, USDA–APHIS/ARS/FS 2020). In general, a semivoltine life cycle is believed to be more common in cooler, northern climates where EAB development is slower, while a univoltine life cycle is dominant in warmer regions (Duan et al. 2018, Jones et al. 2020, USDA–APHIS/ARS/FS 2020). *Tetrastichus planipennisi* and *S. galinae* appear to establish best when susceptible larvae are available beginning in the spring when the adult wasps emerge. This emergence pattern is synchronous with a semivoltine EAB life cycle in which some EAB overwinter as larvae in galleries and resume feeding in the spring. Conversely, *S. agrili* adults emerge later in the season and appear to be best suited for attacking late-instar larvae in the summer and fall, which is consistent with a univoltine EAB life cycle in which susceptible larvae are not available in the spring (Jones et al. 2020, USDA–APHIS/ARS/FS 2020).

Due to variability in the EAB life cycle across regions with different climates, the parasitoid species released for biocontrol must match the voltinism of EAB in a given location. USDA APHIS currently recommends that *S. agrili* be released only in locations where more than 3,500 growing degree days base 50ºF (DD_{50}) are accumulated beginning 1 January. This broadly correlates to regions of the United States below 40ºN latitude (approximately the southern border

of Pennsylvania) where EAB is expected to be univoltine. Conversely, releases of *T. planipennisi* and *S. galinae* are recommended in regions where fewer than 3,500 DD_{50} are accumulated by the end of September and EAB is semivoltine (USDA–APHIS/ARS/FS 2020). These recommendations are based on the fact that *S. agrili* has largely failed to establish in the northern United States, while establishment of *T. planipennisi* and *S. galinae* in this region has been much more successful (Duan et al. 2018, Jones et al. 2020, USDA–APHIS/ARS/FS 2020).

The *S. agrili* holotype and the specimens used in the USDA APHIS biocontrol program were initially collected from Tianjin, China, which lies in the southern, milder portion of its known range in Asia (Yang et al. 2005, Gould et al. 2011, Bauer et al. 2015, Hooie et al. 2015, USDA–APHIS/ARS/FS 2020). Emerald ash borer primarily has a univoltine life cycle in Tianjin, matching the presumed phenology of EAB in the southern United States, and *S. agrili* was found to be the most significant insect natural enemy in this location (Wei et al. 2007, Wang et al. 2010). Unlike in the northern United States, *S. agrili* appears to be well synchronized with the EAB life cycle in Tianjin (Wang et al. 2007, Yang et al. 2010, Jones et al. 2020). Taken together, this evidence supports the belief that the *S. agrili* being released in the United States for EAB biocontrol could be best suited for warmer, southern regions of the United States where EAB is primarily univoltine as opposed to northern regions where most releases and research have taken place thus far (Bauer et al. 2015, Hooie et al. 2015, Jones et al. 2020). Further research on parasitoid establishment in the southern United States is essential, and successful biocontrol of EAB in warmer regions of the United States may also require the identification and importation of additional natural enemy species adapted to warmer regions of the EAB native range (Duan et al. 2018).

Natural Control

Native and Naturalized Parasitoids

In addition to the introduced parasitoid species, there are a variety of insects native to or naturalized in North America that are known to parasitize EAB. Some of the most significant native parasitoids of EAB are members of the genus *Atanycolus* (Hymenoptera: Braconidae). Populations of these solitary ectoparasitoids have been shown to respond strongly to EAB infestations (Duan et al. 2012b, Duan et al. 2014a, Duan et al. 2015). Other notable parasitoids in North America include *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), the naturalized species *Balcha indica* Mani and Kaul (Hymenoptera: Eupelmidae), and native *Spathius* species (Duan et al. 2011, Duan et al. 2012b, Hooie et al. 2015, Gaudon and Smith 2020). Native and naturalized parasitoids recovered in the southern United States include *Atanycolus* spp., *B. indica*, *P. sulcata*, *Spathius* spp., and *Xorides humaralis* Say (Hymenoptera: Ichneumonidae) collected from sites in North Carolina, Tennessee, and Virginia (Hooie et al. 2015, Ragozzino 2020, Nalepa et al. 2021). Rearing and releasing native parasitoids for augmentation biocontrol of EAB is being considered for EAB management in addition to the existing classical biocontrol program (Hooie et al. 2015, Gaudon and Smith 2020).

Avian Predators

Insectivorous birds are also significant natural enemies of EAB. In Tianjin, China, woodpeckers are the foremost EAB predators, attacking about 15% of ash trees and preying on about 25% of EAB larvae in those trees (Wang et al. 2010). Woodpecker predation is typically low in the Russian Far East but has been recorded as high as about 40% (Duan et al. 2012a). The impact of avian predators is significant in North America, and woodpeckers are often the largest cause of immature EAB mortality in North American infestations (Cappeart et al. 2005, Duan et

al. 2010, Jennings et al. 2013, Duan et al. 2019). The most prominent avian predators of EAB in North America are the downy woodpecker (*Dryobates pubescens* L.), hairy woodpecker (*Leuconotopicus villosus* L.), and red-bellied woodpecker (*Melanerpes carolinus* L.) (Cappeart et al. 2005, Lindell et al. 2008, Jennings et al. 2013, Koenig et al. 2013, Jennings et al. 2016, Koenig and Liebhold 2017, Murphy et al. 2018). Larval mortality levels caused by North American woodpeckers often average about 30% with a maximum of about 50% to 90% (Jennings et al. 2013, Jennings et al. 2016, Duan et al. 2019).

REFERENCES

Abell, K. J., J. J. Duan, and P. M. Shrewsbury. 2019. Determining optimal parasitoid release timing for the biological control of emerald ash borer (Coleoptera: Buprestidae). The Florida Entomologist 102(4): 691–694.

Bauer, L. S., R. A. Haack, D. L. Miller, T. R. Petrice, and H. Liu. 2004. Emerald ash borer life cycle, p. 8. *In* V. Mastro and R. Reardon (comps.), Emerald Ash Borer Research and Technology Development Meeting, 30 September–1 October 2003, Port Huron, MI. Publication FHTET-2004-02. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Bauer, L. S., J. J. Duan, and J. R. Gould. 2014. Emerald ash borer, pp. 189–209. *In* R. Van Driesche and R. Reardon (eds.). The Use of Classical Biological Control to Preserve Forests in North America. Publication FHTET-2013-2. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Bauer, L. S., J. J. Duan, J. R. Gould, and R. Van Driesche. 2015. Progress in the classical biological control of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America. Canadian Entomologist 147(3): 300–317.

Belokobylskij, S. A., G. I. Yurchenko, J. S. Strazanac, A. Zaldívar-Riverón, and V. Mastro. 2012. A new emerald ash borer (Coleoptera: Buprestidae) parasitoid species of *Spathius* Nees (Hymenoptera: Braconidae: Doryctinae) from the Russian Far East and South Korea. Annals of the Entomological Society of America 105(2): 165–178.

Bolen, A. 2020. A silent killer: Black ash basket makers are battling a voracious beetle to keep their heritage alive. https://www.americanindianmagazine.org/story/black-ash-basketry. Accessed 8 May 2022.

Bonfil, C. 2010. Present state, use, and perspectives of the genus *Fraxinus* in Mexico. 2010 Proceedings Symposium on Ash in North America. GTR-NRS-P-72. 1 p.

Brown-Rytlewski, D. E., and M. A. Wilson. 2004. Tracking the emergence of emerald ash borer adults, pp. 11. *In* V. Mastro and R. Reardon (comps.), Emerald Ash Borer Research and Technology Development Meeting, 30 September–1 October 2003, Port Huron, MI. Publication FHTET-2004-02. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Cappaert, D., D. G. McCullough, T. M. Poland, and N. W. Siegert. 2005. Emerald ash borer in North America: A research and regulatory challenge. American Entomologist 51(3): 152–165.

Cipollini, D. 2015. White fringetree as a novel larval host for emerald ash borer. Journal of Economic Entomology 108(1): 370–375.

Cipollini, D., and C. M. Rigsby. 2015. Incidence of infestation and larval success of emerald ash borer (*Agrilus planipennis*) on white fringetree (*Chionanthus virginicus*), Chinese fringetree (*Chionanthus retusus*), and devilwood (*Osmanthus americanus*). Environmental Entomology 44(5): 1375–1383.

Cipollini, D., C. M. Rigsby, and D. L. Peterson. 2017. Feeding and development of emerald ash borer (Coleoptera: Buprestidae) on cultivated olive, *Olea europaea*. Journal of Economic Entomology 110(4): 1935–1937.

Cipollini, D., and D. L. Peterson. 2018. The potential for host switching via ecological fitting in the emerald ash borer-host plant system. Oecologia 187(2): 507–519.

Davidson, W., and L. K. Rieske. 2016. Establishment of classical biological control targeting

emerald ash borer is facilitated by use of insecticides, with little effect on native arthropod communities. Biological Control 101: 78–86.

Duan, J. J., M. D. Ulyshen, L. S. Bauer, J. Gould, and R. Van Driesche. 2010. Measuring the impact of biotic factors on populations of immature emerald ash borers (Coleoptera: Buprestidae). Environmental Entomology 39(5): 1513–1522.

Duan J. J., P. B. Taylor, and R. W. Fuester. 2011. Biology and life history of *Balcha indica*, an ectoparasitoid attacking the emerald ash borer, *Agrilus planipennis*, in North America. Journal of Insect Science 11: 127.

Duan, J. J., G. Yurchenko, and R. Fuester. 2012a. Occurrence of emerald ash borer (Coleoptera: Buprestidae) and biotic factors affecting its immature stages in the Russian Far East. Environmental Entomology 41(2): 245–254.

Duan, J. J., L. S. Bauer, K. J. Abell, and R. Van Driesche. 2012b. Population responses of hymenopteran parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded areas in north central United States. BioControl 57(2): 199–209.

Duan, J. J., K. Larson, T. Watt, J. Gould, and J. P. Lelito. 2013. Effects of host plant and larval density on intraspecific competition in larvae of the emerald ash borer (Coleoptera: Buprestidae). Environmental Entomology 42(6): 1193–1200.

Duan, J. J., K. J. Abell, L. S. Bauer, J. Gould, and R. Van Driesche. 2014a. Natural enemies implicated in the regulation of an invasive pest: A life table analysis of the population dynamics of the emerald ash borer. Agricultural and Forest Entomology 16(4): 406–416.

Duan, J. J., T. J. Watt, and K. Larson. 2014b. Biology, life history and laboratory rearing of *Spathius galinae*, a larval parasitoid of the invasive emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 107(3): 939–946.

Duan, J. J., L. S. Bauer, K. J. Abell, M. D. Ulyshen, R. G. Van Driesche, and A. Sheppard. 2015. Population dynamics of an invasive forest insect and associated natural enemies in the aftermath of invasion: Implications for biological control. The Journal of Applied Ecology 52(5): 1246–1254.

Duan, J. J., L. S. Bauer, R. G. Van Driesche, and J. R. Gould. 2018. Progress and challenges of protecting North American ash trees from the emerald ash borer using biological control. Forests 9(3): 142.

Duan, J. J., R. G. Van Driesche, R. S. Crandall, J. M. Schmude, C. E. Rutledge, B. H. Slager, J. R. Gould, and J. S. Elkinton. 2019. Establishment and early impact of *Spathius galinae* (Hymenoptera: Braconidae) on emerald ash borer (Coleoptera: Buprestidae) in the northeastern United States. Journal of Economic Entomology 112(5): 2121–2130.

Duan, J. J., J. M. Schmude, and K. M. Larson. 2021. Effects of low temperature exposure on diapause, development, and reproductive fitness of the emerald ash borer (Coleoptera: Buprestidae): Implications for voltinism and laboratory rearing. Journal of Economic Entomology 114(1): 201–208.

Emerald Ash Borer Information Network. 2022. About emerald ash borer. http://www.emeraldashborer.info/about-eab.php. Accessed 25 Apr 2022.

Federal Register. 2003. Emerald ash borer; quarantine and regulations. 7 CFR Part 301, 68(198): 59082–59091. https://www.govinfo.gov/content/pkg/FR-2003-10-14/pdf/03-25881.pdf. Accessed 7 Dec 2020.

Federal Register. 2020. Removal of emerald ash borer domestic quarantine regulations. 7 CFR Part 301, 85(241): 81085–81095. https://www.govinfo.gov/content/pkg/FR-2020-12-15/pdf/2020-26734.pdf. Accessed 30 Dec 2020.

Filgueira, J. J., L. Franco-Lara, J. E. Salcedo, S. L. Gaitan, and E. R. Boa. 2004. Urapan (*Fraxinus uhdei*) dieback, a new disease associated with a phytoplasma in Colombia. Plant Pathology 53(4): 520.

Flower, C. E., K. S. Knight, and M. A. Gonzalez-Meler. 2013. Impacts of the emerald ash borer (*Agrilus planipennis* Fairmaire) induced ash (*Fraxinus* spp.) mortality on forest carbon cycling and successional dynamics in the eastern United States. Biological Invasions 15(4): 931–944.

Francis, J. K. 1990. *Fraxinus uhdei* (Wenzig) Lingelsh. SO-ITF-SM-28. Rio Piedras, PR. U.S. Department of Agriculture, Forest Service, Institute of Tropical Forestry. 4 p.

Gaudon, J. M., and S. M. Smith. 2020. Augmentation of native North American natural enemies for the biological control of the introduced emerald ash borer in central Canada. BioControl 65(1): 71–79.

Gould, J. R., T. Ayer, and I. Fraser. 2011. Effects of rearing conditions on reproduction of *Spathius agrili* (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 104(2): 379–387.

Gould, J. R., M. L. Warden, B. H. Slager, and T. C. Murphy. 2020. Host overwintering phenology and climate change influence the establishment of *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), a larval parasitoid introduced for biocontrol of the emerald ash borer. Journal of Economic Entomology 113(6): 2641–2649.

(GAO) Government Accountability Office. 2006. Invasive forest pests: Lessons learned from three recent infestations may aid in managing future efforts. Rep. US GAO, GAO-06–353. https://www.gao.gov/assets/250/249776.pdf. Accessed 30 Dec 2020.

Herms, D. A., and D. G. McCullough. 2014. Emerald ash borer invasion of North America:

History, biology, ecology, impacts, and management. Annual Review of Entomology 59(1): 13–30.

Herms, D. A., D. G. McCullough, C. S. Clifford, D. R. Smitley, F. D. Miller, and W. Cranshaw. 2019. Insecticide options for protecting ash trees from emerald ash borer. North Central IPM Center Bulletin. 3rd ed. 16 pp.

Hooie, N. A., G. J. Wiggins, P. L. Lambdin, J. F. Grant, S. D. Powell, and J. P. Lelito. 2015. Native parasitoids and recovery of *Spathius agrili* from areas of release against emerald ash borer in eastern Tennessee, USA. Biocontrol Science and Technology 25(3): 345–351.

IUCN. 2022. The IUCN Red List of Threatened Species. Version 2021-3. https://www.iucnredlist.org. Accessed 6 Jan 2022.

Jennings, D. E, J. R. Gould, J. D. Vandenberg, J. J. Duan, and P. M. Shrewsbury. 2013. Quantifying the impact of woodpecker predation on population dynamics of the emerald ash borer (*Agrilus planipennis*). PLoS One 8(12): e83491.

Jennings, D. E., J. J. Duan, L. S. Bauer, J. M. Schmude, M. T. Wetherington, and P. M. Shrewsbury. 2016. Temporal dynamics of woodpecker predation on emerald ash borer (*Agrilus planipennis*) in the northeastern U.S.A. Agricultural and Forest Entomology 18(2): 174–181.

Jones, M. I., J. R. Gould, H. J. Mahon, and M. K. Fierke. 2020. Phenology of emerald ash borer (Coleoptera: Buprestidae) and its introduced larval parasitoids in the northeastern United States. Journal of Economic Entomology 113(2): 622–632.

Kelly, L. J., W. J. Plumb, D. W. Carey, M. E. Mason, E. D. Cooper, W. Crowther, A. T.

Whittemore, S. J. Rossiter, J. L. Koch, and R. J. A. Buggs. 2020. Convergent molecular evolution among ash species resistant to the emerald ash borer. Nature Ecology & Evolution 4(8): 1116–1128.

Koch, J. L., D. W. Carey, M. E. Mason, T. M. Poland, and K. S. Knight. 2015. Intraspecific variation in *Fraxinus pennsylvanica* responses to emerald ash borer (*Agrilus planipennis*). New Forests 46(5): 995–1011.

Koenig, W. D., A. M. Liebhold, D. N. Bonter, W. M. Hochachka, and J. L. Dickinson. 2013. Effects of the emerald ash borer invasion on four species of birds. Biological Invasions 15(9): 2095–2103.

Koenig, W. D., and A. M. Liebhold. 2017. A decade of emerald ash borer effects on regional woodpecker and nuthatch populations. Biological Invasions 19(7): 2029–2037.

Kovacs, K. F., R. G. Haight, D. G. McCullough, R. J. Mercader, N. W. Siegert, and A. M. Liebhold. 2010. Cost of potential emerald ash borer damage in U.S. communities, 2009–2019. Ecological Economics 69(3): 569–578.

Lindell, C. A., D. G. McCullough, D. Cappaert, N. M. Apostolou, and M. B. Roth. 2008. Factors influencing woodpecker predation on emerald ash borer. The American Midland Naturalist 159(2): 434–444.

Liu, H., L. S. Bauer, D. L. Miller, T. Zhao, R. Gao, L. Song, Q. Luan, R. Jin, and C. Gao. 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biological Control 42(1): 61–71.

Lyons, D. B., G. C. Jones, and K. Wainio-Keizer. 2004. The biology and phenology of the

emerald ash borer, *Agrilus planipennis*, p. 5. *In* V. Mastro and R. Reardon (comps.), Emerald Ash Borer Research and Technology Development Meeting, 30 September–1 October 2003, Port Huron, MI. Publication FHTET-2004-02. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

MacFarlane, D. W., and S. P. Meyer. 2005. Characteristics and distribution of potential ash tree hosts for emerald ash borer. Forest Ecology and Management 213(1): 15–24.

McConnell, T. E., C. L. VanderSchaaf, and S. M. Tanger. 2019. Potential changes to Louisiana hardwood timber industry economic contributions following emerald ash borer (EAB) invasion: An input–output approach. Journal of Economic Entomology 112(6): 2751–2760.

McCullough, D. G., A. Agius, D. Cappaert, T. Poland, D. Miller, and L. Bauer. 2004. Host range and host preference of emerald ash borer, pp. 39–40. *In* V. Mastro and R. Reardon (comps.), Emerald Ash Borer Research and Technology Development Meeting, 30 September–1 October 2003, Port Huron, MI. Publication FHTET-2004-02. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Murfitt, J., Y. He., J. Yang, A. Mui, and K. De Mille. 2016. Ash decline assessment in emerald ash borer infested natural forests using high spatial resolution images. Remote Sensing 8(3): 256.

Murphy, T. C., J. R. Gould, R. G. Van Driesche, and J. S. Elkinton. 2018. Interactions between woodpecker attack and parasitism by introduced parasitoids of the emerald ash borer. Biological Control 122: 109–117.

Nalepa, C.A., K.L.F. Oten, and M.A. Bertone. 2021. Overwintering developmental stages of emerald ash borer in North Carolina. The Florida Entomologist 104(3): 213–217.

(NCDA&CS) North Carolina Department of Agriculture and Consumer Services. 2015. NCDA&CS news release: Entire state now under quarantine for emerald ash borer. http://www.ncagr.gov/paffairs/release/2015/stateEAB.htm. Accessed 1 Jan 2021.

(NCFS) North Carolina Forest Service. 2017. Emerald ash borer frequently asked questions. https://www.ncforestservice.gov/forest_health/fh_eabfaq.htm. Accessed 1 Jan 2021.

(NCFS) North Carolina Forest Service. 2021. Map - Current known distribution of emerald ash borer in North Carolina. https://www.ncforestservice.gov/forest_health/pdf/ Map_EAB_NCTracking.pdf. Accessed 7 Jan 2022.

Orlova-Bienkowskaja, M. J., and M. G. Volkovitsh. 2018. Are native ranges of the most destructive invasive pests well known? A case study of the native range of the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae). Biological Invasions 20(5): 1275–1286.

Oten, K. 2020. Emerald ash borer. https://content.ces.ncsu.edu/emerald-ash-borer. Accessed 1 Jan 2021.

Peterson, D. L., B. Slager, A. C. Anulewicz, and D. Cipollini. 2020. Feeding, survival, and fecundity of adult emerald ash borer (Coleoptera: Buprestidae) on foliage of two novel hosts and implications for host range expansion. Environmental Entomology 49(3): 709–716.

Peterson, D. L., and D. Cipollini. 2021. Attack dynamics and impacts of emerald ash borer on wild white fringetree populations. Biological Invasions 24(1): 9–15.

Poland, T. M., and D. G. McCullough. 2006. Emerald ash borer: Invasion of the urban forest and the threat to North America's ash resource. Journal of Forestry 104(3): 118–124.

Poland, T. M., D. G. McCullough, and A. C. Anulewicz. 2011. Evaluation of double-decker

traps for emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 104(2): 517–531.

Preston Jr., R. J., and R. R. Braham. 2002. North American trees - 5th ed. Iowa State Press, Ames, IA.

Ragozzino, M. L. 2020. Influences of climate, competition, and novel hosts on parasitoids of emerald ash borer, and their establishment in Virginia and North Carolina. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg.

Rebek, E. J., D. A. Herms, and D. R. Smitley. 2008. Interspecific variation in resistance to emerald ash borer (Coleoptera: Buprestidae) among North American and Asian ash (*Fraxinus* spp.). Environmental Entomology 37(1): 242–246.

Robinett, M. A., and D. G. McCollough. 2019. White ash (*Fraxinus americana*) survival in the core of the emerald ash borer (*Agrilus planipennis*) invasion. Canadian Journal of Forest Research 49(5): 510–520.

Rojas-Rodríguez, F., and G. Torres-Córdoba. 2016. Árboles del Valle Central de Costa Rica: reproducción Fresno (*Fraxinus uhdei* (Wenz.)). Revista Forestal Mesoamericana Kurú 14(34): 71–73.

Ryall, K. 2015. Detection and sampling of emerald ash borer (Coleoptera: Buprestidae) infestations. Canadian Entomologist 147(3): 290–299.

Siegert, N. W., D. G. McCullough, A. M. Liebhold, and F. W. Telewski. 2014. Dendrochronological reconstruction of the epicentre and early spread of emerald ash borer in North America. Diversity & Distributions 20(7): 847–858.

Siegert, N. W., R. J. Mercader, and D. G. McCullough. 2015. Spread and dispersal of emerald

ash borer (Coleoptera: Buprestidae): Estimating the spatial dynamics of a difficult-to-detect invasive forest pest. Canadian Entomologist 147(3): 338–348.

Stephens, J. P., K. A. Berven, and S. D. Tiegs. 2013. Anthropogenic changes to leaf litter input affect the fitness of a larval amphibian. Freshwater Biology 58(8): 1631–1646.

Swink, W. G., C. A. Nalepa, and J. P. Basham. 2014. Year-to-year variation in prey capture by *Cerceris fumipennis* (Hymenoptera: Crabronidae) at two sites in North Carolina. Annals of the Entomological Society of America 107(6): 1121–1125.

Taylor, R. A. J., L. S. Bauer, T. M. Poland, and K. N. Windell. 2010. Flight performance of *Agrilus planipennis* (Coleoptera: Buprestidae) on a flight mill and in free flight. Journal of Insect Behavior 23(2): 128–148.

Tobin, P. C., B. L. Strom, J. A. Francese, D. A. Herms, D. G. McCullough, T. M. Poland, K. L. Ryall, T. Scarr, P. J. Silk, and H. W. Thistle. 2021. Evaluation of trapping schemes to detect emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 114(3): 1201–1210.

USDA–APHIS/ARS/FS. 2020. Emerald ash borer biological control release and recovery guidelines. USDA–APHIS–ARS–FS, Riverdale, MD.

Villari, C., D. A. Herms, J. G. A. Whitehill, D. Cipollini, and P. Bonello. 2016. Progress and gaps in understanding mechanisms of ash tree resistance to emerald ash borer, a model for wood-boring insects that kill angiosperms. The New Phytologist 209(1): 63–79.

Wagner, D. L., and K. J. Todd. 2016. New ecological assessment for the emerald ash borer: A cautionary tale about unvetted host-plant literature. American Entomologist 62(1): 26–35.

Wang, X., Z. Yang, G. Liu, and E. Liu. 2007. Relationships between the emergence and

oviposition of ectoparasitoid *Spathius agrili* Yang and its host emerald ash borer, *Agrilus planipennis* Fairmaire. Frontiers of Forestry in China 2(4): 453–458.

Wang, X.-Y., Z.-Q. Yang, J. R. Gould, Y.-N. Zhang, G.-J. Liu, and E.-S. Liu. 2010. The biology and ecology of the emerald ash borer, *Agrilus planipennis*, in China. Journal of Insect Science 10(128): 1–23.

Wang, X. Y., D. E. Jennings, and J. J. Duan. 2015. Trade-offs in parasitism efficiency and brood size mediate parasitoid coexistence, with implications for biological control of the invasive emerald ash borer. Journal of Applied Ecology 52(5): 1255–1263.

Wei, X., Y. Wu, R. Reardon, T.-H. Sun, M. Lu, and J.-H. Sun. 2007. Biology and damage traits of emerald ash borer (*Agrilus planipennis* fairmaire) in China. Insect Science 14(5): 367–373.

Yang, Z.-Q., J. S. Strazanac, P. M. Marsh, C. van Achterberg, and W.-Y. Choi. 2005. First recorded parasitoid from China of *Agrilus planipennis*: A new species of *Spathius* (Hymenoptera: Braconidae: Doryctinae). Annals of the Entomological Society of America 98(5): 636–642.

Yang, Z.-Q., X.-Y. Wang, J. R. Gould, and H. Wu. 2008. Host specificity of *Spathius agrili* Yang (Hymenoptera: Braconidae), an important parasitoid of the emerald ash borer. Biological Control 47(2): 216–221.

Yang, Z.-Q., X.-Y. Wang, J. R. Gould, R. C. Reardon, Y.-N. Zhang, G.-J. Liu, and E.-S. Liu. 2010. Biology and behavior of *Spathius agrili*, a parasitoid of the emerald ash borer, *Agrilus planipennis*, in China. Journal of Insect Science 10(30): 1–13.

Zhang, Y.-Z., D.-W. Huang, T.-H. Zhao, H.-P. Liu, and L. S. Bauer. 2005. Two new species of

egg parasitoids (Hymenoptera: Encyrtidae) of wood-boring beetle pests from China. Phytoparasitica 33(3): 253–260.

CHAPTER 2

Characterization of Emerald Ash Borer Phenology and Voltinism in Central North Carolina

ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), has killed millions of ash (*Fraxinus* spp.) trees across eastern and central North America. Classical biological control using introductions of parasitoid wasps may provide a sustainable approach to managing EAB. However, establishment of parasitoids in the southern United States has been difficult. The phenology of EAB was studied in central North Carolina to inform appropriate parasitoid release timings that align with the seasonal availability of susceptible EAB life stages in the warm climate of this region. Biweekly EAB life stage assessments and adult trapping were conducted in stands of EAB-infested green ash (*Fraxinus pennsylvanica* Marshall) at a site in Garner, North Carolina from June 2019 through August 2021. Based on these collections, EAB exhibits a univoltine (one-year) life cycle. Parasitoid-susceptible larvae (third and fourth instars) are present from late June through October (~1,100–3,000 degree days base 10ºC) and are mostly absent (≤6.2% of EAB from sampled trees) during the remainder of the year. Parasitoid release timings and the life history of selected parasitoid species should be aligned with this window of host availability to be effective. This characterization of EAB phenology and voltinism will help improve the timing and effectiveness of EAB management efforts as this forest pest continues to spread in southern North America.

INTRODUCTION

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is one of the most devastating non-native pests to impact North American forests. Accidentally introduced from northeastern Asia, EAB has likely been present in North America since the

1990s and is attacking ash (*Fraxinus* spp.) trees across the eastern and central portions of the continent (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006, Siegert et al. 2014). Unlike the ash hosts of EAB in its native range, ash species native to North America are highly susceptible to EAB-caused mortality (Liu et al. 2007, Rebek et al. 2008, Villari et al. 2016). The larvae of EAB feed in the vascular tissue of their host plant, impeding the flow of nutrients and water within the tree (Cappaert et al. 2005, Wang et al. 2010, Villari et al. 2016). Millions of ash trees have been killed by EAB in North America, resulting in incalculable losses to the ecological, economic, and cultural values these trees provide (Poland and McCullough 2006, Kovacs et al. 2010, Flower et al. 2013, Stephens et al. 2013, Herms and McCullough 2014, Wagner and Todd 2016, Bolen 2020).

The impacts of EAB created an urgent need to understand the biology of this insect, and its life cycle has since been studied in regions of its native and non-native ranges (Bauer et al. 2004, Cappaert et al. 2005, Liu et al. 2007, Wei et al. 2007, Wang et al. 2010, Jones et al. 2020). Adult beetles lay eggs on host trees in spring or early summer, and larvae bore into the tree after hatching. Larvae develop through four instars as they feed within the tree, creating serpentine galleries in the phloem, cambium, and outer xylem. After feeding is completed, mature larvae bore pupal chambers in the tree. Larvae initially fold into a J-shaped larval stage, and these "J-larvae" later straighten and condense into a prepupal stage. After overwintering, individuals in chambers pupate and emerge from the tree as adults to mate and lay the next generation of eggs. This general progression of the EAB life cycle remains consistent across studied regions of Asia and North America, but the seasonal timing and duration of life stages can vary greatly based on climate (Wei et al. 2007, Duan et al. 2018, Gould et al. 2020, Jones et al. 2020).

Emerald ash borer was first detected in North America in the area of Detroit, Michigan, United States and Windsor, Ontario, Canada in the summer of 2002 (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006). In the ensuing 20 years, this insect has spread outward from this initial infestation and is now confirmed in 35 states of the United States and five provinces of Canada (Emerald Ash Borer Information Network 2022). As a result of this expansion, EAB experiences a variety of climatic conditions across the extent of its non-native range. Activity and development of EAB is dependent on temperature, so the life cycle of this species is influenced by differences in seasonal heat accumulation between the climates of different regions (Duan et al. 2018, Gould et al. 2020, USDA–APHIS/ARS/FS 2020).

In both Asia and North America, EAB exhibits a univoltine (one-year) or semivoltine (two-year) life cycle across a latitudinal gradient. In a univoltine life cycle, individuals overwinter once as J-larvae/prepupae and emerge as adults the subsequent spring. Emerald ash borer can also exhibit a semivoltine life cycle if larvae do not develop to the J-larval/prepupal stage by the end of their first growing season. In this longer cycle, individuals are understood to spend their first winter as larvae in their existing gallery, begin feeding again in the spring, develop into J-larvae/prepupae during the summer, and complete development after overwintering a second time (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006, Wei et al. 2007, Bauer et al. 2014, Gould et al. 2020, Jones et al. 2020, Duan et al. 2021). Faster larval development related to longer or warmer growing seasons would increase the ability of larvae to reach the J-larval/prepupal stage before overwintering, allowing them to complete their development to adulthood after their first winter (Duan et al. 2018, Duan et al. 2021). As a result, a shorter, univoltine life cycle is believed to be more common in warmer, southern regions relative to colder, northern regions because of the more rapid development of

eggs and larvae (Duan et al. 2018). Previous sampling of overwintering life stages in the United States has generally supported this predicted trend (Gould et al. 2020). However, life stage studies in the southern United States have been limited in terms of the duration of sampling and the number of individuals sampled (Abell et al. 2019, Gould et al. 2020, Ragozzino et al. 2020, Nalepa et al. 2021). Larger studies that monitor life stages continuously across years would improve the current understanding of EAB development in understudied southern regions.

The climate-based variation in the EAB life cycle has important implications for the challenging management of this forest pest in North America. This insect has continued to spread despite regulatory control of plant material, and the federal domestic quarantine for EAB was discontinued in the United States in 2021 (Federal Register 2020). Systemic insecticide treatments can successfully protect individual trees but are not practical or economical for sustained control across forested landscapes (Davidson and Rieske 2016, Herms et al. 2019). The development of resistant ash genotypes for forest restoration remains in progress (Koch et al. 2015, Villari et al. 2016, Pike et al. 2021). Of the limited options available now, classical biological control is considered to have high potential for contributing to sustainable management (Bauer et al. 2015, Duan et al. 2018, Jones et al. 2020). This method involves the introduction of several species of parasitoid wasps that attack either EAB eggs or larvae in the native range of the pest in Asia. To date, the egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) and the larval parasitoids *Spathius agrili* Yang (Hymenoptera: Braconidae), *Spathius galinae* Belokobylskij and Strazanac, and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) have been released in the United States for EAB biocontrol (Bauer et al. 2015, Duan et al. 2018).

Successful establishment of biocontrol agents in North America is dependent on synchronizing the life cycle of parasitoids with the temporal availability of susceptible EAB life stages in or on which the parasitoids can successfully attack and develop (Duan et al. 2018, Gould et al. 2020, Jones et al. 2020). The introduced larval parasitoids attack third- and fourth-instar larvae in galleries, and different parasitoid species emerge and oviposit at different times of the year (Jennings et al. 2016, Duan et al. 2018, Gould et al. 2020, Jones et al. 2020). Consequently, understanding the phenology of EAB in a given region is crucial to determining the appropriate parasitoid species and the appropriate release timings for these parasitoids that match when third and fourth instars are present in the environment.

Characterizing EAB phenology is especially important in southern North America. The majority of research on EAB in North America has been conducted in the northern United States and southern Canada because the spread of EAB radiated from this region. As a result, there is a need for foundational research on EAB biology in the warm climate of the southern United States that region-specific EAB management decisions can be based on. If the EAB life cycle in the Southeast differs from that in northern regions, then management approaches developed for the northern United States would not be appropriate for warmer climates. The need for climate-specific management is particularly relevant for biocontrol. In contrast to the Northeast and Midwest regions, establishment of released parasitoids has been very limited in the southern United States (Duan et al. 2018, MapBioControl 2020). Biocontrol efforts in the Southeast are likely to fail if selected parasitoid species and release timings are based on the EAB life cycle in a colder climate, so a deeper understanding of the seasonal availability of parasitoid-susceptible larvae in southern North America is needed to inform effective biocontrol release recommendations (Duan et al. 2018, Gould et al. 2020, Jones et al. 2020). To this end, the

research presented in this chapter focuses on characterizing EAB phenology and voltinism in the warm climate of central North Carolina.

MATERIALS AND METHODS

Site Description

This research was conducted at the Wrenn Road Facility in Garner, North Carolina (35.6437, -78.5802). This site is more than 240 ha in size and consists of grass fields and planted stands of trees, including green ash (*Fraxinus pennsylvanica* Marshall), loblolly pine (*Pinus taeda* L.), sycamore (*Platanus occidentalis* L.), sweetgum (*Liquidambar styraciflua* L.), baldcypress (*Taxodium distichum* (L.) Rich.), and poplar (*Populus* sp.). The site was used for land application treatment of municipal primary wastewater until 2008 (Ghezehei et al. 2015, City of Raleigh 2022). Since that time, land use has been converted to reclaimed water management, and the stands continued to be irrigated by a system of sprinklers at a rate of ~1,368 mm per year (Ghezehei et al. 2015).

Emerald ash borer life stage assessments and adult trapping were conducted in three stands of planted green ash (*Fraxinus pennsylvanica* Marshall) (Figure 1). Stands 1, 2, and 3 were ~4.2, ~4.5, and ~4.8 ha, respectively. These even-aged ash stands were planted in 1993 at a density of 1,359 trees per ha (550 trees per acre) (Ghezehei et al. 2015). The trees were grown from wild-collected green ash seed from the North Carolina Piedmont. The composition of canopy trees in Stand 1 varied between pure green ash areas and mixed green ash and loblolly pine areas. Stands 2 and 3 were nearly pure green ash stands with few scattered loblolly pines and sycamores in the canopy. Based on ash health assessments conducted in Stands 2 and 3, green ash had experienced ~90% mortality from EAB as of 26 July 2021 (Appendix A). The ground cover of each stand was dominated by a thick layer of stiltgrass (*Microstegium vimineum*

(Trin.) A. Camus) during the growing season. The understories of the stands were composed primarily of boxelder (*Acer negundo* L.), dogfennel (*Eupatorium capillifolium* (Lam.) Small), privet (*Ligustrum sinense* Lour.), eastern redcedar (*Juniperus virginiana* L.), and limited green ash regeneration. The understories of Stands 1 and 2 were sparse, while Stand 3 had a much thicker understory dominated by boxelder and dogfennel. Other documented understory species included sea-myrtle (*Baccharis halimifolia* L.), waxmyrtle (*Morella cerifera* (L.) Small), sweetgum, oaks (*Quercus* spp.), and *Melia azedarach* L.

Figure 1. Green ash stands at the Wrenn Road Facility (Garner, North Carolina).

Debarking and Emerald Ash Borer Life Stage Assessments

Standing green ash trees were debarked in each of the three stands at the site to collect EAB specimens. Debarking was conducted every two weeks from 28 June 2019 through 31 August 2021 except the week of 20 December 2020. During each debarking, one ash tree with

signs and symptoms of current EAB infestation (live epicormic sprouts, canopy thinning/dieback, bark splits, woodpecker activity, EAB exit holes) and a diameter at breast height (~1.37 m; DBH) between 18 and 22 cm was pseudo-randomly selected in the interior of each stand. The mean DBH of trees selected between 18 August 2020 and 31 August 2021 was ~19.8 cm.

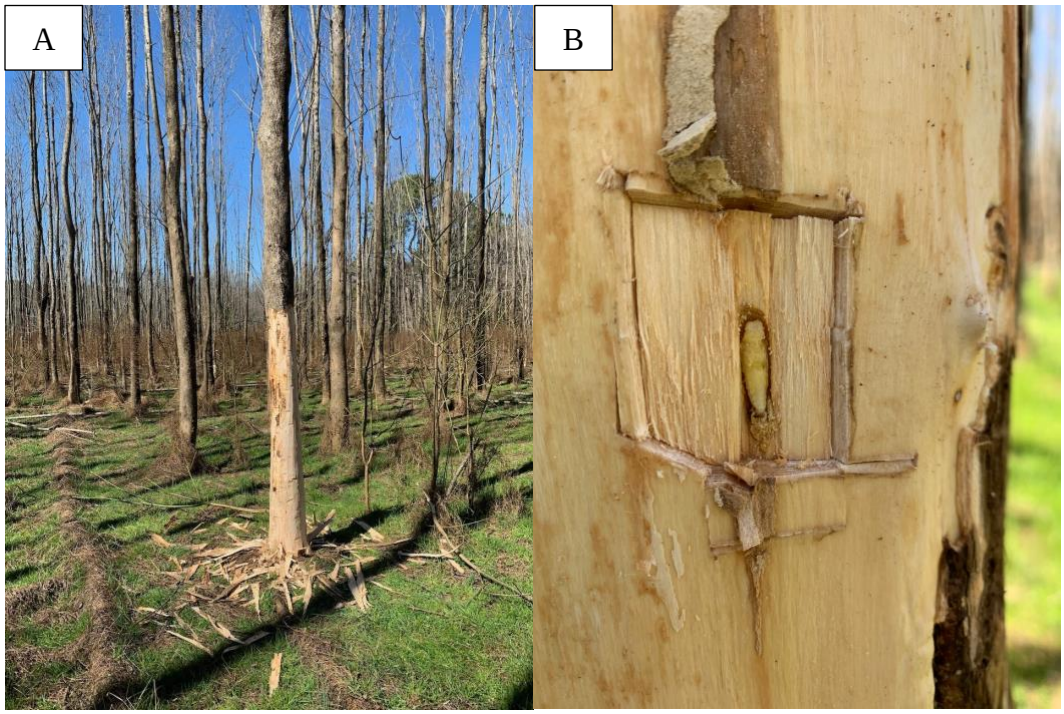

Figure 2. (A) Debarked green ash tree sampled for emerald ash borer life stages; (B) Emerald ash borer pupa inside of an excavated pupal chamber.

On each collection date, EAB larvae, J-larvae/prepupae, pupae, and unemerged adults in pupal chambers were collected from each selected tree. The bark was removed from the lower 1.8 m of each tree using drawknives and machetes (Figure 2A). All EAB larvae found in galleries under the bark were collected and placed into vials containing 89% ethanol, and the vials were labeled with the stand number and collection date. Emerald ash borer pupal chambers were identified by an EAB exit hole filled with frass adjacent to the end of an EAB gallery. Specimens in pupal chambers were excavated by using a chisel and hammer to remove a block

of wood surrounding the chamber (Figure 2B). J-larvae/prepupae found in chambers were kept in separate vials from larvae found in galleries so they could be distinguished when counting and measuring life stages. When large numbers of pupal chambers were present in a tree, a sample of 11 to 20 EAB specimens in chambers were excavated and collected, and the remaining chambers were counted. If no EAB were found in the first tree selected in a stand, up to three additional trees per stand were debarked until EAB were found. Species of wood-boring insects other than EAB that were found in or on ash trees during debarking were also identified and are reported in Appendix B.

Emerald ash borer specimens were transported to a lab at North Carolina State University in Raleigh, and the life stage of each specimen was recorded. Specimens were classified as first- through fourth-instar larvae, J-larvae/prepupae, pupae, or adults. Larvae were measured under a microscope using a 0.50 mm ocular micrometer to determine their instar. The primary characteristic measured for instar determination was the length of a larva's urogomphi, which are paired processes on the last abdominal segment (Figure 3A). If the urogomphi were missing or damaged, the width of the larva's epistome was measured instead (Figure 3B). Measurements were made to the nearest 0.05 mm. Larvae were classified to their appropriate instar based on the measurement ranges determined by Orlova-Bienkowskaja and Bieńkowski (2016). The actual categories of urogomphi length measurements used in this study are similar to those used by Nalepa et al. (2021) and are as follows: ≤0.20 mm (first instar), 0.21 to 0.39 mm (second instar), 0.40 to 0.74 mm (third instar), and ≥0.75 mm (fourth instar). The actual categories of epistome width measurements used in this study are as follows: ≤0.30 mm (first instar), 0.31 to 0.49 mm (second instar), 0.50 to 0.89 mm (third instar), and ≥0.90 mm (fourth instar). J-larvae/prepupae were distinguished from fourth-instar larvae based on their body shape and location in pupal

chambers rather than urogomphi length or epistome width due to the overlap in measurement ranges observed by Orlova-Bienkowskaja and Bieńkowski (2016). Emerald ash borers in counted chambers from a given tree were assigned to an appropriate life stage based on the relative proportions of each life stage recorded for the EAB excavated from chambers in that tree.

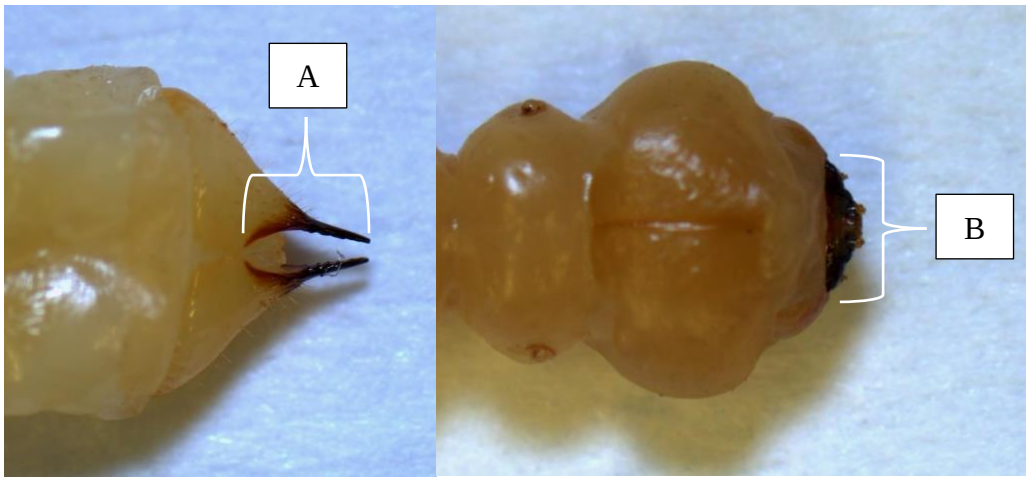

Figure 3. Morphological characteristics of an emerald ash borer larva used for determining its instar: (A) urogomphi length, (B) epistome width.

Adult Emerald Ash Borer Trapping

Emerged adult EAB were also collected at the research site using traps. Three purple prism traps were deployed in each of the three stands in 2019, 2020, and 2021 (Figure 4A). The traps consisted of a purple corrugated plastic sheet folded into a three-sided prism and coated with an adhesive on the outside surface (Francese et al. 2008, Francese et al. 2011, Tobin et al. 2021). The prism traps were arranged in transects, with one trap located at the stand edge and the second and third traps located ~45–60 m progressively further into the stand interior. This prism trap configuration was kept the same in each year of trapping. In addition, two green Lindgren multifunnel traps were deployed in each stand in 2021 (Figure 4B). The multifunnel traps consisted of 12 green plastic funnel units and a removable collection cup containing ~150–200

mL of propylene glycol as a preservative (Francese et al. 2011, Tobin et al. 2021). The two traps were located in the stand interior and were spaced ~30–70 m apart. Prism and multifunnel traps were hung from a metal hook and were placed in an ash tree using a telescoping pole. Prism traps were hung from a branch in the lower canopy ~4.5–5 m from the ground. Multifunnel traps were hung from a fork or large branch about ~2.5–3 m from the ground. Ash tree canopies at this site lacked branches accessible to the telescoping pole due to EAB damage, tall tree heights, and small tree crowns, which limited placement of traps higher in the trees' canopies.

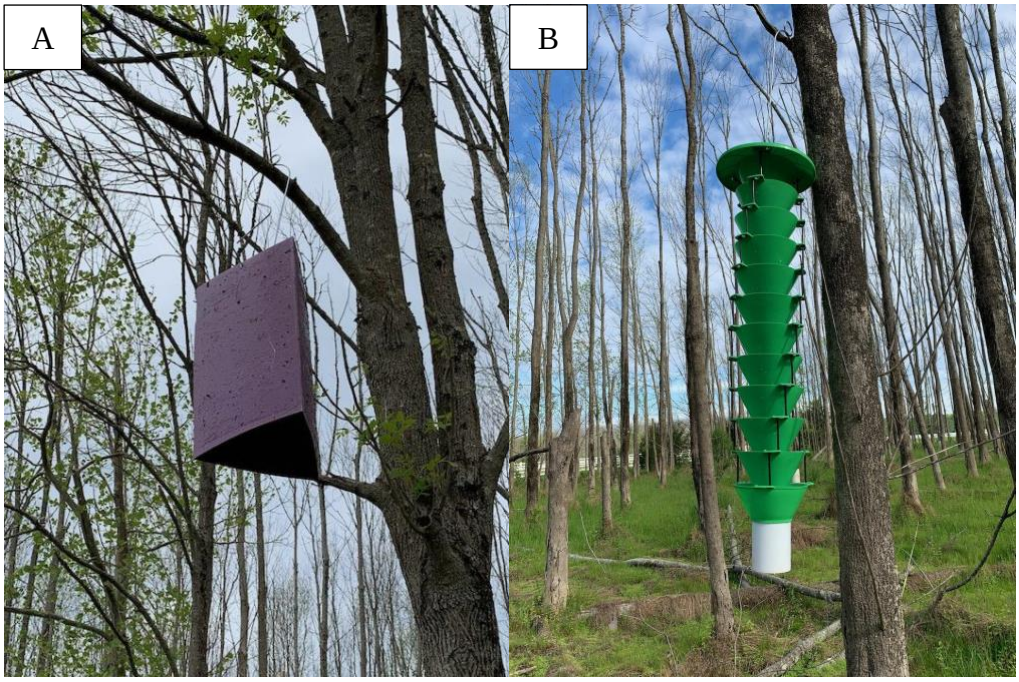

Figure 4. (A) Purple prism trap and (B) green multifunnel trap used to collect adult emerald ash borers.

Prism traps were checked every two weeks from 23 April to 21 June 2019 and 31 March to 23 June 2020. In 2021, prism and multifunnel traps were deployed on 26 March and were checked every two weeks from 2 April to 11 May and weekly from 11 May to 7 July. Each trap was baited with a (Z)-3-hexenol lure. Lures were attached on the day of deployment for all traps in 2021 except the multifunnel traps in Stands 2 and 3, which were baited on 30 March due to supply availability. A fresh lure was added to each trap within the recommended 60-day

expiration period (4 June 2019; 30 April and 16 June 2021). During trap checks, each prism trap was lowered from the tree with the telescoping pole and all adult EAB on the trap surface were removed with forceps, counted, and placed into vials containing 89% ethanol. To check the multifunnel traps, the collection cup was removed and emptied into a labeled paint filter. The filters containing the contents of each trap were then placed into individually labeled zipper storage bags, and the trap contents were sorted later on the same day of collection. All adult EAB from each trap were counted and placed in vials containing 89% ethanol. Separate labeled vials were kept for each unique combination of collection date, stand, and trap type. In addition, representative specimens of wood-boring insect species other than EAB that were caught in the traps were also collected and placed in vials containing 89% ethanol. These specimens were later identified and are reported in Appendix B.

Degree Day Calculation

Seasonal heat accumulation throughout each year of this study was quantified based on degree days, a measure of heat accumulation commonly used to describe and predict the development and activity of insects in agriculture and forestry (Herms 2004, Barker et al. 2020, Crimmins et al. 2020). Degree day accumulation for sampling dates was calculated using the Duarte 2013 OSU OIPMC model (USPEST.ORG 2018). This model was designed for EAB and calculates cumulative degree days beginning 1 January using a single sine method, a lower threshold of 10°C, and an upper threshold of 37.8°C. Cumulative degree days were also calculated with an equivalent lower threshold of 50ºF and upper threshold of 100°F using the same model to aid in comparison to references using these units. Cumulative degree days with lower thresholds of 10°C and 50ºF are referred to as DD_{10} and DD_{50}, respectively. The

calculations were run based on daily maximum and minimum temperatures recorded at Station E1728 (35.6450, -78.5444), which is located ~3 km east of the research site.

Statistical Analysis

Statistical analysis was conducted using R version 4.1.2 (R Core Team 2021). Analysis of variance (ANOVA) F-tests were conducted to test for differences in the counts of all EAB life stages recorded from debarked trees across different stands. ANOVA F-tests were also conducted to test for differences in counts of adult EAB collected on purple prism traps based on stands, trap locations within stands (edge, middle, interior), and the interaction between stand and trap location. Due to the large decrease in EAB counts from collections over the course of the study, counts of EAB were log transformed to help meet the assumptions of normally distributed residuals and homogeneity of variances. Statistical significance was based on an alpha level of 0.05.

Observed proportions of EAB overwintering life stages were also compared to a model created by Gould et al. (2020) to predict the proportion of EAB that overwinter as first- through fourth-instar larvae rather than J-larvae in different climatic regions of the United States. Predicted proportions of overwintering non-J-larvae in Garner in winter 2019–2020 and 2020–2021 were estimated by inputting the DD_{50} accumulated between 1 January and 30 September of 2019 and 2020, respectively, to the inverse-logit equation developed by Gould et al. (2020).

RESULTS

Emerald Ash Borer Life Stage Assessments

In total, 5,506 EAB specimens were recorded from 171 debarked trees between 28 June 2019 and 31 August 2021 (Figure 5). There were no significant differences in log counts of total EAB collected from debarking between the three sampled ash stands (F = 0.543, df = 2, p =

0.582). Larvae in the first two instars were present in June and July (Figures 5, 7, 8). Both first- and second-instar larvae appeared at ~900 DD_{10} (early June) and were not recorded after ~1,500–1,800 DD_{10} (late July). Parasitoid-susceptible, third- and fourth-instar larvae in galleries were mostly present from late June through October (~1,100–3,000 DD_{10}) (Figures 5–8). Collections of third-instar larvae occurred mostly from ~1,100–1,900 DD_{10} (late June to early August) and peaked at ~1,300 DD_{10} (late June or early July) each year. Fourth-instar larvae were first recorded at ~1,300 DD_{10}, peaked at ~1,300–1,800 DD_{10} (late June to early August), and were collected infrequently after ~3,000 DD_{10} (mid-to-late October). Only one larva at a stage before the fourth instar was recorded before 800 DD_{10} (end of May) or after 2,900 DD_{10} (late September to mid-October), and no larvae of any stage were recorded in galleries from 200–800 DD_{10} (mid-March or mid-April through May) (Figures 5, 6). No more than 6.2% of EAB recorded during collections from November through early June (after 3,100 DD_{10} and before 1,000 DD_{10}) were third- or fourth-instar larvae in galleries (Figures 7, 8).

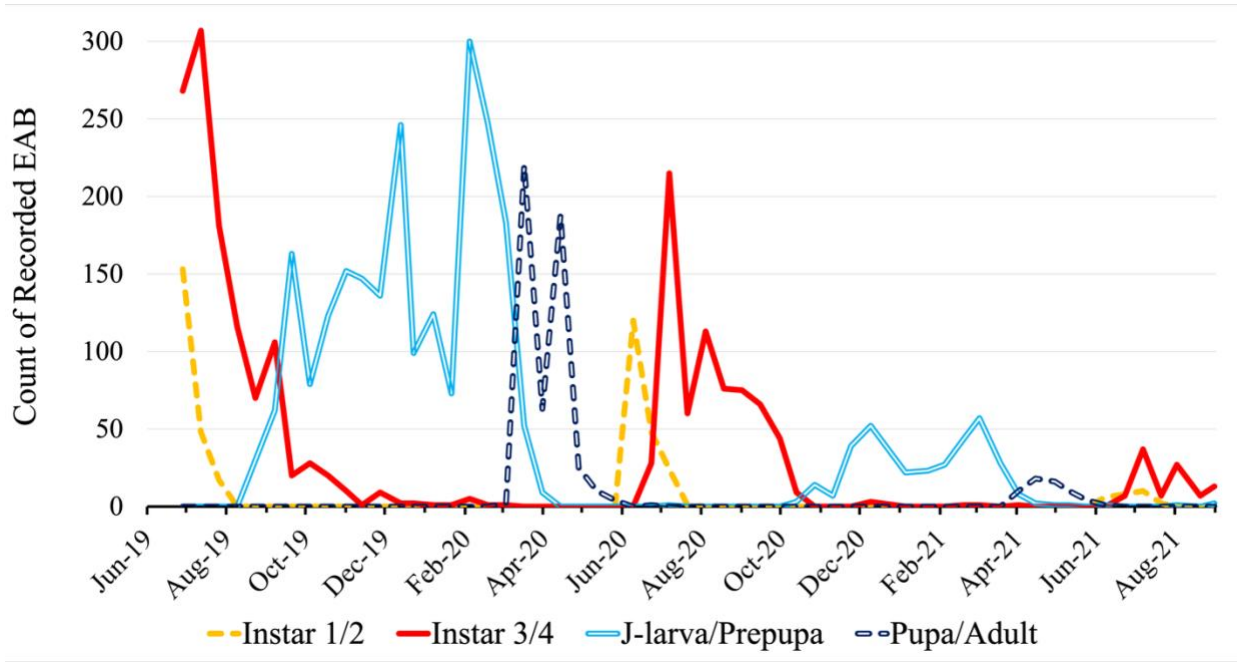

Figure 5. Counts of emerald ash borer (EAB) life stages recorded from biweekly debarking of green ash trees in Garner, North Carolina from June 2019 through August 2021.

Live J-larvae/prepupae in pupal chambers were first recorded on 23 August 2019 (2,331 DD_{10}), 13 October 2020 (2,824 DD_{10}), and 2 August 2021 (1,753 DD_{10}). Collections of live J-larvae/prepupae ceased after 307 DD_{10} (31 March) in 2020 and 510 DD_{10} (11 May) in 2021 with the exception of one potential specimen recovered at 1,366 DD_{10} (7 July) in 2020 (Figures 5, 7). More than 93% of recorded EAB were J-larvae/prepupae by the end of October (~3,000–3,200 DD_{10}) through early to mid-March (~100–200 DD_{10}) (Figures 7, 8). Pupation of EAB was first recorded at 147 DD_{10} (3 March) in 2020 and 181 DD_{10} (2 April) in 2021. Unemerged adults were first recorded in pupal chambers at 404 DD_{10} (14 April) in 2020 and 298 DD_{10} (15 April) in 2021. Pupae and unemerged adults were last recorded at 510 DD_{10} (11 May) and 858 DD_{10} (8 June), respectively, in 2021 (Figures 5, 7). However, unemerged adults collected after 510 DD_{10} appeared to be dead and decaying.

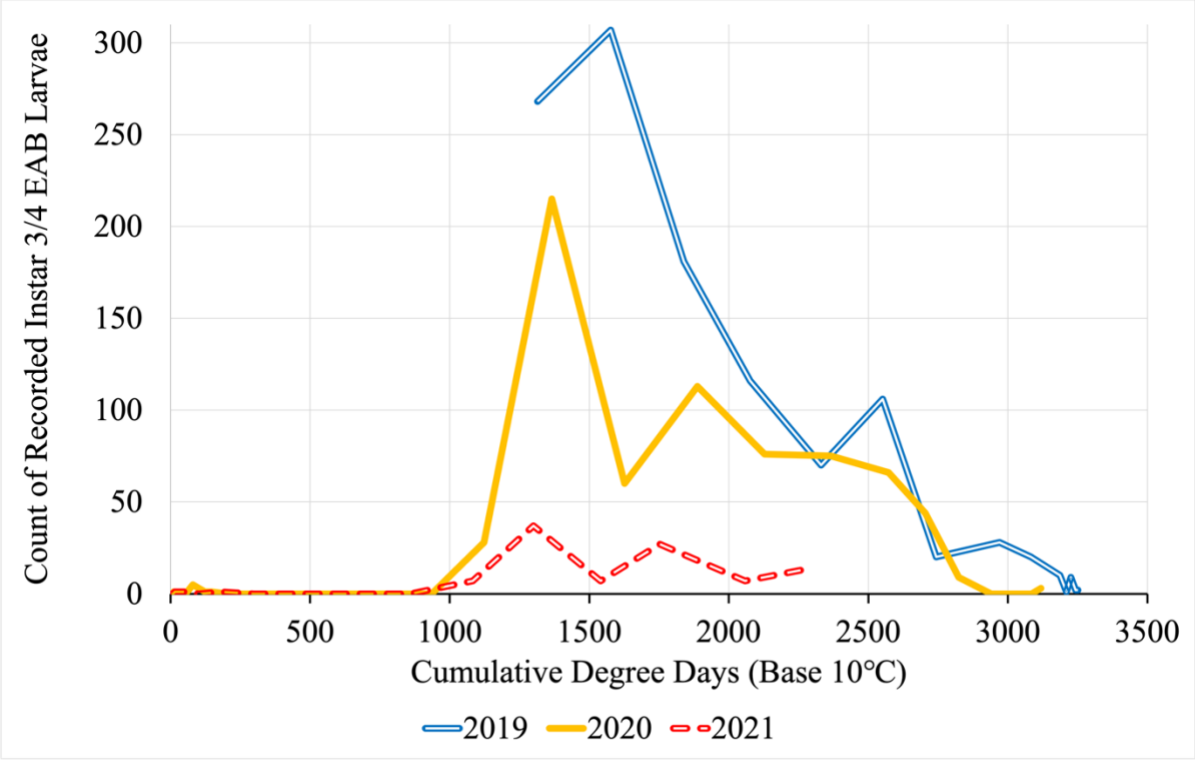

Figure 6. Counts of parasitoid-susceptible, third- and fourth-instar emerald ash borer (EAB) larvae recorded from biweekly debarking of green ash trees in Garner, North Carolina across degree day accumulations in 2019, 2020, and 2021. Sampling was conducted from June 2019 through August 2021.

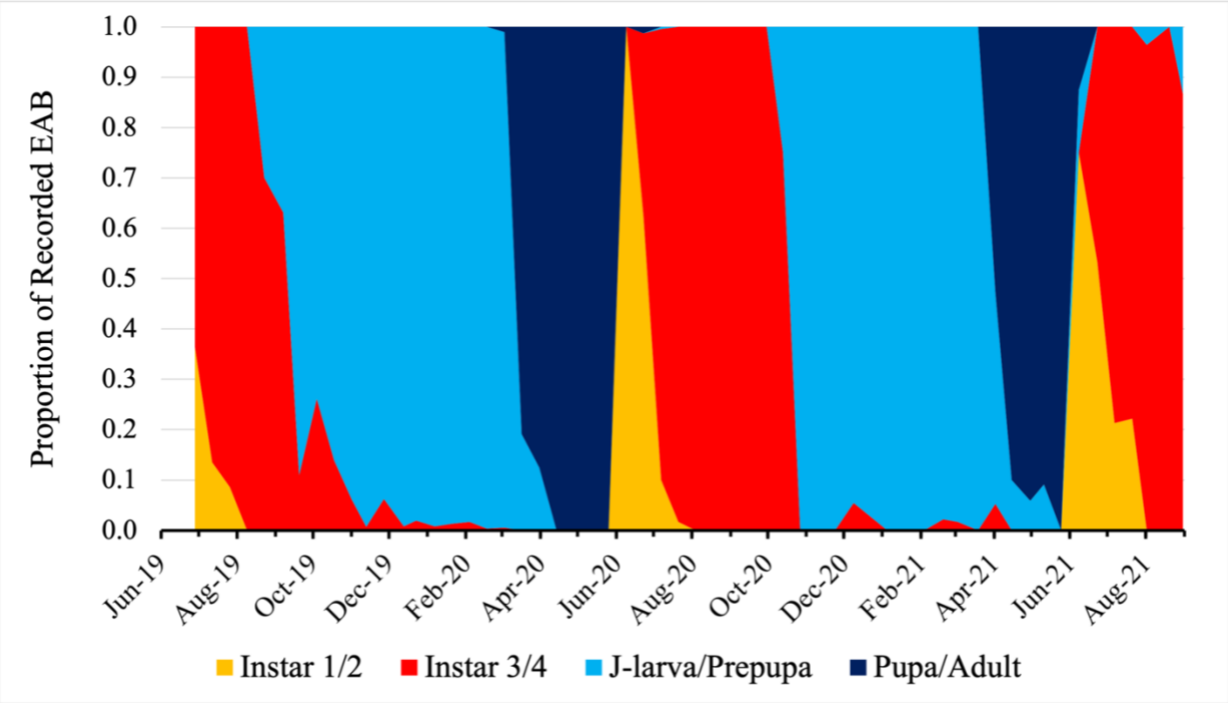

Figure 7. Proportions of emerald ash borer (EAB) life stages recorded from biweekly debarking of green ash trees in Garner, North Carolina from June 2019 through August 2021.

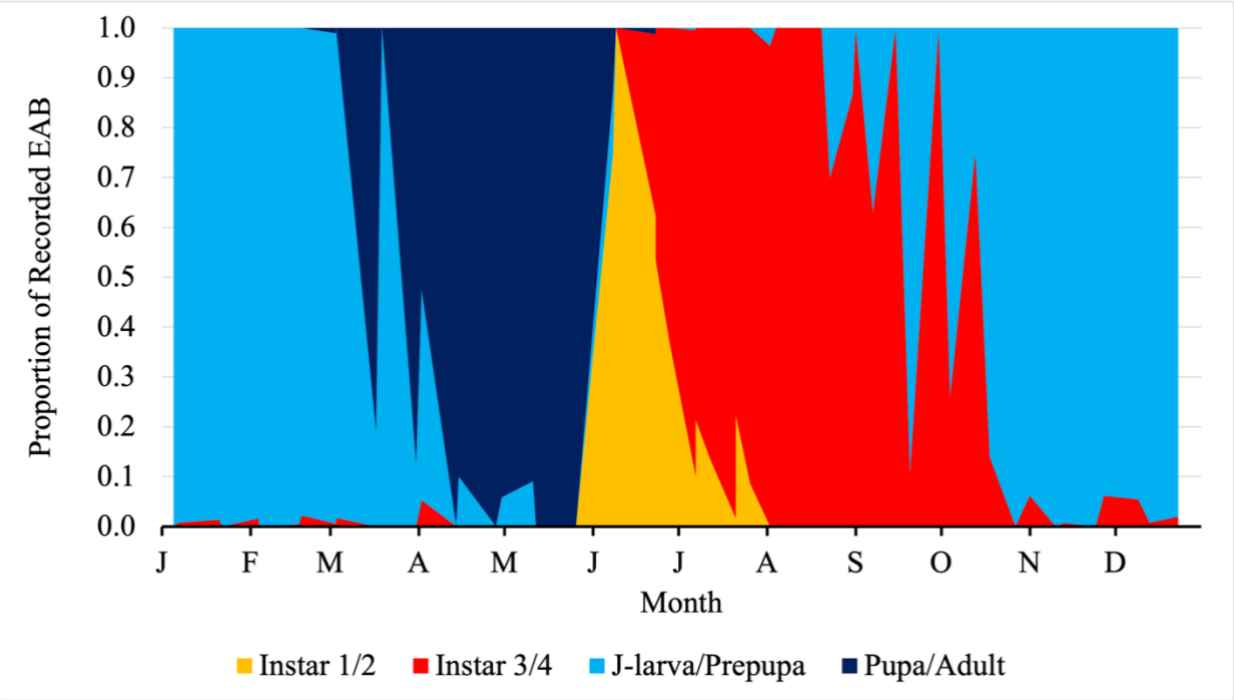

Figure 8. Proportions of emerald ash borer (EAB) life stages recorded from biweekly debarking of green ash trees in Garner, North Carolina over the course of a year. Data are pooled from samples collected from June 2019 through August 2021.

Adult Emerald Ash Borer Activity

A total of 1,773 adult EAB were collected from traps in 2019, 2020, and 2021 (Figures 9, 10). The nine prism traps captured 876 adults in 2019, 552 adults in 2020, and 64 adults in 2021 over eight, 12, and 15 weeks of deployment, respectively. This amounts to a 58.0% decrease in per-week prism trap catch between 2019 and 2020 and a 90.7% decrease between 2020 and 2021. The effects of stand (F = 0.293, df = 2, p = 0.747), trap location (F = 0.078, df = 2, p = 0.925), and their interaction (F = 1.031, df = 4, p = 0.397) on log counts of adult EAB collected from prism traps were not statistically significant. An additional 281 adults were caught in the six multifunnel traps over 15 weeks in 2021. Of these adults from multifunnel traps, 73.3% were collected from the two traps in Stand 1 between 15 April and 11 May (collected 30 April and 11 May).

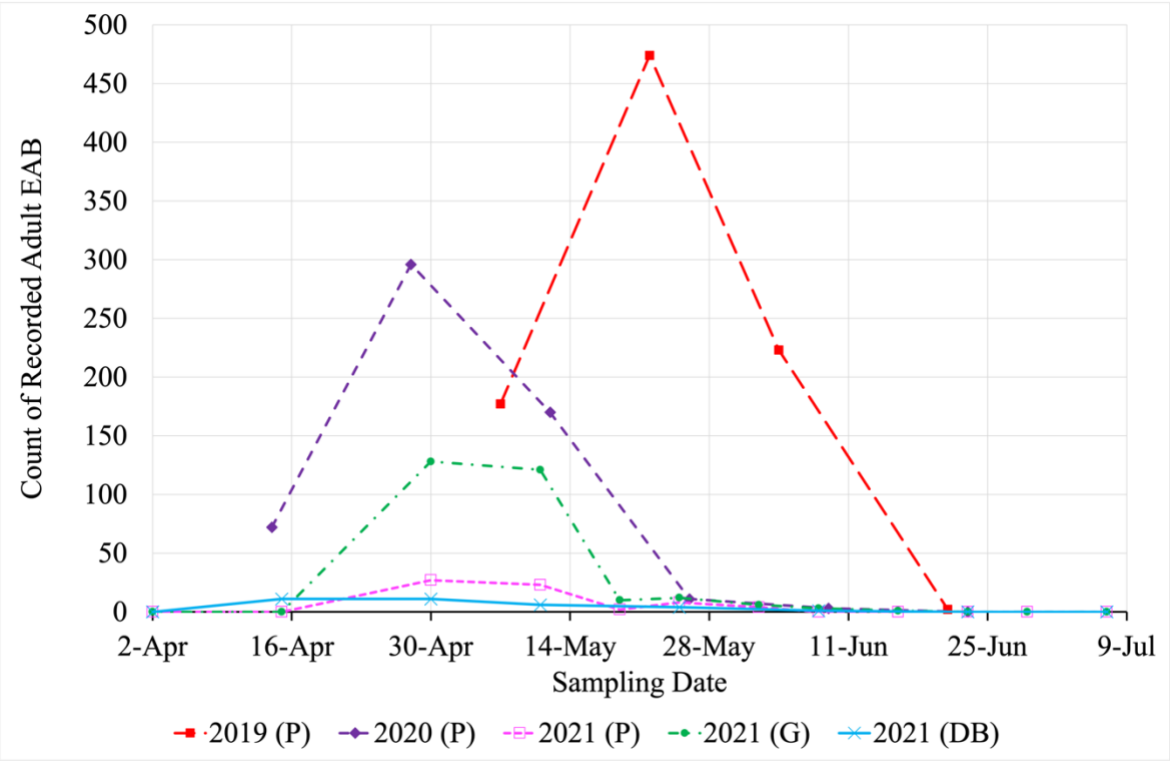

Figure 9. Counts of adult emerald ash borer (EAB) recorded from purple prism traps (P), green multifunnel traps (G), and debarked ash trees (DB) in Garner, North Carolina in 2019–2021.

In 2021, the first emergence of adult EAB was detected on 15 April (298 DD_{10}) based on finding fully developed adults actively emerging from exit holes and flying during debarking. No adults were found on traps that day, and adults were first detected on traps during the next check on 30 April (405 DD_{10}) on prism and multifunnel traps in all three stands. The exact timing of first EAB emergence is uncertain in 2019 and 2020 because adults were already present on traps during the first trap check each year (7 May 2019 (536 DD_{10}) and 14 April 2020 (404 DD_{10})). Based on trapping, adult EAB activity peaked in mid-May (~600–800 DD_{10}) in 2019 and late April to early May (~350–550 DD_{10}) in 2020 and 2021. Trap collection of adult EAB ceased by mid-June (~900–1,200 DD_{10}) each year (Figures 9, 10).

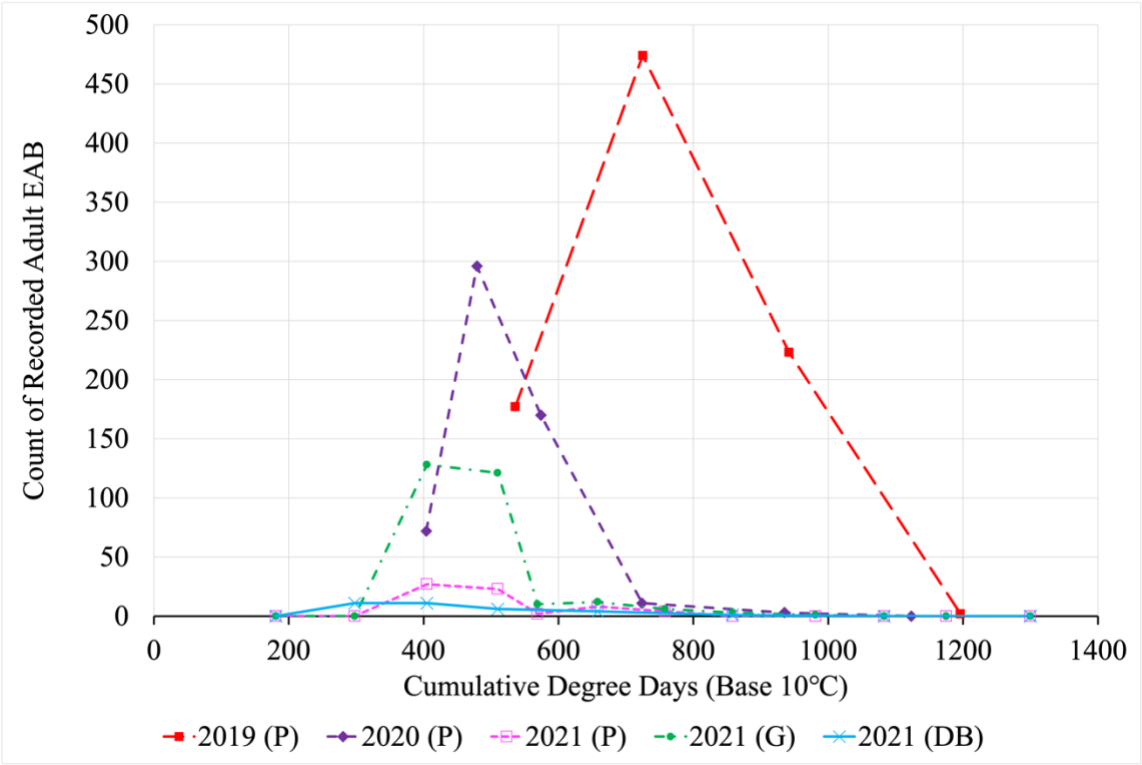

Figure 10. Counts of adult emerald ash borers (EAB) recorded from purple prism traps (P), green multifunnel traps (G), and debarked ash trees (DB) in Garner, North Carolina across degree day accumulations in 2019–2021.

DISCUSSION

Emerald Ash Borer Phenology and Voltinism

Based on year-round collections from green ash trees at the Wrenn Road Facility, EAB exhibited a univoltine life cycle in central North Carolina. In this life cycle, larvae begin feeding and forming galleries in the vascular tissue of host trees in early June (~900 DD_{10}) and feed throughout the summer and fall. By the end of October (~3,000 DD_{10}), nearly all larvae have developed sufficiently to cease feeding and excavate pupal chambers in which they overwinter as J-larvae/prepupae. An average of 1.1% (range = 0.0–6.2%) of EAB recorded from debarking were larvae in galleries (instars 1–4) from the end of October (~2,900–3,200 DD_{10}) through May (~700 DD_{10}) at this site. This very low proportion of overwintering non-J-larvae indicates that nearly all EAB develop through a univoltine life cycle, and it aligns well with the model of EAB development created by Gould et al. (2020) to predict the proportion of overwintering non-J-larvae in different climatic regions of the United States based on degree day accumulation. This model is based on observations of overwintering EAB life stages at latitudes ranging from ~33ºN (Louisiana) to nearly 47ºN (Minnesota and Wisconsin). The model estimates that ≤13% of EAB overwinter as non-J-larvae in areas predicted to accumulate more than 3,892 DD_{50} (~2,162 DD_{10}) between 1 January and 30 September (Gould et al. 2020). This climatic region corresponds to most of the Piedmont, Atlantic Coastal Plain, and Gulf Coastal Plain from southern Delaware through Texas and also aligns with the humid subtropical (Cfa) Köppen-Geiger climate class in southeastern North America (Beck et al. 2018, Gould et al. 2020). Garner, North Carolina accumulated ~5,221 and ~4,868 DD_{50} (~2,901 and ~2,704 DD_{10}) by 30 September 2019 and 2020, respectively. The equation developed by Gould et al. (2020) predicted that 0.5% and 1.3% of EAB would overwinter as non-J-larvae in winter 2019–2020 and 2020–2021, respectively,

based on these degree day accumulations for the proceeding growing season. The observed average proportions of overwintering non-J-larvae between the end of October and the start of the new generation of larvae in June were 1.3% in 2019–2020 and 1.0% in 2020–2021. Consequently, the EAB overwintering stages observed in central North Carolina support the prediction that EAB exhibits a predominantly univoltine life cycle in warm climates of the southeastern United States (Duan et al. 2018, Gould et al. 2020, Jones et al. 2020).

The development of EAB life stages recorded in this study differs from EAB development observed in similar studies in the northern United States. Jones et al. (2020) observed third and fourth instars feeding in galleries throughout their sampling period from May through October in central New York, while no parasitoid-susceptible larvae were detected before late June in central North Carolina (Figures 5, 7). New York also experiences two peaks in parasitoid-susceptible larvae during the growing season corresponding to semivoltine and univoltine development. Third and fourth instars developing in a univoltine life cycle appeared earlier in our study (late June; ~1,100–1,300 DD_{10}) than the beginning of the peak in univoltine parasitoid-susceptible larvae in New York (late August; ~1,200 DD_{10}), which correlates with the faster heat accumulation experienced in North Carolina (Jones et al. 2020; Figures 5, 7). The proportion of parasitoid-susceptible larvae present from June through August is higher in our study (72.3% of all stages) than from observations by Jones et al. (2020) in central New York (39.9% of all stages) and Quinn et al. (2022) in southeastern New York and Connecticut (51.2% of larvae). Conversely, the proportion of overwintering non-J-larvae in our study is lower than that estimated in central New York (≤25%) (Jones et al. 2020). These results suggest that the presence of parasitoid-susceptible larvae peaks and is heavily concentrated in the mid-summer

months (late June–August) in central North Carolina, while in the northern United States a larger proportion of these larvae are present both before and after this period.

The univoltine development of EAB in central North Carolina is more consistent with the southernmost samples of life stages reported in the United States. Abell et al. (2019) determined that EAB is univoltine near Washington, DC, with ~92% of specimens from debarked trees reaching the J-larval stage by late October. In western Virginia, Ragozzino et al. (2020) recorded mostly pupae and minimal observations of larvae in June, suggesting that few larvae overwintered in this location. Although EAB voltinism appears to be consistent between these locations and our study, the progression of instars during the summer may be more rapid in central North Carolina. In early August, >96% of larvae had advanced to the third or fourth instars in central North Carolina, while only ~40% of larvae reached these instars by early August near Washington, DC at similar DD_{10} (Abell et al. 2019). Most specimens had also not reached the third instar by mid-July in western Virginia, while ~78–90% of specimens were third or fourth instars in central North Carolina by this time (Ragozzino et al. 2020). These data suggest that differences in EAB developmental rates across the southern United States influence the temporal availability of parasitoid-susceptible larvae even if populations are consistently univoltine in the region.

The most comparable previous study of EAB life stages in North Carolina was conducted by Nalepa et al. (2021) based on once-yearly harvesting and debarking of EAB-infested ash trees in February in Granville County, which is located in the northern North Carolina Piedmont near the Virginia state line. The EAB life cycle observed in the Granville County study aligns with that observed ~60 km southeast in the present study in that only one of the 466 overwintering larvae collected in 2018 and 2019 was classified as a stage before the fourth instar based on

urogomphi length measurements. However, the Granville County study classified >90% of EAB from debarked trees as fourth-instar, non-J-larvae in early February (~78 DD_{50}) of 2018 and 2019. In contrast, <2% of EAB were observed in a stage before J-larvae at similar dates and DD_{50} in Garner in 2020 and 2021. This apparent discrepancy between studies is likely due to EAB in Granville County being assigned to a life stage primarily based on urogomphi length rather than location in galleries or pupal chambers. Urogomphi lengths of fourth-instar larvae, J-larvae, and prepupae overlap, so prepupae could be classified as fourth-instar larvae if they are not differentiated based on location or body length (Orlova-Bienkowskaja and Bieńkowski 2016, Nalepa et al. 2021). In discussion with the authors, it is likely that many overwintering stages measuring as fourth instars were most likely prepupae (Christine A. Nalepa, personal communication).

Emerald ash borer collections in central North Carolina also provide information about adult emergence and activity in the region. Degree day accumulation at actual emergence in this study (298 DD_{10} on 15 April 2021) was lower than that observed in central New York (~400–450 DD_{10}) (Jones et al. 2020). Adult collections from multifunnel traps in our study also occurred earlier than those from this trap type near Washington, DC. Multifunnel trap catch peaked at ~400 DD_{10} (late April to early May) and ceased after 981 DD_{10} (mid-June) in central North Carolina, while collections peaked at ~823 DD_{10} (early to mid-June) and ended after 1,174 DD_{10} (early July) near Washington, DC (Abell et al. 2019). Despite these differences, adult emergence recorded in central North Carolina (536 DD_{50}) aligns with degree day accumulations used by the USA National Phenology Network (450 DD_{50}), the Spatial Analytic Framework for Advanced Risk Information Systems (400 DD_{50}), and the Duarte 2013 OSU OIPMC model (550 DD_{50}) to generate emergence predictions across the United States (USPEST.ORG 2018, Herms

et al. 2019, SAFARIS 2021, USA-NPN 2022). As is the case with accelerated larval development, faster degree day accumulation in central North Carolina appears to result in adults emerging earlier in the year (by mid-April) than in more northern regions such as southern Indiana and Ohio (early May), Michigan (mid-May to early June), and central New York (June) (Cappaert et al. 2005, Herms et al. 2019, Jones et al. 2020).

The results of our study emphasize the complexity of the interaction between degree day accumulation and date in determining EAB development. As also observed by Nalepa et al. (2021) in the northern North Carolina Piedmont, early season degree day accumulation in North Carolina is highly variable between years. In our study, seasonal heat accumulation was 95 DD_{10} behind on 1 March and 127 DD_{10} behind on 1 April in 2021 compared to 2020. This difference in degree day accumulation is reflected in pupation being recorded a month later in 2021 (2 April) than in 2020 (3 March) (Figures 5, 7). Conversely, adult activity peaked during nearly the same dates (14–28 April 2020 and 15–30 April 2021) despite an average difference of 104 DD_{10} between years from 14–30 April. Interestingly, the sampling dates when third or fourth instars were first recorded (23 June) and peaked (7 July) were the same in both 2020 and 2021. The difference in degree day accumulation between years had decreased to 40 DD_{10} (23 June) and 66 DD_{10} (7 July) by these points in the summer, which could help explain the temporal consistency in parasitoid-susceptible larvae. The consistency in the dates of adult activity, and likely oviposition, between years could also realign the timing of parasitoid-susceptible larval development despite variation in pupal development related to degree days.

The relative importance of degree day accumulation or date in predicting the timing of EAB development across years varied between different life stages, and this pattern also occurs in the spotted lanternfly, *Lycorma delicatula* White (Hemiptera: Fulgoridae), another invasive

forest insect (Dechaine et al. 2021). Degree day calculations are commonly based on ambient air temperatures, and previous studies note that using this measure of heat accumulation to model forest insect development can be challenging. These studies consider that in forests, the actual temperatures experienced by insects are moderated by the canopy and, for borers such as EAB, the bark of trees (Gaylord et al. 2008, Vermunt et al. 2012, Hartshorn et al. 2016). Additional abiotic and biotic factors such as precipitation, humidity, and host resources may also influence the development of forest insect pests (Lantschner et al. 2019, Barker et al. 2020). In particular, host tree stress and high larval densities may accelerate EAB larval development, and these factors may play a role in the univoltine development exhibited by this insect in central North Carolina in addition to warm temperatures (Cappaert et al. 2005, Tluczek et al. 2011, Gould et al. 2020). While degree days serve as a valuable and accessible metric for modeling the phenology of forest pests, these observations indicate the potential limitations of predicting EAB development seasonally and in different climates based only on degree day input.

Previous research has focused on the cold tolerance of EAB at the northern extent of its non-native range (Sobek-Swant et al. 2011, Jones et al. 2017, Christianson and Venette 2018, Duell et al. 2022). Conversely, little is known about limitations to EAB development in warm climates that could determine the extent of its southern spread in the Americas. Based on laboratory study by Duan et al. (2021), development of EAB to adulthood may depend on larvae undergoing obligatory diapause at the end of the growing season. The study indicates that larvae must then experience a period of cool temperatures in fall or winter that terminates their diapause and allows them to complete adult development when warm temperatures return the next year. There is evidence that EAB mostly complete this diapause as J-larvae/prepupae in order to complete adult development, while few adults develop when larvae (instars 1–4) are exposed to

the same simulated overwintering conditions (Duan et al. 2021). The need for EAB to overwinter as J-larvae/prepupae before pupating is also supported by field studies (Cappaert et al. 2005, Wang et al. 2010, Jones et al. 2020). If EAB development does in fact require exposure to a period of cool temperatures, development could be limited in consistently warm, tropical climates if EAB were to spread farther south in the Americas. Duan et al. (2021) proposed exposure to temperatures at 12.8ºC for two months or more is needed to break diapause and allow development to continue. Overwintering EAB in Garner experienced maximum ambient temperatures at or below 12.8ºC for at most eight consecutive days in winter 2019–2020 and 14 consecutive days in winter 2020–2021 and were able to develop successfully. However, average temperatures throughout the day or minimum temperatures may be more relevant to a potential cool-temperature requirement than maximum temperatures. The characterization of the EAB life cycle in central North Carolina adds to the current knowledge of EAB development in warm climates, but further research on the minimum temperature and duration of a cool period needed to complete development, especially in field settings, is needed. The potential expansion of EAB southward beyond the United States should be of concern because EAB would likely encounter suitable host plants in these areas. For example, tropical ash (*Fraxinus uhdei* (Winzig) Lingelsh), a species native to Mexico and Guatemala and cultivated elsewhere in Central and South America, appears to be highly suitable for EAB larval development based on its use in laboratory rearing of EAB (Francis 1990, Filgueira et al. 2004, Bonfil 2010, Duan et al. 2013, Duan et al. 2014, Rojas-Rodríguez and Torres-Córdoba 2016, Duan et al. 2021). In this context, a deeper understanding of EAB phenology and climate-related limitations to development and survival is crucial to understanding risks to potential EAB host plants in warmer regions of the Americas and the ecological and economic values they provide.

Implications for Emerald Ash Borer Biological Control in the Southern United States

The characterization of EAB phenology and voltinism in central North Carolina has major implications for developing a successful biocontrol program in the region. The absence of overwintering larvae in galleries in regions where EAB is univoltine causes minimal availability of parasitoid-susceptible larvae between late fall and the development of new larvae the following summer. Rather than being distributed throughout the year, third and fourth instars are only available to parasitoids during a distinct window in the summer and fall (late June through October; ~1,100–3,000 DD_{10}) (Figures 5–7). As a result, introduced parasitoid wasps must have a life cycle in which emergence and oviposition aligns with this window in order to serve as effective biocontrol agents. Releases of the larval parasitoids *T. planipennisi* and *S. galinae* resulted in successful establishment in the northern United States, but these species emerge and begin attacking hosts in the spring when few susceptible larvae are available in central North Carolina (Bauer et al. 2014, Gould et al. 2020, Jones et al. 2020, Duan et al. 2022, Quinn et al. 2022). The adult female lifespans of *T. planipennisi* and *S. galinae* are about six and four to seven weeks, respectively, so female *T. planipennisi* and *S. galinae* that emerge at or before early to late May (~500–700 DD_{10}) in central North Carolina would be unlikely to encounter hosts that they could successfully exploit for reproduction during their lifespan (Duan et al. 2011, Duan et al. 2014, Ragozzino et al. 2020). The resulting phenological mismatch between parasitoid-susceptible larvae availability and parasitoid activity could make establishment of spring-emerging parasitoids difficult in southern regions where EAB is univoltine. Given the expense of rearing parasitoids and the growing demand for releases across the range of EAB in the United States, the apparently unsuitable conditions observed in our study may support the prioritization of *T. planipennisi* and *S. galinae* releases in colder regions with a greater probability of

successful establishment (Gould et al. 2020). However, establishment and spread of *T. planipennisi* and *S. galinae* occurred in Maryland where a univoltine host life cycle is expected to be common, which could indicate the suitability of these species further south than may be inferred based solely on climate and host voltinism (Jennings et al. 2016, Abell et al. 2019, Gould et al. 2020, Aker et al. 2022).

While *T. planipennisi* and *S. galinae* may be unsuitable for the southern United States, the larval parasitoid *S. agrili* may be more successful in this region. *Spathius agrili* was described and collected from Tianjin, China, which lies near the southern extent of the studied range of EAB in Asia (Yang et al. 2005). *Spathius agrili* is the most important EAB parasitoid in this location, where it has been responsible for parasitism levels of 30–50% and as high as 60–90% (Yang et al. 2005, Wang et al. 2010, Yang et al. 2010). Emerald ash borer exhibits a univoltine life cycle in Tianjin, which could indicate the suitability of *S. agrili* for regions of the southern United States with similar host voltinism (Wei et al. 2007, Wang et al. 2010, Jones et al. 2020). The percentage of EAB overwintering as larvae in galleries in Tianjin was recorded at 5–7% by Wei et al. (2007) and <1% by Wang et al. (2010), which aligns with the percentages observed in central North Carolina (mean = 1.1%, range = 0.0–6.2%). Larvae were observed in galleries from late May or early June through late October or early November in Tianjin, which also closely aligns with larval activity observed in our study (early June through October or early November) and likely indicates similar temporal availability of parasitoid-susceptible larvae (Wei et al. 2007, Wang et al. 2010). Differences in factors such as temperature and precipitation distributions between the climates of Tianjin (humid continental (Dwa)) and central North Carolina (humid subtropical (Cfa)) may influence the suitability of *S. agrili* between these locations (Beck et al. 2018). However, the univoltine life cycle we observed for EAB and its

similarity to the life cycle in Tianjin lends support to the potential success of *S. agrili* in the southern United States.

Comparing the phenology of *S. agrili* and parasitoid-susceptible larvae in central North Carolina further informs the suitability of *S. agrili* for this location. Jones et al. (2020) observed female *S. agrili* emergence at ~1,000–1,100 DD_{10} (late July to early August) in central New York, so *S. agrili* emergence would be well aligned with the appearance of parasitoid-susceptible larvae at ~1,100 DD_{10} (late June) in central North Carolina if degree day timing remains consistent (Figure 6). By defining the period when parasitoid-susceptible larvae are available, our results can also inform optimal release timings for *S. agrili* to exploit univoltine EAB populations in the southern United States. Release timing recommendations for *S. agrili* should consider that this species has a preoviposition period of ~10 days and that overwintering survival of immature *S. agrili* is poor if eggs are oviposited too late in the season (Yang et al. 2010, Jones et al. 2020). Abell et al. (2019) suggested that releases of larval parasitoids should be concentrated between 1,400 and 2,500 DD_{10} to match the univoltine EAB life cycle near Washington, DC. Based on *S. agrili* life history and the availability of parasitoid-susceptible larvae we observed, *S. agrili* releases in central North Carolina may be optimized between ~1,000 and ~2,300 DD_{10} (mid-to-late June to late August) (Figure 6).

By establishing the phenology and voltinism of EAB in the warm climate of central North Carolina, this study aids in determining the appropriate parasitoid species and release timings that align with the host life cycle in this region. Releases of *S. agrili* on a revised schedule informed by these results should be conducted to assess the efficacy of this parasitoid in the southern United States. In addition, these results could potentially inform biocontrol in more northern regions if EAB development were to shift toward a univoltine life cycle as cooler

regions warm with climate change (Gould et al. 2020, Jones et al. 2020). This study provides a foundational characterization of EAB development in the previously understudied southern United States, and future research should consider the climatic variability present within the region to strengthen management prescriptions for this forest pest. In particular, the southern Appalachian Mountains of western North Carolina have a much colder climate than lower-elevation regions of the Southeast. Climate-related differences in EAB development could have important implications for biocontrol, as the parasitoids *T. planipennisi* and *S. galinae* that have been successful in northern regions may be suitable for these colder, high-elevation areas. Together with other potential tools such as chemical treatments and resistance breeding, establishment of effective biocontrol agents in the southern United States offers hope for the future of ash trees across the region.

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REFERENCES

Abell, K. J., J. J. Duan, and P. M. Shrewsbury. 2019. Determining optimal parasitoid release timing for the biological control of emerald ash borer (Coleoptera: Buprestidae). The Florida Entomologist 102(4): 691–694.

Aker, S. A., R. B. de Andrade, J. J. Duan, and D. S. Gruner. 2022. Rapid spread of an introduced parasitoid for biological control of emerald ash borer (Coleoptera: Buprestidae) in Maryland. Journal of Economic Entomology 115: 381–386.

Barker, B. S., L. Coop, T. Wepprich, F. Grevstad, and G. Cook. 2020. DDRP: Real-time phenology and climatic suitability modeling of invasive insects. PLoS One 15(12): e0244005.

Bauer, L. S., R. A. Haack, D. L. Miller, T. R. Petrice, and H. Liu. 2004. Emerald ash borer life cycle, p. 8. *In* V. Mastro and R. Reardon (comps.), Emerald Ash Borer Research and Technology Development Meeting, 30 September–1 October 2003, Port Huron, MI. Publication FHTET-2004-02. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Bauer, L. S., J. J. Duan, and J. R. Gould. 2014. Emerald ash borer, pp. 189–209. *In* R. Van Driesche and R. Reardon (eds.). The Use of Classical Biological Control to Preserve Forests in North America. Publication FHTET-2013-2. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Bauer, L. S., J. J. Duan, J. R. Gould, and R. Van Driesche. 2015. Progress in the classical biological control of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America. Canadian Entomologist 147(3): 300–317.

Beck, H. E., N. E. Zimmermann, T. R. McVicar, N. Vergopolan, A. Berg, and E. F. Wood. 2018.

Present and future Köppen-Geiger climate classification maps at 1-km resolution. Scientific Data 5(1): 180214.

Bolen, A. 2020. A silent killer: Black ash basket makers are battling a voracious beetle to keep their heritage alive. https://www.americanindianmagazine.org/story/black-ash-basketry. Accessed 8 May 2022.

Bonfil, C. 2010. Present state, use, and perspectives of the genus *Fraxinus* in Mexico. 2010 Proceedings Symposium on Ash in North America. GTR-NRS-P-72. 1 p.

Cappaert, D., D. G. McCullough, T. M. Poland, and N. W. Siegert. 2005. Emerald ash borer in North America: A research and regulatory challenge. American Entomologist 51(3): 152–165.

Christianson, L. D. E., and R. C. Venette. 2018. Modest effects of host on the cold hardiness of emerald ash borer. Forests 9(6): 346.

City of Raleigh. 2022. Water & wastewater treatment plants. https://raleighnc.gov/services/content/PubUtilAdmin/Articles/TreatmentPlants.html. Accessed 25 Apr 2022.

Crimmins, T. M., K. L. Gerst, D. G. Huerta, R. L. Marsh, E. E. Posthumus, A. H. Rosemartin, J. Switzer, J. F. Weltzin, L. Coop, N. Dietschler, D. A. Herms, S. Limbu, R. T. Trotter, and M. Whitmore. 2020. Short-term forecasts of insect phenology inform pest management. Annals of the Entomological Society of America 113(2): 139–148.

Davidson, W., and L. K. Rieske. 2016. Establishment of classical biological control targeting emerald ash borer is facilitated by use of insecticides, with little effect on native arthropod communities. Biological Control 101: 78–86.

Dechaine, A. C., M. Sutphin, T. C. Leskey, S. M. Salom, T. P. Kuhar, and D. G. Pfeiffer. 2021.

Phenology of *Lycorma delicatula* (Hemiptera: Fulgoridae) in Virginia, USA. Environmental Entomology 50(6): 1267–1275.

Duan, J. J., C. B. Oppel, M. D. Ulyshen, L. S. Bauer, and J. Lelito. 2011. Biology and life history of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), a larval endoparasitoid of the emerald ash borer (Coleoptera: Buprestidae). The Florida Entomologist 94(4): 933–940.

Duan, J. J., K. Larson, T. Watt, J. Gould, and J. P. Lelito. 2013. Effects of host plant and larval density on intraspecific competition in larvae of the emerald ash borer (Coleoptera: Buprestidae). Environmental Entomology 42(6): 1193–1200.

Duan, J. J., T. J. Watt, and K. Larson. 2014. Biology, life history and laboratory rearing of *Spathius galinae*, a larval parasitoid of the invasive emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 107(3): 939–946.

Duan, J. J., L. S. Bauer, R. G. Van Driesche, and J. R. Gould. 2018. Progress and challenges of protecting North American ash trees from the emerald ash borer using biological control. Forests 9(3): 142.

Duan, J. J., J. M. Schmude, and K. M. Larson. 2021. Effects of low temperature exposure on diapause, development, and reproductive fitness of the emerald ash borer (Coleoptera: Buprestidae): Implications for voltinism and laboratory rearing. Journal of Economic Entomology 114(1): 201–208.

Duan, J. J., R. G. Van Driesche, J. Schmude, R. Crandall, C. Rutlege, N. Quinn, B. H. Slager, J. R. Gould and J. S. Elkinton. 2022. Significant suppression of invasive emerald ash borer by introduced parasitoids: Potential for North American ash recovery. Journal of Pest Science 95: 1081–1090.

Duell, M. E., M. T. Gray, A. D. Roe, C. J. K. MacQuarrie, and B. J. Sinclair. 2022. Plasticity drives extreme cold tolerance of emerald ash borer (*Agrilus planipennis*) during a polar vortex. Current Research in Insect Science 2: 100031.

Emerald Ash Borer Information Network. 2022. About emerald ash borer. http://www.emeraldashborer.info/about-eab.php. Accessed 25 Apr 2022.

Federal Register. 2020. Removal of emerald ash borer domestic quarantine regulations. 7 CFR Part 301, 85(241): 81085–81095. https://www.govinfo.gov/content/pkg/FR-2020-12-15/pdf/2020-26734.pdf. Accessed 30 Dec 2020.

Filgueira, J. J., L. Franco-Lara, J. E. Salcedo, S. L. Gaitan, and E. R. Boa. 2004. Urapan (*Fraxinus uhdei*) dieback, a new disease associated with a phytoplasma in Colombia. Plant Pathology 53(4): 520.

Flower, C. E., K. S. Knight, and M. A. Gonzalez-Meler. 2013. Impacts of the emerald ash borer (*Agrilus planipennis* Fairmaire) induced ash (*Fraxinus* spp.) mortality on forest carbon cycling and successional dynamics in the eastern United States. Biological Invasions 15(4): 931–944.

Francese, J. A., J. B. Oliver, I. Fraser, D. R. Lance, N. Youssef, A. J. Sawyer, and V. C. Mastro. 2008. Influence of trap placement and design on capture of the emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 101(6): 1831-1837.

Francese, J. A., I. Fraser, D. R. Lance, and V. C. Mastro. 2011. Efficacy of multifunnel traps for capturing emerald ash borer (Coleoptera: Buprestidae): Effect of color, glue, and other trap coatings. Journal of Economic Entomology 104(3): 901–908.

Francis, J. K. 1990. *Fraxinus uhdei* (Wenzig) Lingelsh. SO-ITF-SM-28. Rio Piedras, PR. U.S. Department of Agriculture, Forest Service, Institute of Tropical Forestry. 4 p.

Gaylord, M. L., K. K. Williams, R. W. Hofstetter, J. D. McMillin, T. E. DeGomez, and M. R. Wagner. 2008. Influence of temperature on spring flight initiation for southwestern ponderosa pine bark beetles (Coleoptera: Curculionidae, Scolytinae). Environmental Entomology 37(1): 57–69.

Ghezehei, S. B., S. D. Shifflett, D. W. Hazel, and E. G. Nichols. 2015. SRWC bioenergy productivity and economic feasibility on marginal lands. Journal of Environmental Management 160: 57–66.

Gould, J. R., M. L. Warden, B. H. Slager, and T. C. Murphy. 2020. Host overwintering phenology and climate change influence the establishment of *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), a larval parasitoid introduced for biocontrol of the emerald ash borer. Journal of Economic Entomology 113(6): 2641–2649.

Hartshorn, J. A., L. J. Haavik, J. D. Allison, J. R. Meeker, W. Johnson, L. D. Galligan, K. D. Chase, J. J. Riggins, and F. M. Stephen. 2016. Emergence of adult female *Sirex nigricornis* F. and *Sirex noctilio* F. (Hymenoptera: Siricidae) coincides with a decrease in daily minimum and maximum temperature. Agricultural and Forest Entomology 18(3): 206–213.

Herms, D. A. 2004. Using degree-days and plant phenology to predict pest activity, pp. 49–59. *In* V. Krischik and J. Davidson (eds.). IPM (Integrated Pest Management) of Midwest Landscapes. Publication SB-07645. Minnesota Agricultural Experiment Station, St. Paul, MN.

Herms, D. A., and D. G. McCullough. 2014. Emerald ash borer invasion of North America: History, biology, ecology, impacts, and management. Annual Review of Entomology 59(1): 13–30.

Herms, D. A., D. G. McCullough, C. S. Clifford, D. R. Smitley, F. D. Miller, and W. Cranshaw. 2019. Insecticide options for protecting ash trees from emerald ash borer. North Central IPM Center Bulletin. 3rd ed. 16 pp.

Jennings, D. E., J. J. Duan, D. Bean, J. R. Gould, K. A. Rice, and P. M. Shrewsbury. 2016. Monitoring the establishment and abundance of introduced parasitoids of emerald ash borer larvae in Maryland, U.S.A. Biological Control 101: 138–144.

Jones, M. I., J. R. Gould, and M. K. Fierke. 2017. Mortality of overwintering emerald ash borer (Coleoptera: Buprestidae) associated with an extreme cold event in New York, United States of America. Canadian Entomologist 149(4): 482–486.

Jones, M. I., J. R. Gould, H. J. Mahon, and M. K. Fierke. 2020. Phenology of emerald ash borer (Coleoptera: Buprestidae) and its introduced larval parasitoids in the northeastern United States. Journal of Economic Entomology 113(2): 622–632.

Koch, J. L., D. W. Carey, M. E. Mason, T. M. Poland, and K. S. Knight. 2015. Intraspecific variation in *Fraxinus pennsylvanica* responses to emerald ash borer (*Agrilus planipennis*). New Forests 46(5): 995–1011.

Kovacs, K. F., R. G. Haight, D. G. McCullough, R. J. Mercader, N. W. Siegert, and A. M. Liebhold. 2010. Cost of potential emerald ash borer damage in U.S. communities, 2009–2019. Ecological Economics 69(3): 569–578.

Lantschner, M. V., G. de la Vega, and J. C. Corley. 2019. Predicting the distribution of harmful species and their natural enemies in agricultural, livestock and forestry systems: an overview. International Journal of Pest Management 65(3): 190–206.

Liu, H., L. S. Bauer, D. L. Miller, T. Zhao, R. Gao, L. Song, Q. Luan, R. Jin, and C. Gao. 2007.

Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biological Control 42(1): 61–71.

MapBioControl. 2020. https://www.mapbiocontrol.org. Accessed 2 Apr 2022.

Nalepa, C. A., K. L. F. Oten, and M. A. Bertone. 2021. Overwintering developmental stages of emerald ash borer in North Carolina. The Florida Entomologist 104(3): 213–217.

Orlova-Bienkowskaja, M. J., and A. O. Bieńkowski. 2016. The life cycle of the emerald ash borer *Agrilus planipennis* in European Russia and comparisons with its life cycles in Asia and North America. Agricultural and Forest Entomology 18(2): 182–188.

Pike, C. C., J. Koch, and C. D. Nelson. 2021. Breeding for resistance to tree pests: Successes, challenges, and a guide to the future. Journal of Forestry 119(1): 96–105.

Poland, T. M., and D. G. McCullough. 2006. Emerald ash borer: Invasion of the urban forest and the threat to North America's ash resource. Journal of Forestry 104(3): 118–124.

Quinn, N. F., J. S. Gould, C. E. Rutledge, A. Fassler, J. S. Elkinton, and J. J. Duan. 2022. Spread and phenology of *Spathius galinae* and *Tetrastichus planipennisi*, recently introduced for biocontrol of emerald ash borer (Coleoptera: Buprestidae) in the northeastern United States. Biological Control 165: 104794.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ragozzino, M., R. Meyer, J. Duan, B. Slager, and S. Salom. 2020. Differences in early season emergence and reproductive activity between *Spathius agrili* (Hymenoptera: Braconidae) and *Spathius galinae*, larval parasitoids of the invasive emerald ash borer (Coleoptera: Buprestidae). Environmental Entomology 49(2): 334–341.

Rebek, E. J., D. A. Herms, and D. R. Smitley. 2008. Interspecific variation in resistance to emerald ash borer (Coleoptera: Buprestidae) among North American and Asian ash (*Fraxinus* spp.). Environmental Entomology 37(1): 242–246.

Rojas-Rodríguez, F., and G. Torres-Córdoba. 2016. Árboles del Valle Central de Costa Rica: reproducción Fresno (*Fraxinus uhdei* (Wenz.)). Revista Forestal Mesoamericana Kurú 14(34): 71–73.

(SAFARIS) Spatial Analytic Framework for Advanced Risk Information Systems. 2021. Weekly degree day phenology maps. https://safaris.cipm.info/safarispestmodel/ StartupServlet?fieldops. Accessed 10 Apr 2022.

Sobek-Swant, S., J. C. Crosthwaite, D. B. Lyons, and B. J. Sinclair. 2011. Could phenotypic plasticity limit an invasive species? Incomplete reversibility of mid-winter deacclimation in emerald ash borer. Biological Invasions 14(1): 115–125.

Siegert, N. W., D. G. McCullough, A. M. Liebhold, and F. W. Telewski. 2014. Dendrochronological reconstruction of the epicentre and early spread of emerald ash borer in North America. Diversity & Distributions 20(7): 847–858.

Stephens, J. P., K. A. Berven, and S. D. Tiegs. 2013. Anthropogenic changes to leaf litter input affect the fitness of a larval amphibian. Freshwater Biology 58(8): 1631–1646.

Tobin, P. C., B. L. Strom, J. A. Francese, D. A. Herms, D. G. McCullough, T. M. Poland, K. L. Ryall, T. Scarr, P. J. Silk, and H. W. Thistle. 2021. Evaluation of trapping schemes to detect emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 114(3): 1201–1210.

Tluczek, A. R., D. G. McCullough, and T. M. Poland. 2011. Influence of host stress on emerald

ash borer (Coleoptera: Buprestidae) adult density, development, and distribution in *Fraxinus pennsylvanica* trees. Environmental Entomology 40(2): 357–366.

(USA-NPN) USA National Phenology Network. 2022. Emerald ash borer forecast. https://www.usanpn.org/data/forecasts/EAB. Accessed 10 Apr 2022.

USDA–APHIS/ARS/FS. 2020. Emerald ash borer biological control release and recovery guidelines. USDA–APHIS–ARS–FS, Riverdale, MD.

USPEST.ORG. 2018. Online phenology and degree-day models for agricultural and pest management decision making in the US. https://uspest.org/dd/model_app. Accessed 10 Apr 2022.

Vermunt, B., K. Cuddington, S. Sobek-Swant, J. C. Crosthwaite, D. B. Lyons, and B. J. Sinclair. 2012. Temperatures experienced by wood-boring beetles in the under-bark microclimate. Forest Ecology and Management 269: 149–157.

Villari, C., D. A. Herms, J. G. A. Whitehill, D. Cipollini, and P. Bonello. 2016. Progress and gaps in understanding mechanisms of ash tree resistance to emerald ash borer, a model for wood-boring insects that kill angiosperms. The New Phytologist 209(1): 63–79.

Wagner, D. L., and K. J. Todd. 2016. New ecological assessment for the emerald ash borer: A cautionary tale about unvetted host-plant literature. American Entomologist 62(1): 26–35.

Wang, X.-Y., Z.-Q. Yang, J. R. Gould, Y.-N. Zhang, G.-J. Liu, and E.-S. Liu. 2010. The biology and ecology of the emerald ash borer, *Agrilus planipennis*, in China. Journal of Insect Science 10(128): 1–23.

Wei, X., Y. Wu, R. Reardon, T.-H. Sun, M. Lu, and J.-H. Sun. 2007. Biology and damage traits of emerald ash borer (*Agrilus planipennis* Fairmaire) in China. Insect Science 14(5): 367–373.

Yang, Z.-Q., J. S. Strazanac, P. M. Marsh, C. van Achterberg, and W.-Y. Choi. 2005. First recorded parasitoid from China of *Agrilus planipennis*: A new species of *Spathius* (Hymenoptera: Braconidae: Doryctinae). Annals of the Entomological Society of America 98(5): 636–642.

Yang, Z.-Q., X.-Y. Wang, J. R. Gould, R. C. Reardon, Y.-N. Zhang, G.-J. Liu, and E.-S. Liu. 2010. Biology and behavior of *Spathius agrili*, a parasitoid of the emerald ash borer, *Agrilus planipennis*, in China. Journal of Insect Science 10(30): 1–13.

CHAPTER 3

Release and Recovery of Emerald Ash Borer Parasitoids in Central North Carolina

ABSTRACT

In the United States, classical biological control is the primary approach pursued for long-term management of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an invasive forest pest that has killed millions of ash (*Fraxinus* spp.) trees across eastern and central North America. This biocontrol program involves introductions of parasitoid wasps that attack EAB, including the larval parasitoid *Spathius agrili* Yang (Hymenoptera: Braconidae). Establishment of *S. agrili* has been poor in the northern United States, and establishment of all parasitoids is limited in the Southeast. *Spathius agrili* releases and parasitoid recovery efforts were conducted in EAB-infested green ash (*Fraxinus pennsylvanica* Marshall) stands in Garner, North Carolina to assess the ability of *S. agrili* to establish in southern regions and identify existing species of natural enemies. *Spathius agrili* was released in 2019 and 2020, and recovery was attempted in 2021 using felled ash trees in emergence cages, yellow pan traps, and sentinel ash bolts containing EAB larvae. *Spathius agrili* was not detected using any recovery method. Establishment was likely hindered by temporal mismatches between release timings and the susceptibility of EAB to parasitism, the limited number of releases related to the COVID-19 pandemic, and acute declines in EAB and living ash trees. In the future, more numerous releases of *S. agrili* with timings that better align with the seasonal availability of parasitoid-susceptible larvae in univoltine EAB populations should increase the potential for *S. agrili* establishment in the Southeast. The recovery efforts identified a diverse community of native or naturalized natural enemies, including the confirmed EAB parasitoids *Atanycolus* sp.

(Hymenoptera: Braconidae) and *Balcha indica* Mani and Kaul (Hymenoptera: Eupelmidae) and the native *Spathius elegans* Matthews (Hymenoptera: Braconidae).

INTRODUCTION

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is a devastating non-native insect impacting North American forests. Accidentally introduced from its native range in eastern Asia, EAB has likely been present in North America since the 1990s and was first detected in the area of Detroit, Michigan, United States and Windsor, Ontario, Canada in 2002 (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006, Siegert et al. 2014). This beetle has since been documented attacking ash (*Fraxinus* spp.) trees in 35 states of the United States and five provinces of Canada across the eastern and central portions of the continent (Emerald Ash Borer Information Network 2022). Emerald ash borer larvae feed in the vascular tissue of their host plant, cutting off the flow of nutrients and water in the tree as they tunnel through the phloem, cambium, and outer xylem (Cappaert et al. 2005, Wang et al. 2010, Villari et al. 2016).

Unlike the beetle's ash hosts in its native range, North American ash species do not share a coevolutionary history with EAB and possess poor resistance against EAB attack (Liu et al. 2007, Rebek et al. 2008, Villari et al. 2016). These factors leave North American ash highly susceptible to EAB, and millions of ash trees have been killed as EAB expands its non-native range. The loss of ash has far-reaching consequences for the ecosystem functions and biodiversity of North American forests (Flower et al. 2013b, Stephens et al. 2013, Wagner and Todd 2016). The death of urban ash trees is projected to cost many billions of dollars, and the cultural values of ash are incalculable and irreplaceable (Poland and McCullough 2006, Kovacs et al. 2010, Herms and McCullough 2014, Bolen 2020).

Management efforts to limit the spread and impact of EAB in North American forests are challenging. Regulations on the movement of plant material have had limited success, and systemic insecticide treatments are effective at protecting individual trees but impractical for long-term control at a landscape scale (Davidson and Rieske 2016, Herms et al. 2019, Federal Register 2020). In pursuit of sustainable suppression of outbreaks, classical biological control has been adopted as a focus of EAB management in the United States. This biocontrol program is led by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) and consists of the introduction of natural enemies that attack the EAB in its native range in Asia. These natural enemies include the egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) and the larval parasitoids *Spathius agrili* Yang (Hymenoptera: Braconidae), *Spathius galinae* Belokobylskij and Strazanac (Hymenoptera: Braconidae), and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Bauer et al. 2015, Duan et al. 2018).

In the northern United States, *T. planipennisi* and *S. galinae* have successfully established, dispersed, and are associated with suppression of EAB larval densities (Duan et al. 2022, Quinn et al. 2022). Conversely, establishment of *S. agrili* has been poor in the Northeast and Midwest, and establishment of all introduced parasitoids is limited in the Southeast (Duan et al. 2018, Jones et al. 2020, MapBioControl 2020). *Spathius agrili* was identified as the most important parasitoid of EAB in the warmer, southern extent of the known EAB native range in Asia where EAB is univoltine (Yang et al. 2005, Wang et al. 2010, Yang et al. 2010). As a result, *S. agrili* appears to be best adapted to exploiting EAB in warm regions where EAB is univoltine rather than colder, northern regions where many EAB may take two years to develop (Jones et al. 2020, USDA–APHIS/ARS/FS 2020). Emerald ash borer populations are predicted to be

univoltine across most of the southeastern United States, and *S. agrili* releases in North America are now conducted only at locations below 40ºN latitude (approximately the southern border of Pennsylvania) (Abell et al. 2019, Gould et al. 2020, USDA–APHIS/ARS/FS 2020).

Spathius agrili has been recovered from the southernmost sites studied in published research, but widespread establishment has not occurred. Previous release and recovery efforts have recovered a single adult *S. agrili* in eastern Tennessee and a small number of *S. agrili* in a study across Maryland. However, these recoveries have been almost entirely limited to the year after parasitoid releases (Hooie et al. 2015, Jennings et al. 2016a). More recently, *S. agrili* recoveries were reported later than one year after final releases in northern and central Virginia and eastern North Carolina (Ragozzino 2020). Further research on the release and establishment of *S. agrili* in the southern United States is critical to the development of a successful EAB biocontrol program for this region (Duan et al. 2018, Jones et al. 2020).

In addition to the introduced parasitoid species, a diverse assemblage of natural enemies already present in North America exploit EAB in its non-native range. In particular, insectivorous birds and a suite of native and naturalized parasitoid wasps are known to attack EAB larvae (Bauer et al. 2015). Woodpeckers are often the largest cause of immature EAB mortality in North America (Cappeart et al. 2005, Duan et al. 2010, Jennings et al. 2013, Duan et al. 2015). While the impact of woodpeckers can be highly variable between sites, larval mortality often averages ~30% with a maximum of ~50–90% (Duan et al. 2012, Jennings et al. 2013, Duan et al. 2015, Jennings et al. 2016b, Duan et al. 2019). Native larval parasitoids, particularly *Atanycolus* spp. (Hymenoptera: Braconidae) and *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), are also responsible for high levels of EAB parasitism in the northern United States (Duan et al. 2014a, Duan et al. 2015). Although levels of parasitism and

predation by existing, generalist natural enemies have clearly been unable to prevent EAB outbreaks in North America acting alone, natural control by these species can reduce EAB population growth rates and supplement active management approaches such as classical biocontrol with additional mortality (Duan et al. 2014a, Bauer et al. 2015, Duan et al. 2015). The use of native parasitoid species for augmentation biocontrol is also being considered as an additional EAB management tactic (Hooie et al. 2015, Gaudon and Smith 2020). Characterizing the parasitoid and predator communities associated with EAB across different regions of North America will thus provide a better understanding of the potential contributions of these native and naturalized natural enemies to EAB suppression.

The objectives of this study were to assess the establishment of the introduced parasitoid *S. agrili* and identify the native and naturalized natural enemies associated with EAB in stands of ash trees in the eastern Piedmont of North Carolina. These efforts were designed to evaluate the suitability and effectiveness of *S. agrili* as an EAB biocontrol agent for the southern United States and characterize the community of existing natural enemies exploiting EAB infestations in the region.

MATERIALS AND METHODS

Site Description

This research was conducted at the Wrenn Road Facility in Garner, North Carolina (35.6437, -78.5802). This site is more than 240 ha in size and consists of grass fields and planted stands of trees, including green ash (*Fraxinus pennsylvanica* Marshall), loblolly pine (*Pinus taeda* L.), sycamore (*Platanus occidentalis* L.), sweetgum (*Liquidambar styraciflua* L.), baldcypress (*Taxodium distichum* (L.) Rich.), and poplar (*Populus* sp.). The site was used for land application treatment of municipal primary wastewater until 2008 (Ghezehei et al. 2015,

City of Raleigh 2022). Since that time, land use has been converted to reclaimed water management, and the stands have continued to be irrigated by a system of sprinklers at a rate of ~1,368 mm per year (Ghezehei et al. 2015).

Figure 1. Green ash stands at the Wrenn Road Facility (Garner, North Carolina).

Parasitoid release and recovery efforts were conducted in two stands (Stands 2 and 3) of planted green ash at the facility (Figure 1). Stands 2 and 3 were ~4.5 ha and ~4.8 ha in area, respectively. These even-aged ash stands were established in 1993 at a density of 1,359 trees per ha (550 trees per acre) (Ghezehei et al. 2015). The genetic source of the stands is a diverse collection of green ash seed from the North Carolina Piedmont. Stands 2 and 3 were nearly pure green ash stands with few scattered loblolly pines and sycamores in the canopy. Green ash in these stands had experienced ~90% mortality from EAB as of 26 July 2021, 28 years after their planting (Appendix A). The ground cover of each stand was dominated by a thick layer of

stiltgrass (*Microstegium vimineum* (Trin.) A. Camus) during the growing season. The understories of the stands were composed primarily of boxelder (*Acer negundo* L.), dogfennel (*Eupatorium capillifolium* (Lam.) Small), privet (*Ligustrum sinense* Lour.), eastern redcedar (*Juniperus virginiana* L.), and limited green ash regeneration. The understory of Stand 2 was sparse, while Stand 3 had a much thicker understory dominated by boxelder and dogfennel. Other documented understory species included sea-myrtle (*Baccharis halimifolia* L.), waxmyrtle (*Morella cerifera* (L.) Small), sweetgum, oaks (*Quercus* spp.), and *Melia azedarach* L.

Parasitoid Releases

Adult female and male *S. agrili* were released in Stands 2 and 3 during the summers of 2019 and 2020. The parasitoids were produced and supplied by the USDA EAB Parasitoid Rearing Facility in Brighton, Michigan and shipped overnight in plastic cups provisioned with honey. *Spathius agrili* were released in the interior of each stand by opening a cup and gently tapping it against an ash tree with a live canopy and symptoms of current EAB infestation (e.g. recent excavation by woodpeckers, live epicormic sprouts) until all parasitoids had exited (USDA–APHIS/ARS/FS 2020). The sex ratio and total number of *S. agrili* were divided as equally as possible between the two stands during each release. A total of 1,037 adult female *S. agrili* were released between 23 August and 20 September 2019, and 690 females were released between 4 August and 18 September 2020 (Table 1). Fewer releases were able to be conducted in 2020 due to reduced parasitoid production related to the COVID-19 pandemic.

Table 1. Releases of the emerald ash borer parasitoid *Spathius agrili* in Stands 2 and 3 in Garner, North Carolina (MapBioControl 2020). DD_{10} refers to the degree day accumulation (starting 1 January, base 10ºC) at the date of release.

Date of release	**DD_{10} at release**	**No. female *S. agrili* released**	**No. total *S. agrili* released**
23 August 2019	2,331	205	295
30 August 2019	2,424	205	306
7 September 2019	2,551	206	279
13 September 2019	2,654	215	281
20 September 2019	2,743	206	269
4 August 2020	1,887	239	not reported
14 August 2020	2,069	245	311
18 September 2020	2,604	206	388

Parasitoid Recovery

Tree Felling and Emergence Cages

Collections of parasitoids in Stands 2 and 3 were conducted using three recovery methods in 2021. The first of these methods involved felling EAB-infested ash trees and placing log sections into emergence cages. Two ash trees were felled in each of Stands 2 and 3 on 14 June 2021. Felled trees were between 17.5 and 23.1 cm in diameter at breast height (~1.37 m; DBH), had live phloem, and showed symptoms of current EAB infestation such as woodpecker activity, epicormic sprouting, bark splitting, and canopy dieback. After felling, the trees were cut into ~60 cm sections. Logs were labeled with a letter representing the individual tree (Trees A and B in Stand 2, Trees C and D in Stand 3) and numbered from the lowest to highest position in the tree's stem. Logs were transported to the North Carolina Department of Agriculture and

Consumer Services Beneficial Insects Laboratory in Cary, North Carolina on the same day they were harvested. After the cut ends of the log sections were covered with parafilm to reduce moisture loss, the logs were loaded into emergence cages. Each emergence cage was a 61 cm L × 61 cm W × 49 cm H clear Plexiglass box with mesh vents on the sides and top and a tied cloth on the front to allow access inside (Figure 2, Nalepa et al. 2021). Logs from different trees were kept in separate cages, and one to four logs were placed in each cage. The room temperature was kept at 22–25ºC. Emergence cages were monitored every one to two days from 14 June until 16 August 2021. Any potential parasitoids that emerged from the log sections were collected and placed in labeled vials containing ethanol. Representative specimens of longhorned beetles (Coleoptera: Cerambycidae) and bark and ambrosia beetles (Coleoptera: Curculionidae) that emerged from the logs were also collected and are reported in Appendix B.

Figure 2. Emergence cages containing logs from felled green ash trees.

Yellow Pan Traps

Yellow pan traps were also deployed in 2021 to recover EAB parasitoids. Traps were constructed and operated according to USDA program guidelines (USDA–APHIS/ARS/FS 2020). Traps consisted of two stacked yellow plastic bowls secured to each other with binder clips (Figure 3A). A row of six holes was punched under the lip of the top bowl to prevent overflow, and a piece of fine mesh was glued over these holes to prevent trap contents from spilling out. Two large holes were cut in the bottom bowl to allow drainage, and the bowl was attached to a metal shelf bracket with zip ties. The bracket was then mounted to the trunk of an ash tree using wood screws. The top bowl was filled with a 20% solution of clear propylene glycol as a preservative and a drop of clear, unscented dish soap to eliminate surface tension.

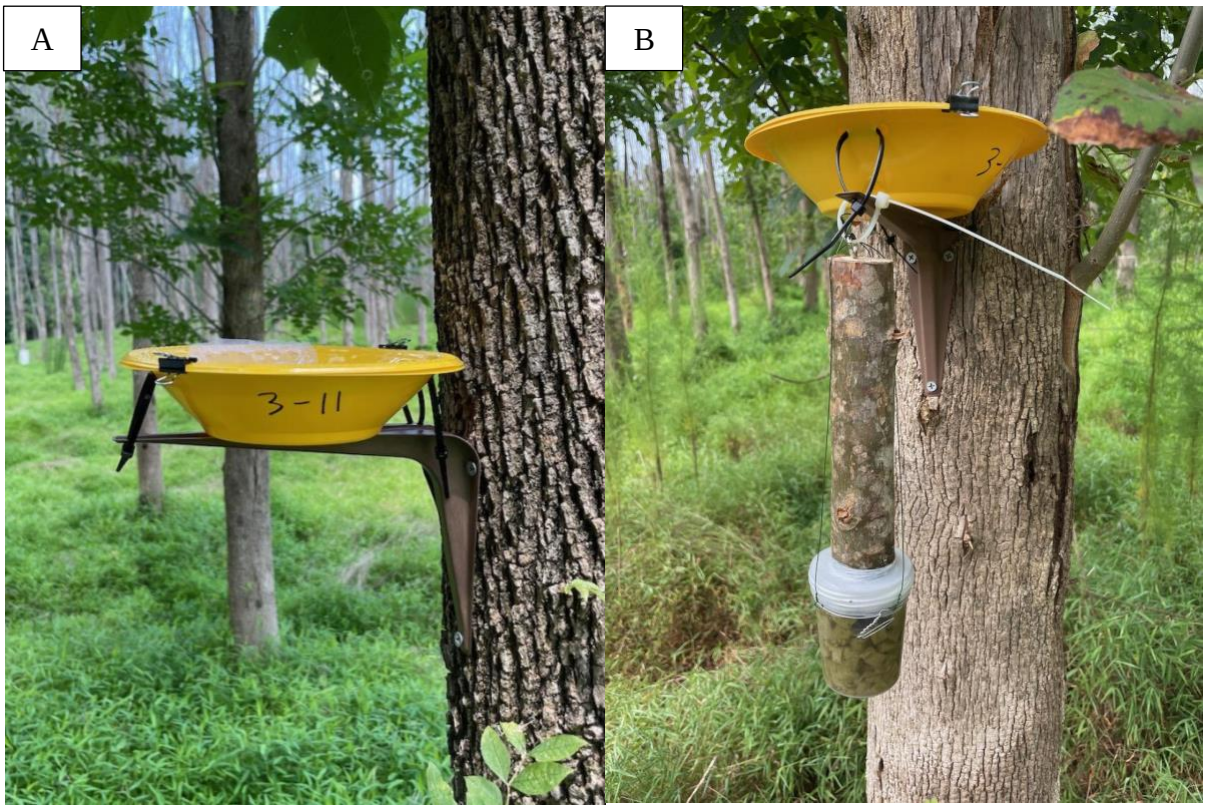

Figure 3. (A) Yellow pan trap; (B) Sentinel bolt hung from a yellow pan trap.

Fifteen traps were installed in each of Stands 2 and 3 on 15 July 2021, and each trap was labeled with an ID number corresponding to the stand number and individual trap number within each stand. Traps were placed on trees that appeared to be both alive and infested with EAB, as evidenced by recent woodpecker activity, live epicormic sprouts, and a canopy class of 3 or 4. Canopy classes were rated using a five-point scale of canopy health, with a rating of 1 indicating a full, healthy canopy and a rating of 5 indicating a completely dead canopy with no foliage (Smith 2006, Flower et al. 2013a, Knight et al. 2014, Appendix A). The DBH of selected trees ranged from 16.3 to 30.0 cm with a mean of 23.1 cm. Traps were mounted ~1.5 m above the ground on the south, southwest, or west side of the tree to increase sunlight exposure. Traps were distributed as evenly within stands as possible, although this was constrained by the lack of living trees, particularly in the interior of Stand 2.

Yellow pan traps were checked weekly through 28 September 2021. During the checks, the top bowl was removed and emptied into a 190-micron paint filter, and the filter containing the trap contents was placed into a zipper bag. Both the filter and bag were labeled with the trap ID number and collection date. The top bowl was then replaced and refilled. After accounting for six samples lost due to trap damage, 324 trap samples were collected from the 30 traps over 11 weeks of trapping. Trap samples were taken to North Carolina State University (NCSU), where they were examined under a microscope. Samples were processed on the day of field collection or were frozen for later processing if necessary. All parasitoids found in the samples that resembled *Spathius* spp. and other species confirmed to be EAB parasitoids in the existing literature were placed in labeled vials containing 95% ethanol. Representative specimens of parasitoid wasp species that did not resemble known EAB parasitoids were also preserved. Hymenopteran specimens resembling *Spathius* spp. were photographed and further identified at

the NCSU Plant Disease and Insect Clinic. Photographs of these specimens were also sent to braconid specialists for further identification. In addition, representative specimens of wood-boring insects other than EAB that were caught in the traps were collected and placed in vials containing ethanol. These specimens were later identified and are reported in Appendix B.

Sentinel Bolts

Sentinel bolts, a method for recovering larval parasitoids of EAB, were also used in 2021. Bolts were deployed at the Garner site as part of a larger parasitoid recovery effort using sentinel bolts described in Chapter 4. Sentinel bolts were made and supplied by the USDA and were constructed similarly to descriptions by Rutledge et al. (2021) and Quinn et al. (2022). Briefly, sentinel bolts are cut tropical ash (*Fraxinus uhdei* (Winzig) Lingelsh) logs measuring ~3–4 cm in diameter and ~20 cm in length (Figure 3B). Each bolt is artificially infested with EAB by inserting EAB larvae under flaps cut in the bark. A cup containing moist rock wool is attached to the bottom end of the bolt to prevent desiccation, and a metal eye hook is screwed into the top end for hanging the bolt (Figure 3B).

One sentinel bolt was deployed in each of Stands 2 and 3 on 4 August 2021, and these bolts were retrieved on 25 August 2021. A new bolt was deployed in each stand on 1 September 2021 and was retrieved on 21 September 2021. Bolts were hung from the shelf bracket of a yellow pan trap near the center of each stand using a zip tie (Figure 3B). After retrieval from the field, each bolt was placed into a separate emergence tube to monitor for parasitoid emergence. An emergence tube consisted of a sealed cardboard tube with a clear glass vial inserted in a hole in the side of the tube near the top (Figure 4A). This vial could be removed to collect insects inside. The tubes were kept indoors and were placed with the vial facing toward a window to provide an attractive light source. The first and second rounds of bolts remained in emergence

tubes from 25 August to 21 September 2021 and 21 September to 21 October 2021, respectively. The tubes were checked for parasitoid emergence several times per week over these durations (first round: 12 checks, second round: 13 checks). After the end of the monitoring periods, bolts were removed from the emergence tubes. The tubes were examined for any emerged insects that had not entered the clear vials. The removed bolts were debarked with a drawknife to determine whether each EAB larva was alive, parasitized, or was dead from another cause (Figure 4B). Emerald ash borer in pupal chambers were excavated using a chisel and hammer. The first and second rounds of bolts were debarked on 23 September and 23 October 2021, respectively (one month after collection from the field).

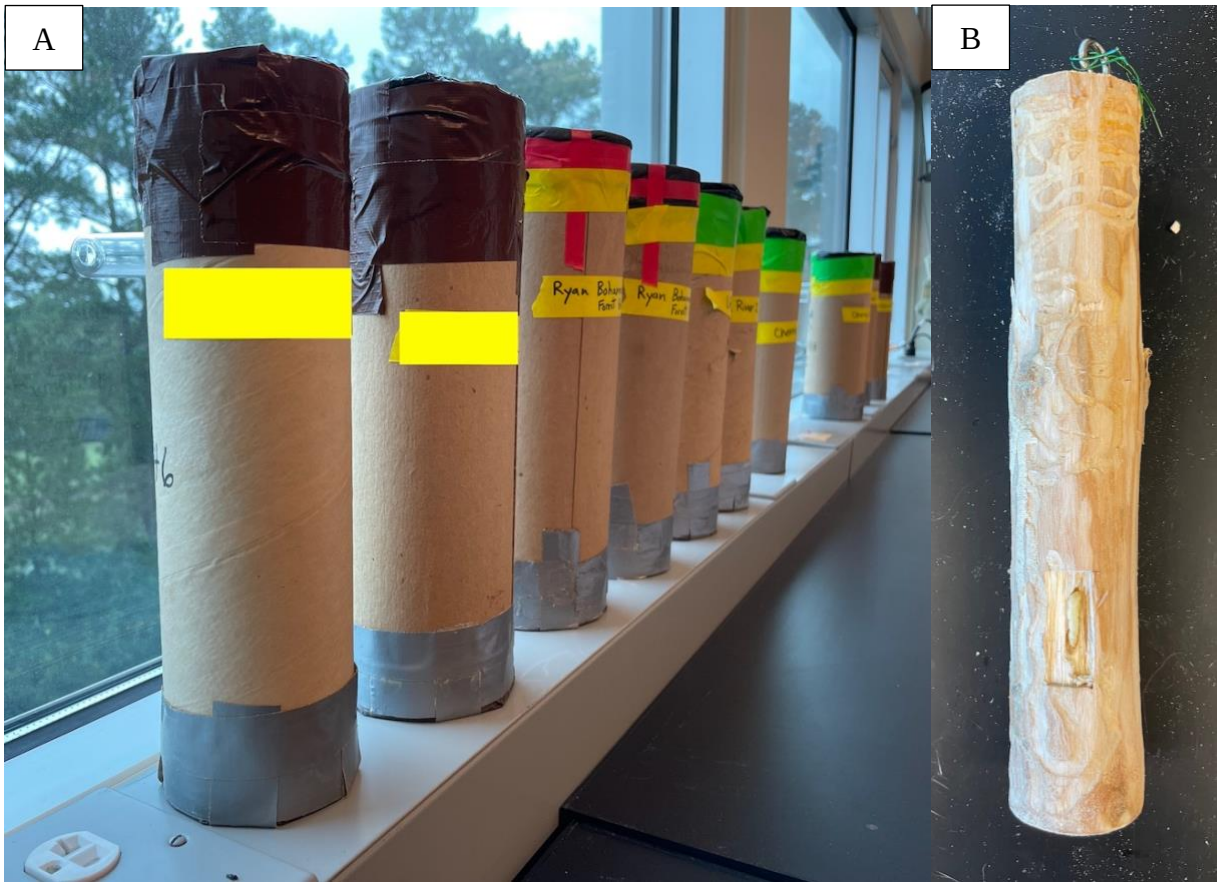

Figure 4. (A) Emergence tubes containing sentinel bolts; (B) Debarked sentinel bolt showing an excavated J-shaped emerald ash borer larva in a pupal chamber.

Degree Day Calculation

Seasonal heat accumulation throughout each year of this study was quantified based on degree days, a measure of heat accumulation commonly used to describe and predict the development and activity of insects in agriculture and forestry (Herms 2004, Barker et al. 2020, Crimmins et al. 2020). Degree day accumulation for sampling dates was calculated using the Duarte 2013 OSU OIPMC model (USPEST.ORG 2018). This model was designed for EAB and calculates cumulative degree days (DD_{10}) beginning 1 January using a single sine method, a lower threshold of 10°C, and an upper threshold of 37.8°C. The calculations were run based on daily maximum and minimum temperatures recorded at Station E1728 (35.6450, -78.5444), which is located ~3 km east of the research site.

RESULTS

Tree Felling and Emergence Cages

The released parasitoid species, *Spathius agrili*, was not recovered from the felled ash logs placed in emergence cages. Two specimens of *Balcha indica* Mani and Kaul (Hymenoptera: Eupelmidae) were recovered from Tree D from Stand 3 (Figure 5B). These two specimens were collected from the same emergence cage (Cage 67) on 17 and 29 June (Table 2).

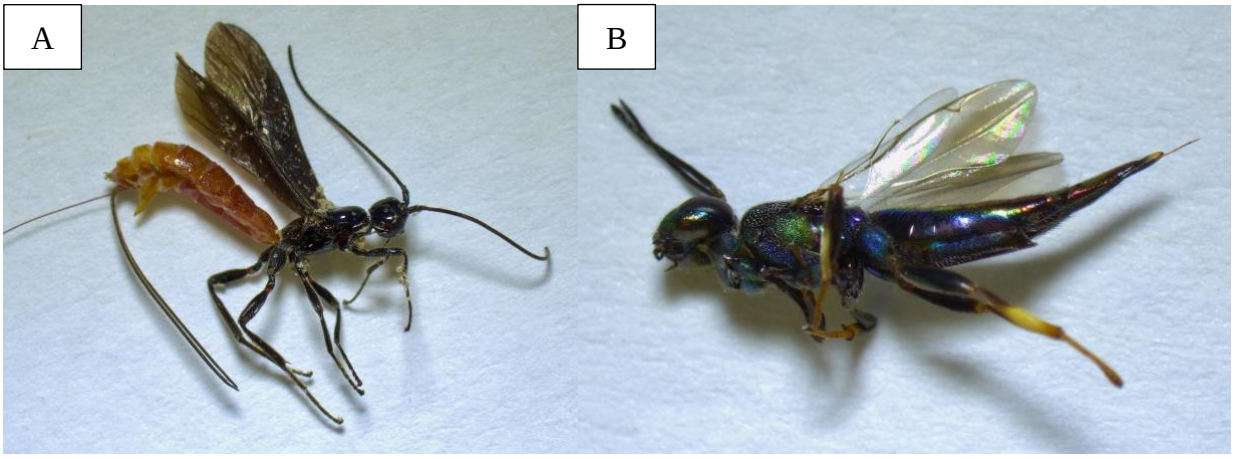

Figure 5. (A) *Atanycolus* sp. (Hymenoptera: Braconidae); (B) *Balcha indica* Mani and Kaul (Hymenoptera: Eupelmidae).

Yellow Pan Traps

Spathius agrili was also not recovered from the yellow pan traps. However, a variety of native or naturalized parasitoids were collected in these traps (Table 2). The most notable potential EAB parasitoids recovered were *Atanycolus* sp., of which 14 specimens were collected over the course of the summer across both stands (Figure 5A). One female *Atanycolus* sp. was recovered on 21 July, and two females were recovered on 28 July. Interestingly, no *Atanycolus* sp. were recovered in August, while 11 specimens were recovered in September. Three males were collected on 7 September, two females and four males were collected on 21 September, and two males were recovered on 28 September.

Three specimens resembling *Spathius* spp. were recovered from yellow pan traps (Table 2). One specimen, recovered from Stand 3 on 17 August, was identified as *Spathius elegans* Matthews (Hymenoptera: Braconidae). The remaining specimens were *Callihormius bifasciatus* Ashmead (Hymenoptera: Braconidae) and an unknown doryctine wasp (Hymenoptera: Braconidae) currently being identified by braconid specialists for full identification. Additional parasitoids of wood-boring insects collected and identified from yellow pan traps include *Agonocryptus discoidaloides* Viereck (Hymenoptera: Ichneumonidae).

Sentinel Bolts

No insects emerged from the sentinel bolts placed in emergence tubes. Excavation of the EAB larvae inside the bolts also did not reveal evidence of parasitism. Debarking of the first round of bolts on 23 September recovered three EAB larvae from the bolt in Stand 2 and four larvae from the bolt in Stand 3. Four EAB larvae were recovered from each bolt in the second round on 23 October. At the time of debarking, every EAB larva in the bolts was a J-shaped mature larva (J-larva) in a pupal chamber. All larvae were alive and apparently healthy.

Table 2. Potential emerald ash borer parasitoids recovered from emergence cages (EC) and yellow pan traps (YPT) in Garner, North Carolina in 2021. DD_{10} refers to the degree day accumulation (starting 1 January, base 10ºC) at the date of collection.

Species	Sex	Method	Location	Date Collected	DD_{10}
Balcha indica	female	EC	Stand 3, Tree D	17 June	-
Balcha indica	female	EC	Stand 3, Tree D	29 June	-
Atanycolus sp.	female	YPT	Stand 3, Trap 10	21 July	1,541
Atanycolus sp.	female	YPT	Stand 2, Trap 7	28 July	1,662
Atanycolus sp.	female		Stand 2, Trap 9		
unknown doryctine	female	YPT	Stand 2, Trap 6	9 August	1,855
Spathius elegans	female	YPT	Stand 3, Trap 5	17 August	2,002
Atanycolus sp.	male	YPT	Stand 2, Trap 9	7 September	2,357
Atanycolus sp.	male		Stand 3, Trap 2		
Atanycolus sp.	male		Stand 3, Trap 2		
Atanycolus sp.	male	YPT	Stand 2, Trap 6	21 September	2,561
Atanycolus sp.	male		Stand 2, Trap 6		
Atanycolus sp.	male		Stand 2, Trap 14		
Atanycolus sp.	female		Stand 3, Trap 4		
Atanycolus sp.	male		Stand 3, Trap 7		
Atanycolus sp.	female		Stand 3, Trap 15		
Callihormius bifasciatus	female		Stand 2, Trap 1		
Atanycolus sp.	male	YPT	Stand 3, Trap 3	28 September	2,633
Atanycolus sp.	male		Stand 3, Trap 3		

DISCUSSION

Evaluation of Release and Recovery Methods for *Spathius agrili*

The introduced parasitoid *S. agrili* was not detected in EAB-infested ash stands in Garner, North Carolina using any recovery method during the summer of 2021 following two years of releases. The lack of *S. agrili* establishment at this location was likely affected in part by asynchrony between release timings and the availability of parasitoid-susceptible EAB larvae. As with the other species of introduced EAB larval parasitoids, *S. agrili* oviposit and develop on

third- and fourth-instar EAB larvae located in galleries under the bark of a host tree (Gould et al. 2020, Jones et al. 2020). Later in development, mature EAB larvae (J-larvae) excavate a pupal chamber typically deeper in the wood of a tree where they are not accessible to parasitoids (Wang et al. 2010, Gould et al. 2020, Jones et al. 2020). Based on biweekly debarking of ash trees to collect EAB life stages in the stands where *S. agrili* was released, parasitoid-susceptible larvae in galleries were present throughout the 2019 release period. However, the proportion of EAB at this site that were susceptible to parasitism was only ~70% on the first release date (2,331 DD_{10}) and had decreased to ~11% by the last release (2,743 DD_{10}) (Chapter 2).

In contrast to 2019, *S. agrili* releases began earlier in 2020 (1,887 DD_{10}) and better matched the availability of susceptible larvae. All EAB larvae recorded from debarked trees during the 2020 release period were parasitoid-susceptible third- or fourth-instar larvae in galleries (Chapter 2). However, the overall EAB population in these ash stands had decreased quickly by this point as trees reached >90% mortality and host plant availability declined (Chapter 2, Appendix A). In a previous study rearing *S. agrili* in outdoor barrels to assess the seasonality and overwintering ability of the species in eastern Tennessee, Hooie (2014) found significant positive correlations between the number of EAB larvae present in ash logs and recovery of overwintering *S. agrili* prepupae, pupae, and adults. Relatedly, Duan et al. (2021) found a significant positive correlation between the abundances of EAB larvae and *S. galinae* broods within debarked sections of pole-size (~8–20 cm DBH) ash trees in the northern United States. The importance of abundant host availability to *S. agrili* success indicated by Hooie (2014) may have influenced the lack of establishment in Garner both in terms of release timings missing the peak abundance of parasitoid-susceptible larvae and the EAB population decline that occurred at the site over the course of the study. The late timing of parasitoid releases also may

have impeded chances of establishment because overwintering survival of immature *S. agrili* can be poor if eggs are oviposited too late in the season (after August in central New York) (Jones et al. 2020).

The limited number of releases conducted in 2020 also likely contributed to the apparent failure of *S. agrili* establishment. Only 690 adult female *S. agrili* were released in 2020, which is less than the minimum number (1,200) recommended by the USDA biocontrol program (USDA–APHIS/ARS/FS 2020). However, the reduced availability of parasitoid releases during the second release year was unavoidable as a consequence of the COVID-19 pandemic.

The efficacy of recovery methods used to assess *S. agrili* establishment also may have influenced the parasitoid collections from this site. Widespread establishment of *S. agrili* has not been achieved in North America, so it is difficult to determine the optimal methods for detecting established *S. agrili* populations. During recovery efforts conducted concurrently with parasitoid releases, Parisio et al. (2017) successfully detected *S. agrili* parasitism or adults using both sentinel bolts and yellow pan traps. The authors found no significant difference in the percentage of all sentinel bolts (14.3%) and yellow pan traps (11.3%) that detected *S. agrili*, and the authors concluded that both methods should be comparably effective for *S. agrili* detection (Parisio et al. 2017). These results lend support to the effectiveness of yellow pan traps for detecting *S. agrili* if adults were active during recovery efforts in Garner, especially given the considerable sampling effort devoted to this method (324 trap samples over 11 weeks).

Recovery of the successfully established and dispersing *S. galinae* may offer additional insights about the effectiveness of sentinel bolts and yellow pan traps for *S. agrili* recovery because these congeners share similar life history traits. *Spathius agrili* and *S. galinae* are both gregarious larval ectoparasitoids that primarily paralyze and oviposit on third- and fourth-instar

EAB larvae (Yang et al. 2005, Yang et al. 2010, Belokobylskij et al. 2012, Duan et al. 2014b). Rutledge et al. (2021) conducted parasitoid monitoring at a site in Connecticut where *S. galinae* had successfully established using green ash sentinel bolts and yellow pan traps deployed together at the same locations. The authors detected *S. galinae* parasitism in 20.8% of deployed sentinel bolts but collected adult *S. galinae* in only 0.7% of yellow pan trap samples (Rutledge et al. 2021). Sentinel bolts also effectively detected *S. galinae* parasitism in a study by Quinn et al. (2022). These studies indicate the effectiveness of sentinel bolts for recovering *Spathius* spp., although debarking of larger, thick-barked trees may quantify the extent of parasitism by *S. galinae*, and potentially *S. agrili*, more accurately than thin-barked sentinel bolts because the long ovipositor of *Spathius* spp. makes them adapted to accessing larvae under thicker bark (Duan et al. 2021, Rutledge et al. 2021, Quinn et al. 2022). While both sentinel bolts and yellow pan traps are capable of detecting *Spathius* spp., the limited number of sentinel bolts deployed in Garner and the low detection rates of *S. galinae* in yellow pan traps observed by Rutledge et al. (2021) may have hindered *S. agrili* recovery in Garner if this parasitoid had in fact established.

The emergence cage protocol used to rear parasitoids from felled EAB-infested trees in this study was previously effective for recovering native parasitoids from logs in North Carolina (Nalepa et al. 2021). The recovery of *B. indica* and other hymenopteran parasitoids from felled trees in emergence cages in the present study indicates the potential for *S. agrili* recovery using this method. However, tree felling and emergence cages have the drawbacks of being labor- and space-intensive, and a previous parasitoid recovery effort in Maryland found rearing EAB-infested logs in barrels to be ineffective at recovering *S. agrili* and *T. planipennisi* compared to tree debarking (Jennings et al. 2016a). Desiccation of logs may have also been a factor in this study because the parafilm used to cover the cut ends of logs did not provide a tight seal on all

logs. The effects of log desiccation over time may also be indicated by the fact that recoveries of known EAB parasitoids (*B. indica*) were limited to the first 15 days of the nine-week period that emergence cages were monitored, and emergence of most insects from the logs ceased by the beginning of July (Christine A. Nalepa, personal communication). A major advantage of operating emergence cages and yellow pan traps in addition to sentinel bolts was the information these methods provided about native parasitoids and wood-boring insects associated with EAB-infested ash. For example, nine *Atanycolus* specimens and *S. elegans* were recovered from yellow pan traps in August and September 2021 during the time that sentinel bolts were deployed in the same stands. *Atanycolus* spp. have previously parasitized EAB larvae in sentinel bolts, so increasing the sample size of bolts relative to other methods may increase the detection of native parasitoids using sentinel bolts (Abell et al. 2016).

The timing of recovery methods could also have influenced parasitoid recovery in this study. In its native range in China, *S. agrili* adults have been observed from late May through early August (Yang et al. 2010). In central New York, Jones et al. (2020) found that female *S. agrili* reared in open-air insectaries emerged at ~1,000–1,100 DD_{10} (late July to early August). This degree day accumulation was reached in mid-to-late June in Garner in 2021. Ash trees for the emergence cage study were felled and placed into emergence cages at 953 DD_{10} (14 June), so the emergence cage recovery method should have been well timed with the predicted emergence of *S. agrili* at this location if degree day timing remains consistent. The sampling periods for yellow pan traps and sentinel bolts were delayed due to logistical constraints, so they did not encompass the entire potential range of *S. agrili* activity based on degree day accumulation. Yellow pan traps were operated at the research site between 1,440 DD_{10} (15 July) and 2,633 DD_{10} (28 September). The two rounds of sentinel bolts were deployed between 1,776 DD_{10} (4

August) and 2,150 DD_{10} (25 August) and between 2,278 DD_{10} (1 September) and 2,561 DD_{10} (21 September), respectively. Operating these recovery methods throughout the predicted flight period of *S. agrili* may increase chances of recovery and detect parasitoid species that are active before the sampling periods in this study. Sentinel bolts deployed in 2021 represented initial efforts in a larger, ongoing parasitoid recovery study. Recovery efforts using sentinel bolts will continue in 2022 and begin earlier in the year.

Native and Naturalized Parasitoids

Parasitoid recoveries from yellow pan traps and emergence cages indicate potential parasitism of EAB by native and naturalized hymenopterans. The prevalence of *Atanycolus* sp. at this site is consistent with extensive native EAB parasitoid recovery efforts conducted in the northern United States. *Atanycolus* spp. have been frequently noted among the most common native parasitoids of EAB in northern areas such as Michigan (Duan et al. 2012, Duan et al. 2014a, Bauer et al. 2015, Duan et al. 2015, Duan et al. 2019). Recoveries of *Atanycolus* spp. are also frequent in parasitoid recovery efforts in the southern United States. *Atanycolus* cf. *cappaerti* Marsh and Strazanac (Hymenoptera: Braconidae) was the most common parasitoid reared by Nalepa et al. (2021) from EAB galleries in infested green ash trees collected from the northern Piedmont of North Carolina. *Atanycolus* spp. have also been collected from EAB-infested stands in eastern North Carolina, eastern Tennessee, and across Virginia (Hooie et al. 2015, Ragozzino 2020). Although parasitism of *Atanycolus* spp. on EAB was not directly quantified in this study, these recoveries and the high incidence of EAB parasitism by this genus in the northern United States suggest that *Atanycolus* spp. are prominent native parasitoids of EAB in North Carolina and the surrounding region.

In addition to *Atanycolus*, the other previously documented EAB parasitoid recovered from the research site was the solitary ectoparasitoid *B. indica* from an emergence cage. *Balcha indica* is confirmed to parasitize EAB larvae, prepupae, and pupae (Duan et al. 2009, Duan et al. 2011). This parasitoid is a naturalized species native to southern and southeastern Asia that was first recorded in Virginia in the mid-1990s (Gibson 2005). Emerald ash borer parasitoid recovery efforts have collected *B. indica* from EAB-infested stands in areas such as Michigan, Pennsylvania, eastern West Virginia, northern Virginia, and north-central North Carolina (Duan et al. 2009, Duan et al. 2013, Duan et al. 2014a, Spinos et al. 2014, Ragozzino 2020, Nalepa et al. 2021). Adults of both EAB and the native longhorned beetle *Tylonotus bimaculatus* Haldeman (Coleoptera: Cerambycidae) emerged from EAB-infested ash logs in emergence cages, so *B. indica* may have been attacking either species at this site (Appendix B).

The recovery of the native parasitoid *S. elegans* from an EAB-infested ash stand is also noteworthy. Native *Spathius* spp. such as *S. floridanus* Ashmead and *S. laflammei* Provancher are confirmed parasitoids of EAB larvae in North America (Duan et al. 2012, Duan et al. 2013, Nalepa et al. 2021). *Spathius elegans* is considered widespread in North America, but the host range of this species is poorly understood (Deyrup 1984, Marsh and Strazanac 2009). *Spathius elegans* was reared from larvae of the wood wasps *Xiphydria maculata* Say and *X. tibialis* Say (Hymenoptera: Xiphydriidae) in branches of sugar maple (*Acer saccharum* Marshall) in Indiana (Deyrup 1984). In addition to maple, *S. elegans* has been reared from birch (*Betula*), hickory (*Carya*), and hackberry (*Celtis occidentalis* L.) (Deyrup 1984, Marsh and Strazanac 2009). The only trees related to these records that were abundant where *S. elegans* was recovered in Garner were small understory boxelders. Hooie et al. (2015) recovered a potential specimen of *S. elegans* from an EAB-infested site in eastern Tennessee using yellow pan traps, although this

identification was not certain. In addition to its potential recovery in Tennessee, the recovery of *S. elegans* in Garner is among the first records in published literature that potentially associate this species with EAB infestations. It is also possible that *S. elegans* has been included in records of unidentified native *Spathius* spp. recovered in previous studies given the difficulty of identifying species in this genus.

Many other species of parasitoid wasps were recovered from yellow pan traps, but it is not possible to determine if parasitoids were attacking EAB based solely on yellow pan trap collections. A variety of longhorned beetles, jewel beetles (Coleoptera: Buprestidae), bark and ambrosia beetles, and the clearwing moth *Podosesia syringae* Harris (Lepidoptera: Sesiidae) were directly observed feeding in EAB-infested ash or recovered from EAB and parasitoid trapping efforts at this site (Appendix B). It is likely that many native, generalist parasitoid species were attacking this community of wood-boring insects that were present and exploiting the stressed and dying ash trees. For example, the ichneumonid wasp *A. discoidaloides* is a previously confirmed parasitoid of *P. syringae* and longhorned beetles and thus cannot be positively associated with EAB from its collection in yellow pan traps (Townes and Townes 1962). Even if not all of the native parasitoid species recovered in these stands were parasitizing EAB, these collections provide insights into the impact of EAB invasions on forest insect communities in North Carolina. Green ash trees infested by EAB were nearly the only mature trees present in the sampled stands, suggesting that parasitoids collected from these recovery efforts are likely associated with EAB-infested ash and the diverse community of native wood-boring insects facilitated by the impact of EAB on these trees.

Conclusion

Although release and recovery efforts for *S. agrili* did not succeed in confirming establishment of this biocontrol agent in the North Carolina Piedmont, these results should not be taken as proof that *S. agrili* cannot establish in this region. A variety of factors are likely to have worked against successful establishment at this site, including temporal mismatches between release timings and the susceptibility of EAB to parasitism, the limited number of releases related to outside factors like the COVID-19 pandemic, and acute declines in the overall numbers of EAB and living ash trees at the site. More numerous releases of *S. agrili* on a schedule that better aligns with the seasonal availability of parasitoid-susceptible larvae in univoltine EAB populations should be conducted in additional EAB-infested stands in the southern United States to determine the potential success of this species in warm climates. While establishment of introduced EAB parasitoids was not confirmed, recoveries of the known EAB parasitoids *Atanycolus* sp. and *B. indica* as well as other parasitoids of wood-boring insects indicate that a variety of native and naturalized natural enemies are responding to the host resources provided by EAB infestations. These results also demonstrate the value of using multiple parasitoid recovery methods simultaneously when characterizing natural enemy communities, as each parasitoid species identified at this site was collected from only one of the three recovery methods. Ultimately, this research contributes to the existing understanding of EAB management and invasion ecology in North Carolina by evaluating the effectiveness of *S. agrili* release and recovery methods and describing the existing natural enemy community associated with EAB infestations in the region.

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REFERENCES

Abell, K. J., L. S. Bauer, D. L. Miller, J. J. Duan, and R. G. Van Driesche. 2016. Monitoring the establishment and flight phenology of parasitoids of emerald ash borer (Coleoptera: Buprestidae) in Michigan by using sentinel eggs and larvae. The Florida Entomologist 99(4): 667–672.

Abell, K. J., J. J. Duan, and P. M. Shrewsbury. 2019. Determining optimal parasitoid release timing for the biological control of emerald ash borer (Coleoptera: Buprestidae). The Florida Entomologist 102(4): 691–694.

Barker, B. S., L. Coop, T. Wepprich, F. Grevstad, and G. Cook. 2020. DDRP: Real-time phenology and climatic suitability modeling of invasive insects. PLoS One 15(12): e0244005.

Bauer, L. S., R. A. Haack, D. L. Miller, T. R. Petrice, and H. Liu. 2004. Emerald ash borer life cycle, p. 8. *In* V. Mastro and R. Reardon (comps.), Emerald Ash Borer Research and Technology Development Meeting, 30 September–1 October 2003, Port Huron, MI. Publication FHTET-2004-02. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Bauer, L. S., J. J. Duan, J. R. Gould, and R. Van Driesche. 2015. Progress in the classical biological control of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America. Canadian Entomologist 147(3): 300–317.

Belokobylskij, S. A., G. I. Yurchenko, J. S. Strazanac, A. Zaldívar-Riverón, and V. Mastro. 2012. A new emerald ash borer (Coleoptera: Buprestidae) parasitoid species of *Spathius* Nees (Hymenoptera: Braconidae: Doryctinae) from the Russian Far East and South Korea. Annals of the Entomological Society of America 105(2): 165–178.

Bolen, A. 2020. A silent killer: Black ash basket makers are battling a voracious beetle to keep their heritage alive. https://www.americanindianmagazine.org/story/black-ash-basketry. Accessed 8 May 2022.

Cappaert, D., D. G. McCullough, T. M. Poland, and N. W. Siegert. 2005. Emerald ash borer in North America: A research and regulatory challenge. American Entomologist 51(3): 152–165.

Crimmins, T. M., K. L. Gerst, D. G. Huerta, R. L. Marsh, E. E. Posthumus, A. H. Rosemartin, J. Switzer, J. F. Weltzin, L. Coop, N. Dietschler, D. A. Herms, S. Limbu, R. T. Trotter, and M. Whitmore. 2020. Short-term forecasts of insect phenology inform pest management. Annals of the Entomological Society of America 113(2): 139–148.

Davidson, W., and L. K. Rieske. 2016. Establishment of classical biological control targeting emerald ash borer is facilitated by use of insecticides, with little effect on native arthropod communities. Biological Control 101: 78–86.

Deyrup, M. A. 1984. A maple wood wasp, *Xiphydria maculata*, and its insect enemies (Hymenoptera: Xiphydriiade). The Great Lakes Entomologist 17(1): 17–28.

Duan, J. J., R. W. Fuester, J. Wildonger, P. B. Taylor, S. Barth, and S. E. Spichiger. 2009. Parasitoids attacking the emerald ash borer (Coleoptera: Buprestidae) in western Pennsylvania. The Florida Entomologist 92(4): 588–592.

Duan, J. J., M. D. Ulyshen, L. S. Bauer, J. Gould, and R. Van Driesche. 2010. Measuring the impact of biotic factors on populations of immature emerald ash borers (Coleoptera: Buprestidae). Environmental Entomology 39(5): 1513–1522.

Duan J. J., P. B. Taylor, and R. W. Fuester. 2011. Biology and life history of *Balcha indica*, an

ectoparasitoid attacking the emerald ash borer, *Agrilus planipennis*, in North America. Journal of Insect Science 11: 127.

Duan, J. J., L. S. Bauer, K. J. Abell, and R. van Driesche. 2012. Population responses of hymenopteran parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded areas in north central United States. BioControl 57(2): 199–209.

Duan, J. J., P. B. Taylor, R. W. Fuester, R. R. Kula, and P. M. Marsh. 2013. Hymenopteran parasitoids attacking the invasive emerald ash borer (Coleoptera: Buprestidae) in western and central Pennsylvania. The Florida Entomologist 96(1): 166–172.

Duan, J. J., K. J. Abell, L. S. Bauer, J. Gould, and R. Van Driesche. 2014a. Natural enemies implicated in the regulation of an invasive pest: A life table analysis of the population dynamics of the emerald ash borer. Agricultural and Forest Entomology 16(4): 406–416.

Duan, J. J., T. J. Watt, and K. Larson. 2014b. Biology, life history and laboratory rearing of *Spathius galinae*, a larval parasitoid of the invasive emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology 107(3): 939–946.

Duan, J. J., L. S. Bauer, K. J. Abell, M. D. Ulyshen, R. G. Van Driesche, and A. Sheppard. 2015. Population dynamics of an invasive forest insect and associated natural enemies in the aftermath of invasion: Implications for biological control. The Journal of Applied Ecology 52(5): 1246–1254.

Duan, J. J., L. S. Bauer, R. G. Van Driesche, and J. R. Gould. 2018. Progress and challenges of protecting North American ash trees from the emerald ash borer using biological control. Forests 9(3): 142.

Duan, J. J., R. G. Van Driesche, R. S. Crandall, J. M. Schmude, C. E. Rutledge, B. H. Slager, J.

R. Gould, and J. S. Elkinton. 2019. Establishment and early impact of *Spathius galinae* (Hymenoptera: Braconidae) on emerald ash borer (Coleoptera: Buprestidae) in the northeastern United States. Journal of Economic Entomology 112(5): 2121–2130.

Duan, J. J., R. G. Van Driesche, J. M. Schmude, N. F. Quinn, T. R. Petrice, C. E. Rutledge, T. M. Poland, L. S. Bauer, and J. S. Elkinton. 2021. Niche partitioning and coexistence of parasitoids of the same feeding guild introduced for biological control of an invasive forest pest. Biological Control 160: 104698.

Duan, J. J., R. G. Van Driesche, J. Schmude, R. Crandall, C. Rutledge, N. Quinn, B. H. Slager, J. R. Gould and J. S. Elkinton. 2022. Significant suppression of invasive emerald ash borer by introduced parasitoids: Potential for North American ash recovery. Journal of Pest Science 95: 1081–1090.

Emerald Ash Borer Information Network. 2022. About emerald ash borer. http://www.emeraldashborer.info/about-eab.php. Accessed 25 Apr 2022.

Federal Register. 2020. Removal of emerald ash borer domestic quarantine regulations. 7 CFR Part 301, 85(241): 81085–81095. https://www.govinfo.gov/content/pkg/FR-2020-12-15/pdf/2020-26734.pdf. Accessed 30 Dec 2020.

Flower, C. E., K. S. Knight, J. Rebbeck, and M. A. Gonzalez-Meler. 2013a. The relationship between emerald ash borer (*Agrilus planipennis* Fairmaire) and ash (*Fraxinus* spp.) tree decline: Using visual ash condition assessments and leaf isotope measurements to assess pest damage. Forest Ecology and Management 303: 143–147.

Flower, C. E., K. S. Knight, and M. A. Gonzalez-Meler. 2013b. Impacts of the emerald ash borer

(*Agrilus planipennis* Fairmaire) induced ash (*Fraxinus* spp.) mortality on forest carbon cycling and successional dynamics in the eastern United States. Biological Invasions 15(4): 931–944.

Gaudon, J. M., and S. M. Smith. 2020. Augmentation of native North American natural enemies for the biological control of the introduced emerald ash borer in central Canada. BioControl 65(1): 71–79.

Gibson, G. A. P. 2005. The world species of *Balcha* Walker (Hymenoptera: Chalcidoidea: Eupelmidae), parasitoids of wood-boring beetles. Zootaxa 1033: 1–62.

Gould, J. R., M. L. Warden, B. H. Slager, and T. C. Murphy. 2020. Host overwintering phenology and climate change influence the establishment of *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), a larval parasitoid introduced for biocontrol of the emerald ash borer. Journal of Economic Entomology 113(6): 2641–2649.

Herms, D. A. 2004. Using degree-days and plant phenology to predict pest activity, pp. 49–59. *In* V. Krischik and J. Davidson (eds.). IPM (Integrated Pest Management) of Midwest Landscapes. Publication SB-07645. Minnesota Agricultural Experiment Station, St. Paul, MN.

Herms, D. A., and D. G. McCullough. 2014. Emerald ash borer invasion of North America: History, biology, ecology, impacts, and management. Annual Review of Entomology 59(1): 13–30.

Herms, D. A., D. G. McCullough, C. S. Clifford, D. R. Smitley, F. D. Miller, and W. Cranshaw. 2019. Insecticide options for protecting ash trees from emerald ash borer. North Central IPM Center Bulletin. 3rd ed. 16 pp.

Hooie, N. A. 2014. The seasonality of two parasitoids (*Spathius agrili* and *Tetrastichus*

planipennisi) of the emerald ash borer, *Agrilus planipennis*, and a survey for native natural enemies of the emerald ash borer in eastern Tennessee. M.S. thesis, University of Tennessee, Knoxville.

Hooie, N. A., G. J. Wiggins, P. L. Lambdin, J. F. Grant, S. D. Powell, and J. P. Lelito. 2015. Native parasitoids and recovery of *Spathius agrili* from areas of release against emerald ash borer in eastern Tennessee, USA. Biocontrol Science and Technology 25(3): 345–351.

Jennings, D. E, J. R. Gould, J. D. Vandenberg, J. J. Duan, and P. M. Shrewsbury. 2013. Quantifying the impact of woodpecker predation on population dynamics of the emerald ash borer (*Agrilus planipennis*). PLoS One 8(12): e83491.

Jennings, D. E., J. J. Duan, D. Bean, J. R. Gould, K. A. Rice, and P. M. Shrewsbury. 2016a. Monitoring the establishment and abundance of introduced parasitoids of emerald ash borer larvae in Maryland, U.S.A. Biological Control 101: 138–144.

Jennings, D. E., J. J. Duan, L. S. Bauer, J. M. Schmude, M. T. Wetherington, and P. M. Shrewsbury. 2016b. Temporal dynamics of woodpecker predation on emerald ash borer (*Agrilus planipennis*) in the northeastern U.S.A. Agricultural and Forest Entomology 18(2): 174–181.

Jones, M. I., J. R. Gould, H. J. Mahon, and M. K. Fierke. 2020. Phenology of emerald ash borer (Coleoptera: Buprestidae) and its introduced larval parasitoids in the northeastern United States. Journal of Economic Entomology 113(2): 622–632.

Knight, K. S., B. P. Flash, R. H. Kappler, J. A. Throckmorton, B. Grafton, and C. E. Flower.

2014. Monitoring ash (*Fraxinus* spp.) decline and emerald ash borer (*Agrilus planipennis*) symptoms in infested areas. Gen. Tech. Rep. NRS-139. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. 18 p.

Kovacs, K. F., R. G. Haight, D. G. McCullough, R. J. Mercader, N. W. Siegert, and A. M. Liebhold. 2010. Cost of potential emerald ash borer damage in U.S. communities, 2009–2019. Ecological Economics 69(3): 569–578.

Liu, H., L. S. Bauer, D. L. Miller, T. Zhao, R. Gao, L. Song, Q. Luan, R. Jin, and C. Gao. 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biological Control 42(1): 61–71.

MapBioControl. 2020. https://www.mapbiocontrol.org. Accessed 2 Apr 2022.

Marsh, P. M. and J. S. Strazanac. 2009. A taxonomic review of the genus *Spathius* Nees (Hymenoptera: Braconidae) in North America and comments on the biological control of the emerald ash borer (Coleoptera: Buprestidae). Journal of Hymenoptera Research 18: 80–112.

Murphy, T. C., J. R. Gould, R. G. Van Driesche, and J. S. Elkinton. 2018. Interactions between woodpecker attack and parasitism by introduced parasitoids of the emerald ash borer. Biological Control 122: 109–117.

Nalepa, C. A., K. L. F. Oten, and M. A. Bertone. 2021. Overwintering developmental stages of emerald ash borer in North Carolina. The Florida Entomologist 104(3): 213–217.

Parisio, M. S., J. R. Gould, J. D. Vandenberg, L. S. Bauer, and M. K. Fierke. 2017. Evaluation of recovery and monitoring methods for parasitoids released against emerald ash borer. Biological Control 106: 45–53.

Poland, T. M., and D. G. McCullough. 2006. Emerald ash borer: Invasion of the urban forest and the threat to North America's ash resource. Journal of Forestry 104(3): 118–124.

Quinn, N. F., J. S. Gould, C. E. Rutledge, A. Fassler, J. S. Elkinton, and J. J. Duan. 2022. Spread and phenology of *Spathius galinae* and *Tetrastichus planipennisi*, recently introduced for biocontrol of emerald ash borer (Coleoptera: Buprestidae) in the northeastern United States. Biological Control 165: 104794.

Ragozzino, M. L. 2020. Influences of climate, competition, and novel hosts on parasitoids of emerald ash borer, and their establishment in Virginia and North Carolina. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg.

Rebek, E. J., D. A. Herms, and D. R. Smitley. 2008. Interspecific variation in resistance to emerald ash borer (Coleoptera: Buprestidae) among North American and Asian ash (*Fraxinus* spp.). Environmental Entomology 37(1): 242–246.

Rutledge, C. E., R. G. Van Driesche, and J. J. Duan. 2021. Comparative efficacy of three techniques for monitoring the establishment and spread of larval parasitoids recently introduced for biological control of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae). Biological Control 161: 104704.

Siegert, N. W., D. G. McCullough, A. M. Liebhold, and F. W. Telewski. 2014. Dendrochronological reconstruction of the epicentre and early spread of emerald ash borer in North America. Diversity & Distributions 20(7): 847–858.

Smith, A. 2006. Effects of community structure on forest susceptibility and response to the emerald ash borer invasion of the Huron River watershed in southeast Michigan. M.S. thesis, Ohio State University, Columbus.

Spinos, T., A. Hill, R. Braud, and R. M. Turcotte. 2014. New distributional record for *Balcha*

indica (Hymenoptera: Eupelmidae) in eastern West Virginia discovered during emerald ash borer parasitoid recovery surveys. The Great Lakes Entomologist 47(1): 73–75.

Stephens, J. P., K. A. Berven, and S. D. Tiegs. 2013. Anthropogenic changes to leaf litter input affect the fitness of a larval amphibian. Freshwater Biology 58(8): 1631–1646.

Townes, H., and M. Townes. 1962. Ichneumon-flies of America north of Mexico: 3. Subfamily Gelinae, tribe Mesostenini. United States National Museum Bulletin 216(3): 1–602.

USDA–APHIS/ARS/FS. 2020. Emerald ash borer biological control release and recovery guidelines. USDA–APHIS–ARS–FS, Riverdale, MD.

USPEST.ORG. 2018. Online phenology and degree-day models for agricultural and pest management decision making in the US. https://uspest.org/dd/model_app. Accessed 10 Apr 2022.

Villari, C., D. A. Herms, J. G. A. Whitehill, D. Cipollini, and P. Bonello. 2016. Progress and gaps in understanding mechanisms of ash tree resistance to emerald ash borer, a model for wood-boring insects that kill angiosperms. The New Phytologist 209(1): 63–79.

Wagner, D. L., and K. J. Todd. 2016. New ecological assessment for the emerald ash borer: A cautionary tale about unvetted host-plant literature. American Entomologist 62(1): 26–35.

Wang, X.-Y., Z.-Q. Yang, J. R. Gould, Y.-N. Zhang, G.-J. Liu, and E.-S. Liu. 2010. The biology and ecology of the emerald ash borer, *Agrilus planipennis*, in China. Journal of Insect Science 10(128): 1–23.

Yang, Z.-Q., J. S. Strazanac, P. M. Marsh, C. van Achterberg, and W.-Y. Choi. 2005. First recorded parasitoid from China of *Agrilus planipennis*: A new species of *Spathius* (Hymenoptera: Braconidae: Doryctinae). Annals of the Entomological Society of America 98(5): 636–642.

Yang, Z.-Q., X.-Y. Wang, J. R. Gould, R. C. Reardon, Y.-N. Zhang, G.-J. Liu, and E.-S. Liu. 2010. Biology and behavior of *Spathius agrili*, a parasitoid of the emerald ash borer, *Agrilus planipennis*, in China. Journal of Insect Science 10(30): 1–13.

CHAPTER 4

Assessment of Parasitoid Establishment at Initial Release Sites in North Carolina Using Sentinel Bolts

ABSTRACT

Classical biological control involving the release of egg and larval parasitoids holds promise for sustainable management of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), a highly destructive, non-native forest pest of ash (*Fraxinus* spp.) trees in North America. Parasitoid wasp establishment in release areas is assessed through a variety of recovery methods, including the deployment of small ash bolts containing EAB larvae that target larval parasitoid recovery. In the late summer of 2021, these sentinel bolts were deployed in Granville and Wayne counties in North Carolina to assess parasitoid establishment at sites where EAB parasitoids had been released between 2013 and 2019. While these initial efforts did not detect introduced parasitoids, they provide a foundation for ongoing, more extensive sentinel bolt deployments to assess the establishment and effectiveness of EAB biological control efforts in North Carolina.

INTRODUCTION

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is one of the most devastating non-native insects to impact North American forests. Accidentally introduced from its native range in northeastern Asia, EAB has been documented attacking ash (*Fraxinus* spp.) trees in 35 states of the United States and five provinces of Canada across the eastern and central portions of the continent since its initial detection in 2002 (Bauer et al. 2004, Cappaert et al. 2005, Poland and McCollough 2006, Emerald Ash Borer Information Network 2022). Feeding by EAB larvae cuts off the flow of nutrients and water in a tree as the larvae tunnel through the vascular tissue of their host plant (Cappaert et al. 2005, Wang et al. 2010,

Villari et al. 2016). North American ash species do not share a coevolutionary history with EAB and possess poor resistance against EAB attack, which has resulted in the deaths of millions of ash trees across this insect's expanding non-native range (Liu et al. 2007, Rebek et al. 2008, Villari et al. 2016). These impacts of EAB cause severe losses to the ecological, economic, and cultural values that ash trees provide in North America (Poland and McCullough 2006, Kovacs et al. 2010, Flower et al. 2013, Stephens et al. 2013, Herms and McCullough 2014, Wagner and Todd 2016, Bolen 2020).

Management efforts to limit the spread and impact of EAB in North American forests are challenging. Insecticide treatments used to protect individual trees from EAB are not practical across forested landscapes, so a classical biological control program has been pursued in the United States to provide sustainable management of this forest pest (Bauer et al. 2015, Davidson and Rieske 2016, Duan et al. 2018). This biocontrol program is led by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) and consists of the introduction of natural enemies that attack the EAB in its native range in Asia. These natural enemies include the egg parasitoid *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) and the larval parasitoids *Spathius agrili* Yang (Hymenoptera: Braconidae), *Spathius galinae* Belokobylskij and Strazanac (Hymenoptera: Braconidae), and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Bauer et al. 2015, Duan et al. 2018).

In the northern United States, *T. planipennisi* and *S. galinae* have successfully established, dispersed, and are associated with suppression of EAB larval densities (Duan et al. 2022, Quinn et al. 2022). Conversely, establishment of introduced parasitoids has been limited in the southern United States (Duan et al. 2018, MapBioControl 2020). Previous release and recovery efforts have recovered a single adult *S. agrili* in eastern Tennessee and a small number

of *S. agrili* in a study across Maryland, but these recoveries have been almost entirely limited to the year after parasitoid releases (Hooie et al. 2015, Jennings et al. 2016). More recently, recoveries of *S. agrili* were reported later than one year after final releases in northern and central Virginia and eastern North Carolina (Ragozzino 2020). While these results may indicate that *S. agrili* can persist in the southern United States, widespread establishment of introduced parasitoids has not occurred in the Southeast (MapBioControl 2020). Further research on the release and establishment of *S. agrili* and other introduced parasitoids in the southern United States is critical to the development of a successful EAB biocontrol program for this region (Duan et al. 2018, Jones et al. 2020).

A variety of methods have been developed to monitor the establishment and dispersal of introduced EAB parasitoids in North America (Parisio et al. 2017, USDA–APHIS/ARS/FS 2020, Rutledge et al. 2021). Destructive sampling of ash trees can be used to detect parasitoids by debarking trees to record evidence of parasitism or rearing adult parasitoids from EAB-infested logs (Jennings et al. 2016). Yellow pan traps can also be operated at previous release areas during the flight period of parasitoids to attract and collect adult parasitoids. While both destructive sampling and yellow pan traps can successfully recover introduced parasitoids, these methods have disadvantages in terms of their labor, time, and space requirements and/or lack of specificity (Rutledge et al. 2021). For example, yellow pan traps are attractive to a wide variety of insects, and large amounts of bycatch must be sorted through to detect the small parasitoids of interest in trap samples. In addition, the presence of a parasitoid in yellow pan trap collections cannot confirm its association with EAB.

More recently, a sentinel bolt method was developed to provide a more targeted approach to larval parasitoid recovery (Abell et al. 2016, Rutledge et al. 2021, Quinn et al. 2022). Sentinel

bolts consist of small ash logs that are artificially infested with EAB larvae and hung at previous release sites to attract oviposition by EAB parasitoids. After removal from the field, bolts are reared to allow emergence of parasitoids developing on the EAB larvae. Bolts are then debarked to determine the fate of the EAB inside and detect further evidence of successful parasitism. Sentinel bolts have been effective at detecting and monitoring dispersal of established *S. galinae* and *T. planipennisi* populations in the northern United States (Rutledge et al. 2021, Quinn et al. 2022). While the process of constructing sentinel bolts is labor and time intensive, the demonstrated efficacy and efficiency of bolts relative to the other recovery methods make sentinel bolts a promising tool for EAB parasitoid recovery efforts in North America (Rutledge et al. 2021). This study was conducted to initiate assessments of introduced larval parasitoid establishment at biocontrol release sites in North Carolina using sentinel bolts.

MATERIALS AND METHODS

Site Descriptions

Parasitoid establishment was assessed at four sites in North Carolina where EAB biocontrol releases had occurred two to eight years previously (Figure 1). Two parasitoid release sites were located in Granville County in the northern Piedmont near the Virginia state line. Two additional release sites were located about 130 km southeast in Wayne County near the inland extent of the Coastal Plain. Releases at both sites in Granville County and one site in Wayne County (Little River) were conducted by the North Carolina Forest Service (MapBioControl 2020). Parasitoid releases at the remaining site in Wayne County (Cherry Research Station) were conducted by Virginia Tech and reported by Ragozzino (2020). The parasitoids were produced and supplied by the USDA EAB Parasitoid Rearing Facility in Brighton, Michigan. Full records of all releases are provided in Appendix C.

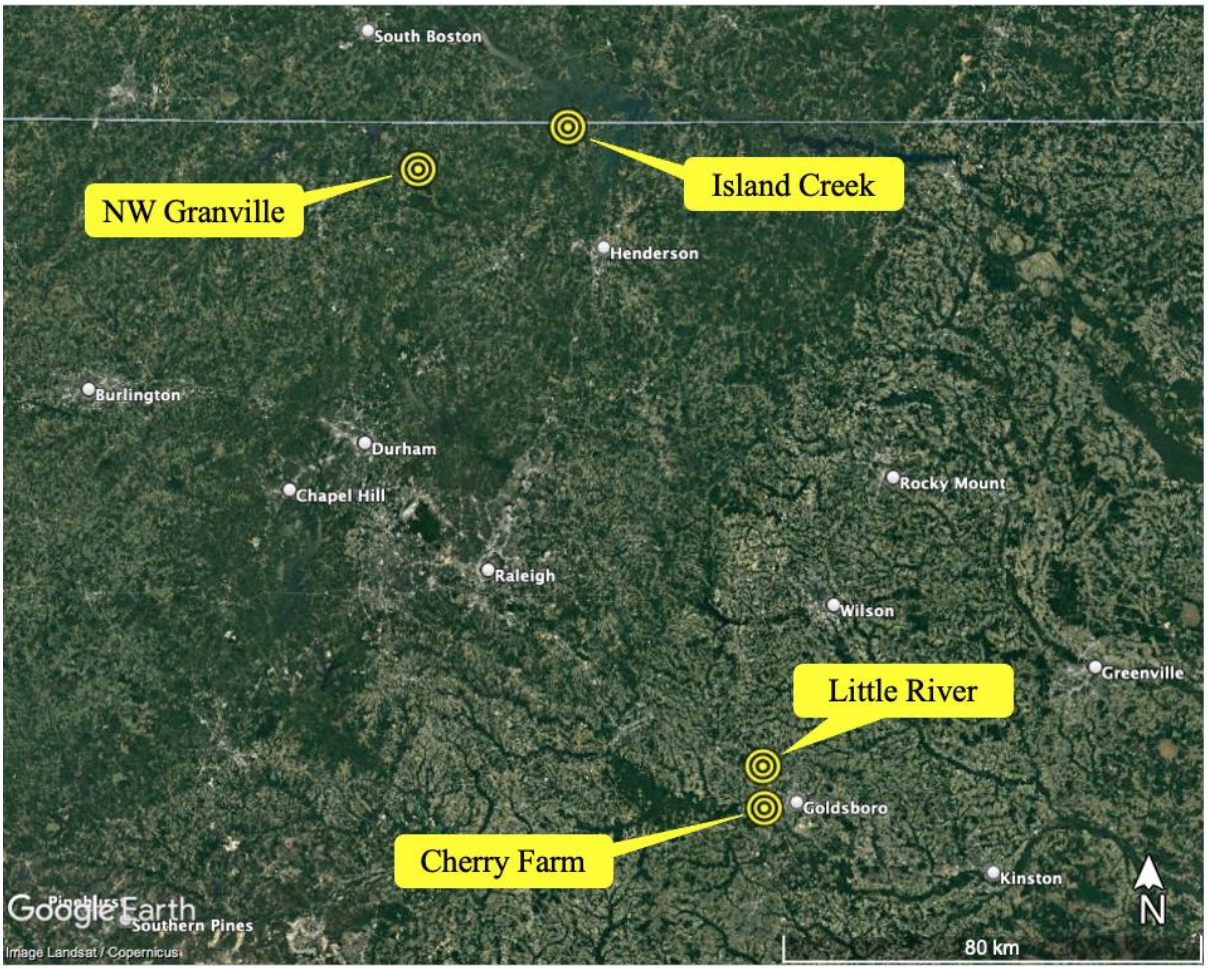

Figure 1. Map of sentinel bolt deployment sites in Granville County (NW Granville and Island Creek) and Wayne County (Little River and Cherry Farm), North Carolina.

Granville County

The first site (NW Granville) in Granville County is located on private property in the northwestern section of the county. This site is characterized by mature upland hardwood forest with oak (*Quercus* spp.) and hickory (*Carya* spp.) as primary tree species. The understory of the site is sparse. Mature ash at this site has experienced near-total mortality from EAB, and ash seedlings and young trees were observed in the understory. A total of 6,978 *O. agrili*, 2,782 *S. agrili*, and 34,049 *T. planipennisi* were released at this site between September 2013 and July 2015 (Table 1 in Appendix C). Parasitoids were released as pupae and adults.

The second site (Island Creek) in Granville County is located ~30 km northeast of the NW Granville site at the Island Creek Wildlife Management Area, which is managed by the United States Army Corps of Engineers. This site is located along Island Creek and is composed of mature bottomland hardwood forest with a sparse understory. Mature ash at this site has experienced near-total mortality from EAB. A total of 6,329 *O. agrili*, 2,962 *S. agrili*, and 35,543 *T. planipennisi* were released at this site between September 2013 and April 2019 (Table 2 in Appendix C). Parasitoids were released as pupae and adults.

Wayne County

The first site (Little River) in Wayne County is located on private property northwest of Goldsboro along Little River. This site is characterized by seasonally wet bottomland hardwood forest. Woody vines and grasses are plentiful in the understory and as ground cover. Mature ash at this site has experienced near-total mortality from EAB. A total of 2,750 *O. agrili*, 436 *S. agrili*, and 7,388 *T. planipennisi* were released at this site between July 2015 and May 2017 (Table 3 in Appendix C). *Oobius agrili* were released as pupae, *S. agrili* were released as adults, and *T. planipennisi* were released as pupae and adults.

The second site (Cherry Farm) in Wayne County is located ~8 km south of the Little River site at Cherry Research Station, which is managed by the North Carolina Department of Agriculture and Consumer Services. Unlike the other study sites, the Cherry Farm site is a pure, row-planted stand of green ash (*Fraxinus pennsylvanica* Marshall) with a thick ground cover and understory of grasses, forbs, and shrubs. This stand was planted in 1999 from a diverse collection of wild green ash seeds collected throughout North Carolina. The stand is ~1.27 ha (~40 m x ~295 m) in size and is bordered by corn (*Zea mays* L.) fields and loblolly pine (*Pinus taeda* L.) stands. This stand is severely impacted by EAB, as evidenced by extensive epicormic sprouting,

canopy dieback, bark splits, and tree mortality. A total of 1,004 *T. planipennisi* had been released at this site on 8 September 2016, and 212 female *S. agrili* and 215 female *S. galinae* had been released on 22 August 2017 (Table 4 in Appendix C). Male *S. agrili* and *S. galinae* were also included in the release at an assumed sex ratio of three females to one male. *Spathius agrili* and *S. galinae* were released as adults, and *T. planipennisi* were released as pupae in infested bolts (Ragozzino 2020).

Parasitoid Recovery

To assess parasitoid establishment at the release sites, sentinel bolts were deployed at all sites in 2021. Sentinel bolts are designed for recovering larval parasitoids of EAB, making this method appropriate for targeting *S. agrili*, *S. galinae*, and *T. planipennisi* but unlikely to recover the egg parasitoid *O. agrili*. Sentinel bolts were made and supplied by the USDA and were constructed similarly to descriptions by Rutledge et al. (2021) and Quinn et al. (2022). Briefly, sentinel bolts are cut tropical ash (*Fraxinus uhdei* (Winzig) Lingelsh) logs measuring ~3–4 cm in diameter and ~20 cm in length. Each bolt is artificially infested with EAB by inserting EAB larvae under flaps cut in the bark. A cup containing moist rock wool is attached to the bottom end of the bolt to prevent desiccation, and a metal eye hook is screwed into the top end for hanging the bolt (Figure 2).

Sentinel bolts were placed in the field for three to four weeks. Two sentinel bolts were deployed at each Wayne County site on 4 August and at each Granville County site on 5 August. This first round of bolts was retrieved on 1 September, and two new bolts were deployed at each site on 1 September. This second round of bolts at the Wayne County sites was retrieved on 20 September, and those at the Granville County sites were retrieved on 22 September. Bolts were hung from a nail hammered into a tree ~1.75 m above the ground (Figure 2). Both bolts at

Cherry Farm and one bolt at Little River were hung from ash trees, but the remaining bolts were hung from tree species other than ash (Little River: musclewood (*Carpinus caroliniana* Walter); NW Granville: both hickories (*Carya* sp.); Island Creek: red maple (*Acer rubrum* L.) and musclewood) due to the scarcity of mature ash with a main stem capable of supporting the weight of the bolts. Brightly colored flagging was tied to the tree that each bolt was hung from to help locate the bolts during subsequent site visits. Bolts were placed ~25–90 m apart at each site.

Figure 2. Sentinel bolt deployed in northwestern Granville County, North Carolina (NW Granville site). Photo by Kelly Oten.

After retrieval from the field, each bolt was placed into a separate emergence tube to monitor for parasitoid emergence over three to four weeks. Emergence tubes consisted of a sealed cardboard tube with a clear glass vial inserted in a hole in the side of the tube near the top (Figure 3A). This vial could be removed to collect insects inside. The tubes were kept indoors and were placed with the vial facing toward a window to provide an attractive light source. The

first and second rounds of bolts from the Wayne County sites remained in emergence tubes from 1 September to 20 September and 20 September to 21 October, respectively. The first and second rounds of bolts from the Granville County sites remained in emergence tubes from 1 September to 22 September and 22 September to 21 October, respectively. The tubes were checked for parasitoid emergence several times per week over these durations (Granville: first round: 9 checks, second round: 12 checks; Wayne: first round: 7 checks, second round: 14 checks). A potential emerged parasitoid specimen was photographed and shipped to the USDA-APHIS-PPQ-S&T Forest Pest Methods Laboratory in Buzzards Bay, Massachusetts for identification.

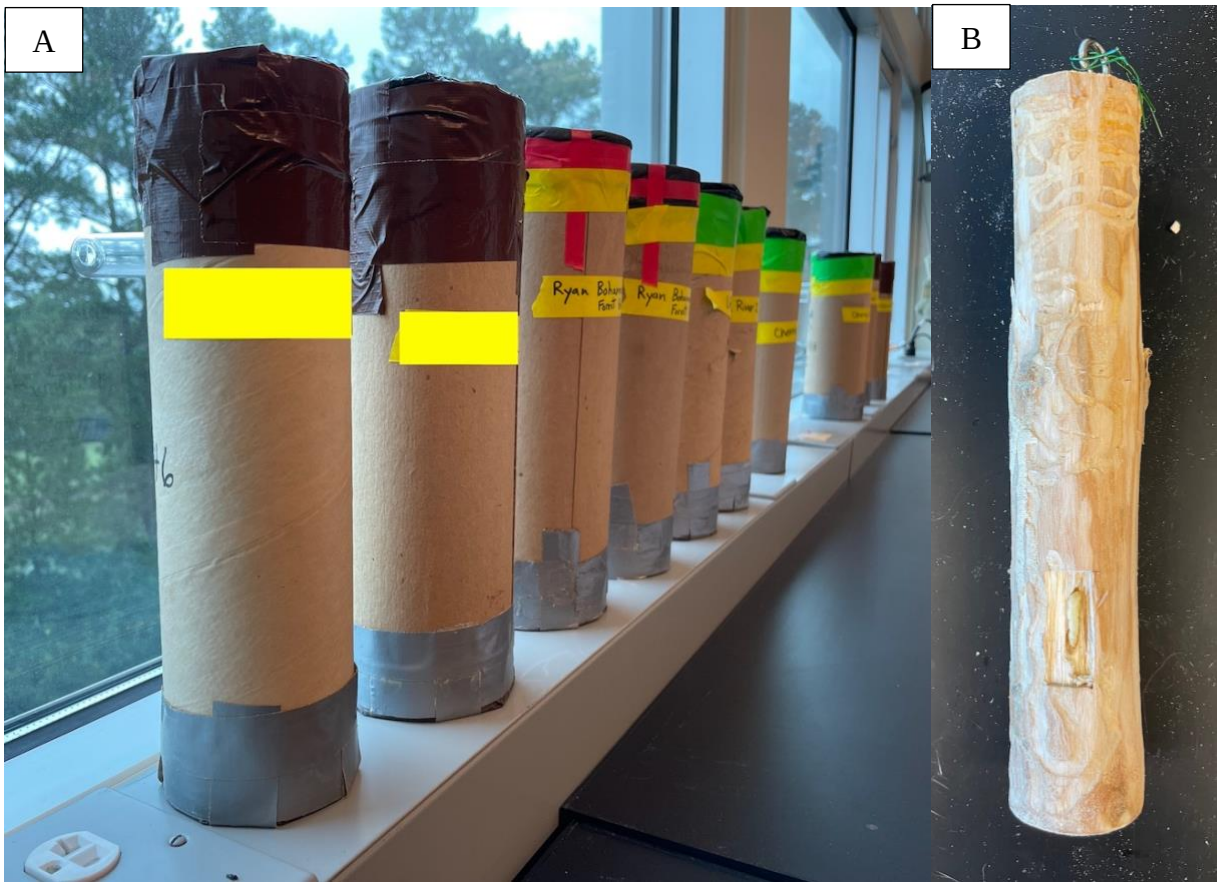

Figure 3. (A) Emergence tubes containing sentinel bolts; (B) Debarked sentinel bolt showing an excavated J-shaped emerald ash borer larva in a pupal chamber.

After the end of the monitoring periods, bolts were removed from the emergence tubes. The tubes were examined for any emerged insects that had not entered the clear vials. The removed bolts were debarked with a drawknife to determine whether each EAB larva was alive, parasitized, or was dead from another cause (Figure 3B). Emerald ash borer in pupal chambers were excavated using a chisel and hammer. The first and second rounds of bolts were debarked on 2 October and 23 October, respectively (one month after collection from the field).

RESULTS

One hymenopteran specimen closely resembling *T. planipennisi* was collected from the clear vial of an emergence tube containing a second-round sentinel bolt from Cherry Farm on 6 October, 16 days after removal from the field (Figure 4). This specimen was determined not to be *T. planipennisi* based on setae length, eye color, and the fact that *T. planipennisi* is a gregarious parasitoid and multiple specimens would be expected to emerge from a parasitized bolt (Juli R. Gould, personal communication). Two thrips (Thysanoptera) were also collected from the same Cherry Farm bolt on 18 October. No insects emerged from the sentinel bolts placed in emergence tubes from the remaining sites.

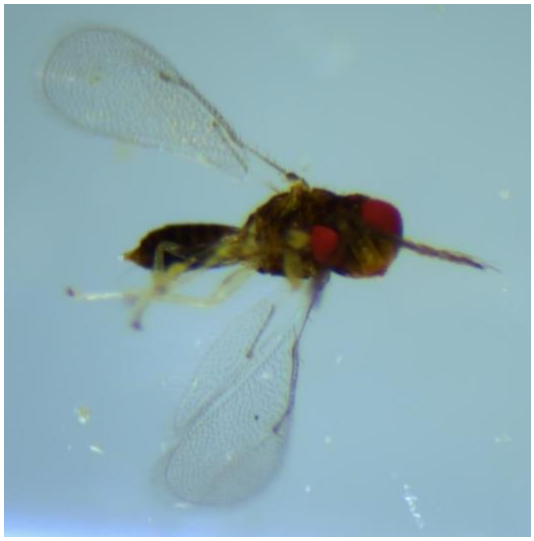

Figure 4. Hymenopteran specimen recovered from an emergence tube containing a sentinel bolt deployed at Cherry Research Station in Wayne County, North Carolina.

Excavation of EAB larvae inside sentinel bolts did not reveal evidence of parasitism from any site. Debarking of the first round of bolts on 2 October recovered a total of 33 EAB larvae from the four bolts, with two to six larvae recovered from each bolt (mean = 4.1). At the time of debarking, all larvae from the first round of bolts were live J-shaped larvae in pupal chambers. A total of 28 EAB larvae were recovered from the second round of bolts during debarking on 23 October. One to five larvae were recovered from each bolt in the second round (mean = 3.5). Twenty (71.4%) of the EAB recovered from the second round of bolts were J-larvae in pupal chambers at the time of debarking. The remaining eight (28.6%) larvae were non-J-larvae in galleries, and they were recovered from one bolt at each site. One non-J-larva and one J-larva from an Island Creek bolt were dead of unknown causes but did not exhibit signs of parasitism. All other larvae from the second round of bolts were recovered alive and without evidence of parasitism.

DISCUSSION

Sentinel bolts were not operated at release sites throughout a full season of parasitoid activity in this pilot study, so it is too early to draw conclusions about parasitoid establishment at the study sites until more extensive recovery efforts are conducted in the future. These sentinel bolt deployments in the late summer of 2021 represented initial efforts in a larger, ongoing research project investigating parasitoid establishment in North Carolina. Recovery efforts using sentinel bolts will continue in 2022 and include an earlier seasonal start to field deployments.

The extensive mortality of ash trees at the study sites indicates that EAB populations are likely to be low. This is especially probable for the sites other than Cherry Farm where ash is present as a smaller component of mixed hardwood forests rather than as a dense, pure stand. If introduced parasitoid species have established and persisted at these sites, they are likely present

in low numbers that correspond to the low availability of EAB hosts. Small parasitoid population levels could make recovery of larval parasitoids more difficult, although sentinel logs may also be more attractive at sites where few EAB larvae are otherwise available.

The primary objective of the ongoing parasitoid recovery efforts initiated in this study is to detect the presence of larval parasitoids at previous release sites in North Carolina where establishment has not been confirmed. While tree debarking may provide a more accurate quantification of parasitism levels at a site, sentinel bolts have been effective for the detection of both *S. galinae* and *T. planipennisi* (Rutledge et al. 2021, Quinn et al. 2022). Sentinel bolts would also be advantageous compared to debarking for future detection efforts in North Carolina because of the much lower field work time necessary at each site, which would allow for a greater number of sites and/or more remote sites to be assessed in a shorter time frame. Relatedly, sentinel bolts may be preferable to yellow pan traps because they do not have to be checked and reset weekly. This is an important consideration for monitoring of remote sites or in studies across a large geographic area where weekly visits to all sites may not be practical.

While the data provided by the sentinel bolts in this study are limited, perhaps the most important result of this effort is in providing the groundwork for continuing parasitoid recovery efforts. By establishing infrastructure (e.g. emergence tubes) and experience with deployment, rearing, and debarking methods, this study will hopefully provide a helpful foundation for future sentinel bolt operations to assess the establishment and effectiveness of EAB biocontrol efforts in North Carolina.

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REFERENCES

Abell, K. J., L. S. Bauer, D. L. Miller, J. J. Duan, and R. G. Van Driesche. 2016. Monitoring the establishment and flight phenology of parasitoids of emerald ash borer (Coleoptera: Buprestidae) in Michigan by using sentinel eggs and larvae. The Florida Entomologist 99(4): 667–672.

Bauer, L. S., R. A. Haack, D. L. Miller, T. R. Petrice, and H. Liu. 2004. Emerald ash borer life cycle, p. 8. *In* V. Mastro and R. Reardon (comps.), Emerald Ash Borer Research and Technology Development Meeting, 30 September–1 October 2003, Port Huron, MI. Publication FHTET-2004-02. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Bauer, L. S., J. J. Duan, J. R. Gould, and R. Van Driesche. 2015. Progress in the classical biological control of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) in North America. Canadian Entomologist 147(3): 300–317.

Bolen, A. 2020. A silent killer: Black ash basket makers are battling a voracious beetle to keep their heritage alive. https://www.americanindianmagazine.org/story/black-ash-basketry. Accessed 8 May 2022.

Cappaert, D., D. G. McCullough, T. M. Poland, and N. W. Siegert. 2005. Emerald ash borer in North America: A research and regulatory challenge. American Entomologist 51(3): 152–165.

Davidson, W., and L. K. Rieske. 2016. Establishment of classical biological control targeting emerald ash borer is facilitated by use of insecticides, with little effect on native arthropod communities. Biological Control 101: 78–86.

Duan, J. J., L. S. Bauer, R. G. Van Driesche, and J. R. Gould. 2018. Progress and challenges of

protecting North American ash trees from the emerald ash borer using biological control. Forests 9(3): 142.

Duan, J. J., R. G. Van Driesche, J. Schmude, R. Crandall, C. Rutlege, N. Quinn, B. H. Slager, J. R. Gould and J. S. Elkinton. 2022. Significant suppression of invasive emerald ash borer by introduced parasitoids: Potential for North American ash recovery. Journal of Pest Science 95: 1081–1090.

Emerald Ash Borer Information Network. 2022. About emerald ash borer. http://www.emeraldashborer.info/about-eab.php. Accessed 25 Apr 2022.

Flower, C. E., K. S. Knight, and M. A. Gonzalez-Meler. 2013. Impacts of the emerald ash borer (*Agrilus planipennis* Fairmaire) induced ash (*Fraxinus* spp.) mortality on forest carbon cycling and successional dynamics in the eastern United States. Biological Invasions 15(4): 931–944.

Herms, D. A., and D. G. McCullough. 2014. Emerald ash borer invasion of North America: History, biology, ecology, impacts, and management. Annual Review of Entomology 59(1): 13–30.

Hooie, N. A., G. J. Wiggins, P. L. Lambdin, J. F. Grant, S. D. Powell, and J. P. Lelito. 2015. Native parasitoids and recovery of *Spathius agrili* from areas of release against emerald ash borer in eastern Tennessee, USA. Biocontrol Science and Technology 25(3): 345–351.

Jennings, D. E., J. J. Duan, D. Bean, J. R. Gould, K. A. Rice, and P. M. Shrewsbury. 2016. Monitoring the establishment and abundance of introduced parasitoids of emerald ash borer larvae in Maryland, U.S.A. Biological Control 101: 138–144.

Jones, M. I., J. R. Gould, H. J. Mahon, and M. K. Fierke. 2020. Phenology of emerald ash borer

(Coleoptera: Buprestidae) and its introduced larval parasitoids in the northeastern United States. Journal of Economic Entomology 113(2): 622–632.

Kovacs, K. F., R. G. Haight, D. G. McCullough, R. J. Mercader, N. W. Siegert, and A. M. Liebhold. 2010. Cost of potential emerald ash borer damage in U.S. communities, 2009–2019. Ecological Economics 69(3): 569–578.

Liu, H., L. S. Bauer, D. L. Miller, T. Zhao, R. Gao, L. Song, Q. Luan, R. Jin, and C. Gao. 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biological Control 42(1): 61–71.

MapBioControl. 2020. https://www.mapbiocontrol.org. Accessed 4 Jan 2022.

Parisio, M. S., J. R. Gould, J. D. Vandenberg, L. S. Bauer, and M. K. Fierke. 2017. Evaluation of recovery and monitoring methods for parasitoids released against emerald ash borer. Biological Control 106: 45–53.

Poland, T. M., and D. G. McCullough. 2006. Emerald ash borer: Invasion of the urban forest and the threat to North America's ash resource. Journal of Forestry 104(3): 118–124.

Quinn, N. F., J. S. Gould, C. E. Rutledge, A. Fassler, J. S. Elkinton, and J. J. Duan. 2022. Spread and phenology of *Spathius galinae* and *Tetrastichus planipennisi*, recently introduced for biocontrol of emerald ash borer (Coleoptera: Buprestidae) in the northeastern United States. Biological Control 165: 104794.

Ragozzino, M. L. 2020. Influences of climate, competition, and novel hosts on parasitoids of emerald ash borer, and their establishment in Virginia and North Carolina. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg.

Rebek, E. J., D. A. Herms, and D. R. Smitley. 2008. Interspecific variation in resistance to

emerald ash borer (Coleoptera: Buprestidae) among North American and Asian ash (*Fraxinus* spp.). Environmental Entomology 37(1): 242–246.

Rutledge, C. E., R. G. Van Driesche, and J. J. Duan. 2021. Comparative efficacy of three techniques for monitoring the establishment and spread of larval parasitoids recently introduced for biological control of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae). Biological Control 161: 104704.

Stephens, J. P., K. A. Berven, and S. D. Tiegs. 2013. Anthropogenic changes to leaf litter input affect the fitness of a larval amphibian. Freshwater Biology 58(8): 1631–1646.

USDA–APHIS/ARS/FS. 2020. Emerald ash borer biological control release and recovery guidelines. USDA–APHIS–ARS–FS, Riverdale, MD.

Villari, C., D. A. Herms, J. G. A. Whitehill, D. Cipollini, and P. Bonello. 2016. Progress and gaps in understanding mechanisms of ash tree resistance to emerald ash borer, a model for wood-boring insects that kill angiosperms. The New Phytologist 209(1): 63–79.

Wagner, D. L., and K. J. Todd. 2016. New ecological assessment for the emerald ash borer: A cautionary tale about unvetted host-plant literature. American Entomologist 62(1): 26–35.

Wang, X.-Y., Z.-Q. Yang, J. R. Gould, Y.-N. Zhang, G.-J. Liu, and E.-S. Liu. 2010. The biology and ecology of the emerald ash borer, *Agrilus planipennis*, in China. Journal of Insect Science 10(128): 1–23.

APPENDICES

Appendix A

Assessment of Canopy Health in Green Ash Stands Infested by Emerald Ash Borer in Central North Carolina

MATERIALS AND METHODS

Surveys of ash tree health and mortality were conducted in Stands 2 and 3 of the Wrenn Road Facility in Garner, North Carolina (35.6437, -78.5802) on 26 July 2021. Green ash trees (*Fraxinus pennsylvanica* Marshall) were assessed along two intersecting transects running across each stand. Transects were arranged between opposite corners of the stands and avoided areas of non-ash vegetation. Transects 1 and 2 in Stand 2 were 260 m and 270 m in length, respectively. Transects 1 and 2 in Stand 3 were 280 m and 170 m in length, respectively. Trees within 5 m of either side of the transects were included in the surveys, and care was taken to avoid double counting trees where the transects crossed. Each tree included in the surveys was assigned a canopy condition rating developed by Smith (2006) for the assessment of canopy health of trees attacked by emerald ash borer. This rating system uses a five-point scale of canopy health, with a rating of 1 indicating a full, healthy canopy and a rating of 5 indicating a completely dead canopy with no foliage (Smith 2006, Flower et al. 2013, Knight et al. 2014). Ratings were assigned based on photographs and descriptions provided in Figure 2 and Table 1 in Knight et al. (2014) (Table 1). The percentage of trees assigned to each rating was calculated for each transect, stand, and across both stands, and a 95% confidence interval was calculated for each percentage.

Table 1. Canopy condition rating system used for the ash health assessment. Table from Knight et al. (2014).

Rating	Description
1	Canopy is full and healthy
2	Canopy has started to lose leaves (thinning), but no dieback (dead top canopy twigs without leaves) is present
3	Canopy has less than 50% dieback
4	Canopy has more than 50% dieback
5	Canopy has no leaves, epicormic sprouts may be present on the trunk

RESULTS

A total of 549 and 333 green ash trees were surveyed in Stands 2 and 3, respectively (Table 2). 91.62% (± 2.32%) of trees in Stand 2 and 88.29% (± 3.45%) of trees in Stand 3 showed complete canopy mortality without any live foliage (canopy condition rating of 5) (Table 3). Only 2.91% (± 1.41%) of trees in Stand 2 and 3.00% (± 1.83%) of trees in Stand 3 showed no dieback (canopy condition rating of 1 or 2) as defined by Knight et al. (2014) (Table 1).

Table 2. Counts of surveyed green ash trees assigned to each canopy condition rating.

	Canopy Condition Rating					
	1	2	3	4	5	**Total**
Stand 2	**8**	**8**	**18**	**12**	**503**	**549**
Transect 1	3	4	11	6	226	250
Transect 2	5	4	7	6	277	299
Stand 3	**5**	**5**	**14**	**15**	**294**	**333**
Transect 1	5	4	10	8	196	223
Transect 2	0	1	4	7	98	110
Total	**13**	**13**	**32**	**27**	**797**	**882**

Table 3. Percentages of surveyed green ash trees assigned to each canopy condition rating.

	Canopy Condition Rating				
	1	2	3	4	5
Stand 2	**1.46%**	**1.46%**	**3.28%**	**2.19%**	**91.62%**
Transect 1	1.20%	1.60%	4.40%	2.40%	90.40%
Transect 2	1.67%	1.34%	2.34%	2.01%	92.64%
Stand 3	**1.50%**	**1.50%**	**4.20%**	**4.50%**	**88.29%**
Transect 1	2.24%	1.79%	4.48%	3.59%	87.89%
Transect 2	0.00%	0.91%	3.64%	6.36%	89.09%
Total	**1.47%**	**1.47%**	**3.63%**	**3.06%**	**90.36%**

REFERENCES

Flower, C. E., K. S. Knight, J. Rebbeck, and M. A. Gonzalez-Meler. 2013. The relationship between emerald ash borer (*Agrilus planipennis* Fairmaire) and ash (*Fraxinus* spp.) tree decline: Using visual ash condition assessments and leaf isotope measurements to assess pest damage. Forest Ecology and Management 303: 143–147.

Knight, K. S., B. P. Flash, R. H. Kappler, J. A. Throckmorton, B. Grafton, and C. E. Flower. 2014. Monitoring ash (*Fraxinus* spp.) decline and emerald ash borer (*Agrilus planipennis*) symptoms in infested areas. Gen. Tech. Rep. NRS-139. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. 18 p.

Smith, A. 2006. Effects of community structure on forest susceptibility and response to the emerald ash borer invasion of the Huron River watershed in southeast Michigan. M.S. thesis, Ohio State University, Columbus.

Appendix B

Wood-Boring Insects Recovered from Green Ash Stands Infested by Emerald Ash Borer in Central North Carolina

Table 1. Wood-boring insect species recovered from debarked ash trees (DB), purple prism traps (Prism), green Lindgren multifunnel traps (Funnel), yellow pan traps (YPT), and ash logs in emergence cages (EC) from the Wrenn Road Facility in Garner, North Carolina. DB, Prism, and Funnel methods are described in Chapter 2. YPT and EC methods are described in Chapter 3.

	Recovery Method				
	DB	**Prism**	**Funnel**	**YPT**	**EC**
COLEOPTERA					
Brentidae (straight-snouted weevils)					
Arrenodes minutus (oak timberworm)			x		
Buprestidae (jewel beetles)					
Agrilus planipennis (emerald ash borer)	x	x	x		x
Dicerca sp.			x		
unidentified buprestid species		x			
Cerambycidae (longhorned beetles)					
Elaphidion mucronatum (spined oak borer)			x		
Neoclytus acuminatus (redheaded ash borer)			x	x	
Tylonotus bimaculatus (ash and privet borer)	x		x	x	x
unidentified cerambycid species			x		
unidentified cerambycid species			x		
unidentified cerambycid species			x		
Curculionidae (snout and bark beetles)					
Cnestus mutilatus (camphor shot borer)			x		
Euplatypus compositus (pinhole borer)				x	
Hylesinus sp. (ash bark beetle)					x
Scolytinae, cf. *Xylosandrus*			x		x
Trogossitidae (bark-gnawing beetles)*					
*Temnoscheila acuta/virescens**			x	x	

*Predators of wood borers

Table 1 (continued).

LEPIDOPTERA					
Sesiidae (clearwing moths)					
Podosesia syringae (lilac borer)	x				

Appendix C

Previous Releases of Emerald Ash Borer Parasitoids at Sites Monitored for Establishment in Granville and Wayne Counties, North Carolina

Table 1. Previous releases of emerald ash borer parasitoids at the NW Granville site in Granville County, North Carolina (MapBioControl 2020).

	Number of Parasitoids Released			
Release Date	***Oobius agrili***	***Spathius agrili***	***Spathius galinae***	***Tetrastichus planipennisi***
23-Sep-13	0	0	0	300
26-Sep-13	0	0	0	300
30-Sep-13	0	0	0	600
7-Oct-13	0	0	0	600
7-Oct-13	0	0	0	1325
11-Oct-13	0	0	0	1325
14-Oct-13	0	0	0	1800
18-Oct-13	0	0	0	1800
21-Oct-13	0	0	0	2250
24-Oct-13	0	0	0	2250
4-Nov-13	0	0	0	2000
6-Nov-13	0	0	0	2000
17-Apr-14	0	500	0	1700
21-Apr-14	0	200	0	1200
24-Apr-14	0	200	0	1200
28-Apr-14	0	0	0	0
5-May-14	0	200	0	450

Table 1 (continued).

8-May-14	0	200	0	450
12-May-14	650	200	0	534
16-May-14	715	200	0	600
2-Jun-14	1300	260	0	1185
4-Jun-14	1300	260	0	1185
9-Jun-14	300	0	0	794
13-Jun-14	300	80	0	830
30-Jun-14	200	0	0	796
1-Jul-14	200	0	0	796
28-Jul-14	534	0	0	434
1-Aug-14	179	0	0	434
22-Sep-14	0	0	0	1472
24-Sep-14	0	0	0	1239
20-May-15	0	210	0	400
25-Jun-15	950	272	0	1000
30-Jul-15	350	0	0	800

Table 2. Previous releases of emerald ash borer parasitoids at Island Creek Wildlife Management Area in Granville County, North Carolina (MapBioControl 2020).

	Number of Parasitoids Released			
Release Date	***Oobius agrili***	***Spathius agrili***	***Spathius galinae***	***Tetrastichus planipennisi***
23-Sep-13	0	0	0	300
26-Sep-13	0	0	0	300
30-Sep-13	0	0	0	600
7-Oct-13	0	0	0	600
7-Oct-13	0	0	0	1350
11-Oct-13	0	0	0	1350
14-Oct-13	0	0	0	1800
18-Oct-13	0	0	0	1800
21-Oct-13	0	0	0	2250
24-Oct-13	0	0	0	2250
4-Nov-13	0	0	0	2000
6-Nov-13	0	0	0	2000
15-Apr-14	0	500	0	1700
21-Apr-14	0	200	0	1200
24-Apr-14	0	200	0	1200
28-Apr-14	0	0	0	0
5-May-14	0	200	0	400
8-May-14	0	200	0	400
12-May-14	650	200	0	533

Table 2 (continued).

16-May-14	600	200	0	500
2-Jun-14	1300	260	0	1185
4-Jun-14	1300	260	0	1185
9-Jun-14	300	200	0	793
13-Jun-14	300	60	0	775
30-Jun-14	200	0	0	796
1-Jul-14	200	0	0	796
28-Jul-14	0	0	0	434
1-Aug-14	179	0	0	434
22-Sep-14	0	0	0	1473
24-Sep-14	0	0	0	1239
20-May-15	0	210	0	400
25-Jun-15	950	272	0	1000
30-Jul-15	350	0	0	800
17-Apr-19	0	0	0	1700

Table 3. Previous releases of emerald ash borer parasitoids at the Little River site in Wayne County, North Carolina (MapBioControl 2020).

	Number of Parasitoids Released			
Release Date	***Oobius agrili***	***Spathius agrili***	***Spathius galinae***	***Tetrastichus planipennisi***
7-Jul-15	600	205	0	1956
29-Jul-15	350	0	0	1508
8-Oct-15	0	0	0	1227
15-Oct-15	0	0	0	1230
22-Jun-16	400	0	0	451
18-Aug-16	1200	0	0	703
23-May-17	200	231	0	313

Table 4. Previous releases of emerald ash borer parasitoids at Cherry Research Station in Wayne County, North Carolina (Ragozzino 2020).

	Number of Parasitoids Released			
Release Date	***Oobius agrili***	***Spathius agrili***	***Spathius galinae***	***Tetrastichus planipennisi***
8-Sep-16	0	0	0	1004
22-Aug-17	0	212	215	0

REFERENCES

MapBioControl. 2020. https://www.mapbiocontrol.org. Accessed 4 Jan 2022.

Ragozzino, M. L. 2020. Influences of climate, competition, and novel hosts on parasitoids of emerald ash borer, and their establishment in Virginia and North Carolina. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg.