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An Evaluation of Potential Biocontrol Agents for Anoplophora Glabripennis (Coleoptera: Cerambycidae) in South Carolina, U.S.A.

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AN EVALUATION OF POTENTIAL BIOCONTROL AGENTS FOR *ANOPLOPHORA GLABRIPENNIS* (COLEOPTERA: CERAMBYCIDAE) IN SOUTH CAROLINA, U.S.A.

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
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Master of Science
Forest Resources

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by
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ABSTRACT

The Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky, hereafter ALB) was named one of the world's top 100 worst invasive species by the Global Invasive Species Database, as it threatens several species of hardwood trees in North America. In May of 2020, the southernmost infestation of ALB in North America was discovered near Hollywood, South Carolina, U.S.A. Current eradication efforts focus on tree removal; however, in ecosystems with rugged or flooded terrain, or otherwise inaccessible and vulnerable natural areas, tree removal is a costly and potentially environmentally damaging endeavor that may not be feasible as a management tactic. In these situations, biological control may be an economically and ecologically advantageous management strategy in the ongoing efforts to eradicate ALB. Three methods were used to determine rates of parasitization from naturally occurring parasitoids on ALB larvae: infested material collection, sentinel log deployment, and releases of a native parasitoid, *Ontsira mellipes* Ashmead. Very low natural parasitization rates occurred. In fact, no ALB larvae were parasitized by native parasitoids out of the 224 ALB larvae extracted from infested material and sentinel logs. After *O. mellipes* releases, 2 out of 56 ALB larvae were confirmed to be parasitized by the wasp. This represents the first field validation of *O. mellipes* as a potential ALB biocontrol agent. Overall, natural populations of parasitoids do not appear to be sufficient for management of ALB in South Carolina, though targeted field releases of *O. mellipes* may have potential as a supplemental management tactic.

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CHAPTER ONE

OVERVIEW OF THE IMPACTS OF INVASIVE SPECIES IN FOREST SYSTEMS AND THE ASIAN LONGHORNED BEETLE IN SOUTH CAROLINA

Invasive species global impact

Throughout history invasive species issues have arisen because of colonization, economic development, and the increase in our global economy (Gentili et al. 2021). Items are transported all over the world for a plethora of reasons by aircraft, ships, railways, and other methods, all which all have potential to move organisms on or in a vector without detection (e.g. Greenwood et al. 2023). Invasive species – those which are the most impactful non-native species – are responsible for a conservatively estimated global impact of $1.29 trillion over the course of the last several decades (Diagne et al. 2021). This includes both the cost of the damage inflicted on the global economy and cost of the management protocols enacted to mitigate their effects (Diagne et al. 2021).

Forests in the eastern U.S. have endured catastrophic changes and losses since European settlement (Thompson et al. 2013). A major contributing factor to this has been the introduction and establishment of invasive species, which can outcompete native species or directly damage forest species – sometimes resulting in functional extinction. The total impact of invading pests and diseases on ecosystems is not fully understood, as most of these cases have been documented thoroughly only in the past century (Gentili et al. 2021). Invasive species can have significant negative effects on native biodiversity, thereby reducing ecosystem resiliency when facing other environmental catastrophes (McMillan et al. 2023). *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), *Sirex noctilio* F. (Hymenoptera: Siricidae), *Anoplophora* *glabripennis* Motschulsky (Coleoptera: Cerambycidae), and *Adelges tsugae* Annand (Hemiptera: Adelgidae) are all invasive forest pests which have shown this potential (Davidson et, al. 1999, Herms and McCullough 2014, Dodds and de Groot 2011, Havill et al. 2021, Ellison et al. 2018, Wear and Greis 2013). A classic example is the spongy moth, *Lymantria dispar* L. (Lepidoptera: Erebidae), which feeds on over 18 species of trees, including those in the genera *Betula*, *Larix*, *Liquidambar*, *Populus*, *Pyrus*, *Quercus*, *Salix*, *Tilia*, *Carpinus*, *Corylus*, *Crataegus*, *Hamamelis*, and *Ostrya* (Davidson et al. 1999). When forest stands are mixed with resistant genera, the frequency, duration, and intensity of invasive species outbreaks may be suppressed. Initial outbreaks of *L. dispar* in susceptible stands (predominantly consisting of *Quercus*) resulted in a 43% total tree mortality rate, and the leading edge of these infestations are deemed more devastating than other infested areas or subsequent outbreaks (Davidson et al. 1999). The balsam wooly adelgid, *Adelges piceae* Ratzeburg (Hemiptera: Adelgidae), has eliminated 95% of mature *Abies fraseri* (Pursh) Poir (Pinales: Pinaceae), and if trends continue with no management, natural stands could be gone within 50 years (Wear and Greis, 2013). This same scenario is true for *Tsuga,* where trees in this genus generally die within 5 years of *A*. *tsugae* infestation; *Tsuga* may be absent from southern forests within the next 50 years (Wear and Greis 2013). *Agrilus planipennis* has been arguably the most devastating invasive forest pest in North America. Its feeding has almost eliminated an entire genus of trees (*Fraxinus*) from urban and rural landscapes across the continent. Only one *Fraxinus* species, *F. quadrangulata* Michx., has shown moderate resistance (Herms and McCullough 2014). In North America, *A*. *planipennis* has infested *Fraxinus* trees in 36 states and 5 provinces. The predicted costs from *A. planipennis* damage and economic effects were one billion U.S. dollars in treatment, removal, and replacement of trees, per year from 2009 to 2019 (Kovacs et al. 2010).

Asian longhorned beetle

One of the most destructive pests introduced to North America is the Asian longhorned beetle (*Anoplophora glabripennis*, or ALB). Originally from China and the Korean peninsula, it was named one of the top 100 worst invasive species in the world and became widely distributed in Europe and North America by the late 1900s (Global Invasive Species Database 2020, Byeon et al. 2021, Coyle et al. 2021). *Populus* monocultures were planted in China in the late 1900s for windbreaks, and this abundance of a preferred host may have contributed to high concentrations of ALB populations prior to their transport to the U.S. (Greenwood et al. 2023). Asian longhorned beetle was first detected in North America in Brooklyn, NY, in August 1996 and it is thought to have arrived in the 1980's in solid wood packing material (SWPM) from China (Haack et al. 1997). SWPM includes wooden pallets, wooden packaging spools, crates, and dunnage (Greenwood et al. 2023). Asian longhorned beetle is currently found in Worcester County, MA; Suffolk and Nassau Counties in NY; Clermont County, OH; and most recently Charleston and Dorchester Counties, SC (Figure 1.1). Infested areas in which ALB was eradicated include several in Illinois, all of New Jersey, several in New York, Boston in Massachusetts, several in Ohio, and in Ontario, Canada (USDA Federal Quarantine 2024, Turgeon et al. 2022).

Asian longhorned beetle biology

In ALB’s native range, *Populus* and *Salix* are preferred hosts, however, in North America *Acer* is preferred*,* particularly *A*. *rubrum* in South Carolina (Coyle et al. 2021). Despite its preferences, ALB can exploit hosts in several different genera including *Aesculus*, *Betula*, *Albizia*, *Cercidiphyllum*, *Fraxinus*, *Koelreuteria*, *Platanus*, *Sorbus*, and *Fagus*. Because ALB is a polyphagous xylophage, most damage is inside the stem and branches. External damage (exit holes and egg niches) may be visible and detected by ground surveyors with binoculars. They typically start to infest the upper canopy and move their way into the main stem over time. Adults feed minimally on the midrib of leaves and are active from May-October in South Carolina (Coyle et al. 2021, Schmitt 2023). Most of its life span (1 year in South Carolina, Schmitt 2023) is spent in the xylem of host trees, and this larval feeding in the phloem and xylem compromises the structural integrity of the tree (Figure 1.8) (Hu et al. 2009, Schmitt 2023). Asian longhorned beetle is a holometabolous insect with four life stages (Figure 1.2). Adult females chew the outer bark layer and oviposit their eggs outside of the cambium in the fall. Eggs hatch and overwinter as larvae. Asian longhorned beetle may be univoltine or bivoltine depending on the region and may have a generation in which the larvae do not overwinter (Schmitt 2023). After pupation, the pupae darken and prepare to exit by chewing a 6-18 mm perfect circle in the bark (Lingafelter and Hoebeke 2002). The adults range from 17-39 mm in length, with females usually being larger and with a smaller antenna to body ratio (Meng 2001).

Asian longhorned beetle impacts and management

Asian longhorned beetle is a major pest due to its propensity to infest healthy trees, with the potential to cause the loss of 35% of urban tree cover across the U.S., which would have a $670 billion U.S. dollar impact in the U.S. (Global Invasive Species Database 2020, Pedlar et al. 2020). Asian longhorned beetle can disperse between 106 to 1000 meters per year, depending on environmental factors and resource availability (Hu et al. 2006). Typical city planning regarding tree diversity and proximity has proven to increase probability of accelerating pest and disease dispersal throughout history, e.g. in the cases of *Ulmus* and *Fraxinus* monoculture plantings along streets (Hu et al. 2009, Faccoli et al. 2016). It creates an abundant food source for invading pests, accelerating their spread and population growth. *Acer rubrum*, due to its resiliency and ability to tolerate a wide range of environmental conditions, has been widely planted to replace these genera targeted by invasive pests and pathogens in the past (Burns 1990).

In May of 2020, the southernmost discovery of an ALB population in North America occurred in Hollywood, SC, U.S. It was presumably introduced to via infested material, and genetic analysis revealed this population closely matches the population in Ohio (Coyle et al. 2021). Current eradication efforts are dominated by tree removal in heavily infested areas, though alternative management strategies are being investigated (Ratcliff 2022). For high value susceptible tree species not yet infested, imidacloprid may be applied via basal trunk injection or soil injection (APHIS 2011).

Biological control as an invasive species management strategy

The Enemy Release Hypothesis suggests that when invasive species become established in a new area, they are freed from the predation, parasitism, and disease pressures faced in their native range. This can result in the invasive species quickly colonizing hosts and extirpating native species in invaded areas. Managing invasive species through biological control seeks to fill those empty niches with organisms that target the pest while causing minimal damage to non-target species (Lockwood 2011). A biological control agent is defined as a predator, parasite, parasitoid, or pathogen that will target and reduce the population or fecundity of a pest species. In a classical biological control approach, the biocontrol agent may be imported from the pest's native range (Lockwood 2011). Control rates of the invasive pest in the new range varies widely. For example, in the case of *A. planipennis*, native parasitism rates were less than 5% in some areas of Michigan (Duan et al. 2023), but there have been records in Michigan and Ontario's most infested areas seeing 71% and 40% parasitism by the native generalists *Atanycolus capperti* Marsh and Strazanac (Hymenoptera: Braconidae) and *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), respectively (Duan et al. 2023). *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), and *Spathius galinae* Belokobylskij and Strazanac (Hymenoptera: Braconidae) are three parasitoids from China which are being released through a federal biological control program and have established in parts of the *A. planipennis* invasive range (Duan et al. 2023). *Spathius agrili* Yang is another non-native parasitoid released to combat *A. planipennis* as it has a higher tolerance to warmer temperatures; however, it has not been sufficiently recovered to suggest establishment (Duan et al. 2023).

Asian longhorned beetle potential for biological control

In ecosystems with rugged, flooded terrain, or otherwise inaccessible and vulnerable forests, tree removal for ALB eradication is a costly and environmentally damaging endeavor that may not be able to solely eradicate the target pest population and to prevent further spread. In these cases, biological control may be an economically and ecologically advantageous management strategy in the ongoing efforts to eradicate ALB (Coyle et al. 2021).

In historical cases of invasive species management involving biological control, the controlling agent co-evolved with the invasive pest in its native range and is imported to the invasive range for release. This type of biocontrol agent is typically accepted as most effective due to their evolutionary relationships. Native parasitoids in the invaded region may not recognize the invasive species or have the means to overcome host defenses. For these reasons, biologists may be reluctant to choose native species as potential biocontrol agents, except in cases where the pest lacks a successful controlling agent in its native region.

In the case of ALB, there are a few potential natural enemies. In China and the Korean peninsula, woodpeckers (D*endrocopos major* L. [Piciformes: Picidae] and *Picus canus* Gmelin [Piciformes: Picidae]) are the main predators of ALB (Haack 2010). Asian longhorned beetle has one potential native fungal pathogen with potential as a mycoinsecticide in the US: *Metarhizium brunneum* Petch (Hypocreales: Clavicipitaceae). The high humidity and warmer climate in the South may be more conducive to its application than in the northeast U.S. There is a 100% mortality rate in the laboratory trials using *M. brunneum* F52, which is currently available for commercial purchase in the U.S. (Clifton et al. 2020). In lab flight tests, the fungus decreased flight capacity of ALB females (Clifton et al. 2019).

Introducing foreign biocontrol components takes years, has a high cost, and is legally required to go through rigorous risk assessment prior to consideration for release (Barratt et al. 2009). Therefore, using native species in biocontrol programs allows managers to expedite the process from research to implementation in the field. Augmentative biological control programs eliminate the risk of the agent becoming invasive, hybridizing with native species, or having a devastating, unprecedented ecological effect (Collier and Steenwyk 2004).

Ontsira mellipes

One candidate for biological control of ALB in North America is the native ectoparasitoid wasp *Ontsira mellipes* Ashmead (Hymenoptera: Braconidae). It is distributed across North America and its habitat ranges from woodlands to meadows (Kula and Marsh 2014). *Ontsira mellipes* adults oviposit on host larvae just beneath the bark of trees (Figures 1.3, 1.4). Eggs hatch in 1-2 days and the host larvae become paralyzed. After going through five instars, the wasp larva forms a golden pubescent pupa inside a cocoon. Adults emerge about 3 weeks after the eggs are laid (Figure 1.7) (Golec et al. 2016). Golec et al. (2016), showed that *O. mellipes* females produced 25.6 ± 4.6 (mean ± SE) progeny throughout their lives, and 7.8 ± 0.8 offspring per ALB larva. The female to male ratio was 6:1 over 50 generations. Female median longevity was 17 days, and male median longevity was 18 days. It may take up to 48 hours for host larvae to become completely immobilized. *Ontsira mellipes* has five instars. The first instar is translucent, bearing apparent antennae which gradually reduce with each instar. The second instar is opaque white. The third instar is cream or yellow in color. In the fourth instar, dorsal ridges became apparent and remain until the fifth instar. Larvae pupate 12 days after oviposition (Figure 1.7).

Ontsira mellipes is the most commonly emerging parasitoid across various hardwood species with cerambycid infestations, and exclusively targets cerambycids (Golec et al. 2020, Duan et al. 2016). It showed no host preference when compared with six common North American cerambycids (*Elaphidion mucronatum* Say*,* *Monochamus carolinensis* Olivier*, M. notatus* Drury*, Neoclytus scutellaris* Olivier*, Xylotrechus colonus* Fabricius*, and X. sagittatus* Germar) and one Asian cerambycid (*Anoplophora chinensis* Forster [Coleoptera: Cerambycidae])*. Ontsira mellipes* attacked ALB and *M*. *caroliniensis* at the same rates and higher than the rest of the cerambycids tested (Wang et al. 2019). *Ontsira mellipes* ovipositors are <4 mm in length, potentially limiting parasitization of larvae that are deeper into the xylem (Wang et al. 2019). This suggests that ideal releases should occur with early instar ALB, when they are closest to the bark. Further, parasitization rates increased as the host size of ALB larvae increased (Wang et al. 2019).

In a laboratory setting, 21% of *O. mellipes* pairs (1:1 M: F) were successful in parasitizing ALB larvae. Golec et al. (2020) states that of those that are successful, they parasitized 15 to 46% (mean of 35%) of larvae that were presented to them throughout their lifetimes. It took a median of 23 days for eggs to reach adulthood in favorable conditions. In this laboratory study, 69% of *O. mellipes* larvae were able to reach adulthood on ALB larvae. With no larvae, water, or honey, male adults only lived 5 days, and females lived 8 days (Golec et al. 2016). *Ontsira mellipes* has only been shown to parasitize ALB in the laboratory thus far, and field trials have yet to take place.

Thesis objectives

My thesis objective was to determine if biological control could be a viable component of an integrated pest management plan for ALB in South Carolina. Specifically, my first objective was to determine if any native organisms parasitize ALB larvae in natural conditions in the South Carolina infested area. My second objective was to determine whether targeted releases of *O. mellipes* is a viable option for ALB biocontrol in South Carolina.

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CHAPTER TWO

FIELD SURVEYS FOR NATIVE PARASITOIDS OF THE ASIAN LONGHORNED BEETLE IN SOUTH CAROLINA

Introduction

All classical biological control endeavors require a thorough search for potential predators, parasitoids, and pathogens before candidates can be chosen for mass rearing and release. Biocontrol projects have proven successful in the past, and most of these successes have been through the introduction of specialists (Stiling and Cornelissen 2005). In a meta-analysis using biocontrol agents from all taxa, there was a 130% decrease in pest abundance and a 159% increase in mortality of target pests compared to control groups (Stiling and Cornelissen 2005). How to define success in biological control has been widely debated as there are many taxa that are utilized – including plants, fungi, insects, and microorganisms – and the question of success is answered when the goals are understood for each respective system (Van Klinken et al. 2006). In the system with *Agrilus plannipennis* Fairmaire [emerald ash borer (EAB); Coleoptera: Buprestidae], the goal of biocontrol is to establish a population of host specific parasitoids that will significantly decrease the population of EAB. Using these criteria, it is a system that has begun to see success. Four parasitoids from EAB's native range have been reared and releases in the U.S. Of these, the egg parasitoid, *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), and two larval parasitoids, *Tetrastichus plannipennisi* Yang (Hymenoptera: Eulophidae) *and Spathius galinae*

Belokobylskij and Strazanac (Hymenoptera: Braconidae), have been consistently recovered in the field two years after their release, meeting one of these criteria (Duan et al. 2023). *Oobius agrili* has been established and spreading, with a parasitism of 1-4% from 2008 to 2011 and 28% by 2014, indicating success. *Tetrastichus plannipennisi* showed similar results, with a 30% parasitism rate in release plots by 2014 (Duan et al. 2023). Efforts to release and recover *Spathius agrili* Yang (Hymenopera: Braconidae) are ongoing. In areas of release and successful establishment, such as Michigan, the mortality of *Fraxinus* spp. has been significantly reduced (Duan et al. 2023). There is some evidence which suggests that a multi-species approach can decrease pest population abundance and increase pest mortality when compared to single species releases (Stiling and Cornelissen 2005).

The Asian longhorned beetle, *Anoplophora glabripennis* Motschulsky (ALB; Coleoptera: Cerambycidae), is a xylophagous invasive insect native to China and the Korean peninsula. Asian longhorned beetle was first discovered in the U.S. in New York in 1996 and has been since discovered in several areas in eastern North America, including South Carolina in 2020 (Coyle et al. 2021). It can use 12 major tree genera in the U.S.. as hosts, but prefers *Acer, Betula, Populus, Salix,* and *Ulmus* (Wang 2015). Management for ALB involves monitoring and tree removal. Chemical treatments using imidacloprid are reserved for high value trees. Asian longhorned beetle has been eradicated from several areas in North America (USDA Federal Quarantine 2024). However, the ability to eradicate ALB may be more difficult in South Carolina, as a warmer climate may facilitate faster ALB population growth and the region includes many swampy areas, inhibiting access by heavy machinery to conduct tree removal

(Krout et al. 2023). Biocontrol may provide supplemental support to the management plan in South Carolina and potentially to infested areas in other states.

While many parasitoids have been observed on ALB in its native range, there has not been sufficient evidence to suggest they are appropriate candidates for biocontrol in China or the U.S.. (Golec et al. 2016). An ideal candidate for biocontrol typically occupies a small niche and is as host specific as possible. Duan et al. (2016) examined in-lab parasitism rates of several North American braconid species on ALB larvae and found that *Ontsira mellipes* Ashmead (Hymenoptera: Braconidae) parasitized 43% of the larvae on which it was tested, *Rhoptrocentrus piceus* Marshall 23%, *Spathius laflammei* Provancher 47%, *Heterospilus* spp. 32%, and *Atanycolus* spp. 32%. However, we do not know the rates at which these candidates parasitize ALB in South Carolina, and there is no record of other native parasitoids in South Carolina that may be using ALB as a host. Of the parasitoids tested, *O. mellipes* is the only braconid known to exclusively attack cerambycids (Duan et al. 2016). In South Carolina, a survey for ALB biocontrol agents has never been conducted.

The objective of this study was to identify naturally occurring parasitoids of ALB in the South Carolina infestation using infested tree collections and sentinel bolts, and to quantify their rate of parasitization compared to rates in a published laboratory study (Duan et al. 2016). This work will serve as an early assessment of whether biocontrol using native or naturalized parasitoids might be an effective management tactic for ALB in South Carolina.

Methods

Study location. The South Carolina ALB quarantine zone is in Charleston and Dorchester counties, totaling 197.8 km² (Figure 2.1). The quarantine zone is situated in a subtropical coastal wetland with mixed softwood and hardwood forests (Table 2.1). Summers are hot and humid, and the winters are relatively mild and breezy. The recorded high and low temperatures for Charleston in 2023 were 36.7 °C and -2.8°C, respectively (NCEI 2023). From 2010 to 2019, the 10-year average low was 3°C in January and the average high was 33°C in July (Charleston SC Average Temperatures by Month, n.d.). Charleston has an average annual precipitation of 113 cm, calculated from 2006-2020 (NCEI 2024).

Parasitoid survey using infested material. Twenty-one infested *Acer rubrum* L. (Sapindaceae) trees within the quarantine zone were collected from three different sites: the USDA Stono Facility (32.744451, -80.181749), Royal Harbor Road (32.777707, -80.136601), and McCombs Road (32.780365, -80.145751). Trees were felled and inspected in 2022 and 2023 (Table 2.1). Trees exhibiting signs of ALB infestation, categorized by weeping sap, exit holes, egg niches, and cracked bark indicating galleries (Figure 1.3, 1.4, 1.5 and 1.6), were chosen for sampling. Trees were then cut into ~1 m long sections and those with damage (indicating potential larvae) were transported to the USDA Stono Facility. All ALB life stages were extracted using a log splitter (Central Machinery, Calabasas, CA), chisel, and mallet. Asian longhorned beetle larvae were visually inspected for ectoparasitoids and placed on ALB specific diet developed by the USDA (Dubois et al. 2002). The diet was modified from these methods by removing phloem-cambium and increasing the amount of cellulose. Asian longhorned beetle larvae were then inspected after two weeks for additional parasitoid activity.

Sentinel log assays. Sentinel logs are artificially infested sections of sealed wood with a predetermined diameter and length range which allow the insertion of lab grown host larvae of a particular size. They are placed in the field for a predetermined period, then removed and the inserted larvae inspected for parasitization. They have been used in parasitoid recovery surveys, including ALB and EAB (Lee et al. 2023, Abell et al. 2016). Sentinel logs (25.4 cm long and 6.6-10.2 cm in diameter) were created from healthy, locally collected *A*. *rubrum* logs. The phloem and xylem were cut in a 2 x 6 cm rectangle on three sides to create a flap that secured each larva, and a 6.5 x 0.5 x 0.3 cm groove was cut into the logs. Asian longhorned beetle larvae, obtained from the USDA-APHIS-PPQ Forest Pest Methods Laboratory in Buzzards Bay, MA, were weighed (sizes ranged from 285-1500 mg) and placed inside the notch and the bark was stapled back onto the log (Figure 1b). Six larvae were placed in each log. The logs were collected and prepared all in the same day to limit variability in frass production (Figure 2.2) which is a potential cue for parasitoids (Golec et al. 2016). To contain potentially emerging adult ALB and prevent rodent or bird entry, a 23-gauge 0.635 cm mesh was wrapped around to log as a cage. Logs were installed in the field 24 hours after larval insertion (Figure 2.2).

Sentinel logs were deployed at the USDA Stono Facility near Hollywood, SC (32.744451, -80.181749). Areas with relatively high *A. rubrum* density were selected. Nine sentinel logs were hung per trial at 14-20 m high. Because parasitoids may search for larvae on infested wood, logs were hung from trees either marked indicating ALB damage or in sight distance of marked trees. They were hung approximately 20 meters apart. This procedure was repeated monthly for one month in 2022 and four months in 2023 (Table 2.3).

After the larvae were extracted, they were placed on artificial diet and checked approximately every two to four weeks for emerging parasitoids. As larvae became pupae and adults, they were assessed for parasitization and preserved in 95% ethanol. If they became contaminated by mites, fungi, or nematodes, they were discarded and counted as contaminated.

Results

Parasitoid survey using infested material. From 21 sampled trees throughout the growing season, 16 out of 98 total larvae were contaminated in the diet or logs by unidentified phorid flies, mites, or fungi (Figure 2.4). Other larvae were predated on by generalists including larval *Alaus oculatus* L. (Coleoptera: Elateridae) (Figures 2.5). After two weeks, 59 larvae either pupated or emerged as adults. Twenty-three larvae did not pupate or become adults and were alive and moving after 31 days, so were counted as non-parasitized (Table 2.3). A total of 17 fly pupae (Diptera: Tachinidae) were collected, which are generalist parasitoids (Figure 2.3).

Sentinel log assays. Over the course of five trials, 215 ALB larvae were deployed. Of these, 165 larvae were alive at the end of their deployment period and zero were parasitized. In 2022, Trial 1 (10 September) resulted in 25 non-parasitized (NP) and 29 contaminated (CT) larvae. In 2023, Trial 1 (11 March) resulted in 45 NP and 5 CT larvae, trial 3 (15 April 2023) resulted in 46 NP and 8 CT larvae, trial 4 (25

May 2023) resulted in 46 NP and 8 CT, and trial 5 (8 June 2023) reared 3 NP and 0 CT. A total of 50 larvae were contaminated during these trials.

Discussion

At this stage, we suggest that naturally occurring populations of parasitoids are insufficient to serve as effective biocontrol for ALB in South Carolina. If a biocontrol program for ALB is to be established, our results indicate either a classical or augmentative biocontrol approach is better suited for ALB.

It is possible that during the duration of this study there were factors not considered which could have hindered the discovery of associations between ALB and parasitoids. Parasitoids may have been undocumented due to the relatively short period in which our surveys were conducted compared with other studies. For instance, Duan et al. (2016) conducted 59 trials of two different methods over a five-year period to find *O. mellipes*, *R. piceus*, *S. laflammei*, *Heterospilus* spp., and *Atanycolus* spp. parasitizing cerambycids (including ALB) and buprestids in *A. rubrum* trees in Delaware, U.S. Another study using sentinel logs in Korea had a sample size of 3,032 ALB larvae from 2019 to 2022, leading to the discovery of *Spathius ibarakius* Belokobylskij & Maetô, which parasitized 21 out of 51 larvae in 2022 (Lee et al. 2023). This illustrates the importance of prolonged parasitoid surveys, considering there can be thousands of larvae sampled and still low or no parasitism may be found. Moreover, two growing seasons may be too short given variable weather and site conditions which may not reflect the long-term conditions of the sites. Perhaps a longer, ongoing project may have greater success.

The methods used could have also contributed to the lack of parasitoid recovery. For one, many larvae were lost to contamination. Contamination in sentinel log and infested material collections was frequent, with 21.3% of ALB larvae contaminated in the parasitoid survey by quickly reproducing mites, scuttle flies, or nematodes which may be already present on or around the larvae or are able to get inside of the diet cups afterwards (Figure 2.4). Future studies may consider using rearing chambers or exclusion cages to limit contamination. The diet used was designed for laboratory use and may have been conducive to contamination by larvae from the field. Diet was stored in freezing temperatures, and then at ambient temperatures when in use. Nevertheless, placing ALB larvae on diet was preferred to emergence chambers: while emergence chambers minimize the contamination risk and the risk that larvae will be killed upon extraction, there is no guarantee that only parasitoids targeting ALB would emerge.

Additionally, sentinel bolts may not be optimal for parasitoid recovery. Parasitoids use several cues to locate their hosts, such as volatile compounds in host frass, vibrations in the tree caused by larval feeding, or host-plant cues like visual cues or volatile compounds. It is unknown which of these cues are necessary for *O. mellipes* host detection (Golec et al. 2016). Therefore, this may be a limitation in sentinel log trials, as these larvae were not so well established in the artificial bolts and were only given 24 hours to develop frass.

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CHAPTER 3

NATIVE PARASITOID RELEASES FOR AUGMENTATIVE BIOLOGICAL CONTROL OF THE ASIAN LONGHORNED BEETLE

Introduction

Classical biological control programs involve searching for parasitoids in the target pest's native range and introducing them to the invaded region to control the target pest. Generalist parasitoids – while used in the past – can have unforeseen impacts. In the case of *Lymantria dispar* L. (Lepidoptera: Erebidae)*,* several parasitoids were released, one of which is the generalist multivoltine tachinid, *Compsilura concinnata* Meigen (Diptera: Tachinidae) (Elkinton and Boettner 2012). This parasitoid also uses several native saturniid moths as hosts, one of which is the revered *Hylaophora cecropia* L. (Lepidoptera: Saturniidae), North America's largest moth. After these parasitoids were released to control *L. dispar*, *H. cecropia* became notably rarer and has been shown to support the fly's population later in the season, when *L*. *dispar* is not present (Elkinton and Boettner 2012). While many classical biocontrol programs now focus on specialists rather than generalists, using native parasitoids for augmentative biocontrol programs eliminates this risk altogether. Biocontrol research analyzing a wide range of pests and their parasitoids determined new associations can be up to 75% more effective than introduced parasitoids (Hokkanen et al. 1984).

A recent successful case of classical biocontrol implemented in North America is for *Agrilus plannipennis* Fairmaire [emerald ash borer (EAB); Coleoptera: Buprestidae]. Success is illustrated through the successful establishment of several agents which are spreading and shown to significantly reduce EAB populations in release plots (Duan et al. 2023). For this pest, numerous biocontrol agents have been discovered. Native or naturalized species include *Atanycolus* spp. (Hymenoptera: Braconidae), *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), the naturalized *Balcha indica* Mani and Kaul (Hymenoptera: Eupelmidae), *Spathius* spp. (Hymenoptera: Braconidae), and *Xorides humaralis* Say (Hymenoptera: Ichneumonidae). Introduced agents, as part of a federally led biocontrol program, include *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), *Spathius agrili* Yang (Hymenoptera: Braconidae), *S. galinae* Belokobylskij and Strazanac, and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Gould et al. 2021). Three of these meet the criteria for success, and the management plan depends on both season and location. *Oobius agrili*, *T. planipennisi,* and *S. galinae* all have documented establishment and spread two years after their release. For *O. agrili* and *T. planipennisi,* the parasitism rate reached 28 to 30% respectively over the course of 3 years (2011-2014) in a study in Michigan, significantly reducing EAB populations (Duan et al. 2023).

Anoplophora glabripennis Motschulsky [Asian longhorned beetle (ALB); Coleoptera: Cerambycidae], is an invasive wood boring pest native to China and the Korean peninsula, which likely arrived in the U.S. in wood packing material. It was first discovered in New York in 1996 and has since been detected in multiple states. It can use 112 species of trees as hosts, however, in the U.S. it prefers *Acer, Betula, Populus, Salix,* and *Ulmus* (Wang 2015). Of the infested trees documented in South Carolina, about 98% are *Acer rubrum* L.(Coyle et al. 2021)

Management of this pest has been dominated by surveys and tree removal, which has resulted in successful eradication in 10 quarantine zones in North America (USDA APHIS 2024). In remote areas where a large portion of the tree composition is *A. rubrum*, there are new challenges for ALB management. *Acer rubrum* is a highly adaptable species, able to survive a wide range of conditions. It is common in swamps, as in the case in the ALB-infested zone in South Carolina. In this newest quarantine zone, established in 2020, tree removal is difficult, as heavy machinery cannot access many sites and can degrade the habitat in its wake. Moreover, detection of ALB is challenging. Previous studies have concluded that detecting ALB damage on trees via visual inspections is only 33-60% effective (Golec et al. 2016). As such, biocontrol may help the USDA and other agencies to stay ahead and slow the spread by aiding in ALB population control on trees that have avoided detection by humans.

Additionally, the warmer climate of the southern U.S. allows ALB to develop from egg to adult more rapidly than in colder climates (Schmitt 2023). This suggests that the rate of ALB development and its management, which was successful in the northern infestations, may not be compatible for eradication in the southern ones. Biological control for ALB would aid in control in areas where ALB has spread but has not yet been reflected in the growing quarantine zone. It would help to control major outbreaks by slowing the rate at which the infestation develops. It would also aid in controlling the tail end of infestations while the last of the infested trees are removed and the area is monitored. In all three of these applications, tree removal would still need to be the main method of management, as biocontrol projects have much variation in their percent parasitism. The research on ALB phenology is ongoing, and this will help in the development of a comprehensive ALB management plan which may also utilize biocontrol (Schmitt 2023).

Previous studies have identified the native parasitoid *O. mellipes* as a promising biocontrol agent for ALB in the U.S. due to its short generation time and wide distribution. A short generation time will allow for the population to grow more quickly in heavily infested areas. In the laboratory, it parasitized ALB at a 70% rate in a no choice test using naturally infested ALB material (Duan et al. 2016). It was the most abundant parasitoid captured in the northeastern U.S., and it has known distributions recorded throughout much of eastern North America (Golec et al. 2020). Rearing methods have already been developed for this species, and it is being actively reared for research in the USDA office in Delaware (Wang et al. 2020). As such, my goal was to evaluate *O. mellipes* as a biocontrol agent for field populations of ALB in South Carolina.

Methods

Study location. The South Carolina ALB quarantine zone established by the United States Department of Agriculture is in Charleston and Dorchester counties, covering 197.8 km^2. The quarantine zone is in a subtropical coastal wetland consisting of mixed softwood and hardwood forests. Trees present in the quarantine zone include but are not limited to trees listed in Table 2.1. Charleston summers are hot and humid, and the winters are mild and breezy. The National Oceanic and Atmospheric Administration states the recorded high and low temperatures for Charleston in 2023 were 36.7°C and -2.8°C, respectively (NCEI 2024). The most precipitation in one event was 94 mm while the total average annual precipitation was 138.7 cm in 2024 (NCEI

2024). Specific study sites were located at the USDA Stono Facility (32°44'41.4"N 80°10'56.3"W) and Royal Harbor Road (32°46'40.7"N 80°08'08.6"W).

Tree selection. In July and August 2023, 15 trees were chosen for each of two trials. Trees chosen exhibited several visual cues of ALB infestation including weeping, egg niches, cracked bark indicating galleries, and adult exit holes (Figures 1.3-1.6) Trees over 10.16 cm dbh and below 66.04 cm dbh were selected for the study.

Ontsira mellipes releases. Ontsira mellipes adults were shipped overnight on two separate occasions during the summer of 2023 to the USDA Stono Facility from the USDA laboratory in Newark, DE. For each trial, ten trees were chosen for wasp releases and five controls were selected and received no releases. The first release was conducted on 10 July 2023, at the USDA Stono Facility and Royal Harbor Road (Figure 3.1). A total of 1,224 adults were released on 10 trees across both sites by securing the vials sideways at breast height using zip ties. The time was 2:16 p.m. at the first tree and 7:57 p.m. at the 10th tree. At 2:16 p.m., the temperature was 35.6°C and the 90-min maximum was 36°C. The relative humidity was 45%, the 6-h minimum temperature was 26.1°C, and the lowest temperature that night was 23.9°C. The average temperature was 36°C and there were 22 cooling degree days. The precipitation was 3 mm. The sky was mostly sunny. The high temperatures likely influenced their performance upon release as they became visibly inert. The time taken for 80% of the wasps to leave the vial was recorded (Table 3.1). Time to leave was not recorded for trees 7-10 due to time constraints during sampling before sundown. The wasps appeared lethargic and inactive during the duration of the release, compared to the time of their arrival two days prior. The wasps arrived with honey and were not given any supplemental water or honey before release.

Parasitoid recovery. Study trees were felled and left on site for 24 h for inspection. Trees were then labeled, cut, and infested pieces were brought to the USDA Stono Facility for processing. Larvae were extracted by cutting through the bark with a chisel and mallet, so that the galleries were exposed. The bark was stripped away at and around egg sites. When ALB larvae were identified, they were weighed and placed on an artificial diet. Larvae that had tunneled into the pupal chamber were left uncounted due to the large quantities of wood to process. The ovipositor of *O. mellipes* is also not long enough to reach into the pupal chamber, so efforts were prioritized to get as many larvae out of the shallow galleries. Most of tree 6 was left in the field due to resource limitations. The trees were felled 26 days after the parasitoid release (15 August 2023). Asian longhorned beetle larvae were found in 13 out of the 15 total trees and extracted and placed on diet. Tree 10 was left standing and could not be retrieved due to time constraints (Table 3.1). Larvae tunneled deeper into the wood were only counted as tunneled and not in the live category as *O. mellipes* cannot reach past the depth of their ovipositors. Moreover, ALB deeper within the wood are likely not parasitized, as *O. mellipes* paralyzes its host upon egg laying.

The second release took place on 17 August 2023 from 8:30 a.m. to 11:30 a.m. at Royal Harbor Road. These trees were cut and processed 25 days later, on 11 September 2023, using the same methods as Trial 1. During this release, the temperature was 23.9°C and the lowest temperature during the night was 23°C. The 90-

min. maximum was 24.4°C. The relative humidity was 94% and partly cloudy. The releases started at 8:50 a.m. with tree 1 and ended at 11:30 a.m. with tree 10.

Molecular techniques. An Oxford NanoPore Technologies MinION portable sequencing device (Oxford NanoPore Technologies, Oxford, United Kingdom) was used to perform low- coverage whole genome amplification using the DNA obtained from these wasps at the University of Massachusetts Amherst, in the Department of Environmental Conservation. Fifteen thousand bp were generated from the two samples. These were compared to sequences in the NCBI GenBank Database (Benson et al. 2013) using the BLASTN algorithm (Altschul et al. 1990). These samples were consistent (a 90% match) with published COI sequences from the genus *Ontsira* (Hymenoptera: Braconidae). A multi-sequence alignment of all published *Ontsira* COI sequences was created, in comparison to the MinION sequences using the alignment tool MUSCLE (Edgar 2004) and designed novel primers specific to be specific to the genus *Ontsira* and excluding published Wolbachia sequences using Primer3 v. 2.3.7 (Untergasser et al. 2012) both implemented in Geneious Prime® v. 2003.2.1 (Biomatters, LTD, Auckland, New Zealand). Forward (Ont-Forward: 5'-TAGTGGGATTATCTATGAGA – 3') and reverse (Ont-Reverse: 5'-GCAGTAATTAAGATAGATCAC – 3') primers to amplify fragments from three unidentified individuals (one male and two females). Amplified fragments were then cleaned using Exo-SAP digestion and sent to the Keck DNA Sequencing Core at Yale University to be sequenced. The resulting sequences were edited by eye, forward and reverse compliments aligned in Geneious Prime, and the resulting consensus sequences were compared to published sequences in the NCBI GenBank database using the BLASTN algorithm.

Results

During Trial 1, two out of 56 field-collected ALB larvae (3%) were parasitized by *O. mellipes*. One of these ALB larva (from tree #4) had 7 *O. mellipes* larvae attached to it on a 76 mm diameter branch (Figure 3.1). One *O. mellipes* larva was found in another egg niche on tree #4 with a missing ALB larva, also on a 76 mm diameter branch. There were also 17 tachinid pupae or pupa casings present in the ALB galleries (Table 3.1)

During Trial 2, 14 out of 15 trees were felled, and there were no ALB larvae present for inspection, therefore no claims may be made about how many were parasitized during the period of release.

After primers were aligned and trimmed, a 464 base pair fragment of COI was amplified from three unidentified samples. A multisequence alignment indicated that there were no differences between samples. As per the MinION sequencing results, all three mtDNA sequences had high percentage matches (90%) to published sequences from both identified and unidentified species in the genus Ontsira, with the highest percentage match (93.24%) being to an unidentified specimen collected from Kejimkujik National Park, Nova Scotia (GenBank Accession: HQ928872). Generated sequences from this study have been uploaded to GenBank (Accession numbers pending).

Discussion

This study represents the first record of *O. mellipes* successfully parasitizing ALB in the field. This is a promising development, but more work is needed to determine optimal release time, number and interval of releases, and number of wasps sufficient to control ALB populations. In the summer of 2023, we released 1,936 *O. mellipes* adults in addition to a third, unrecorded release of an estimated 400-1,200 wasps. Our releases are a contribution to the ongoing work to evaluate *O. mellipes* as an ALB biocontrol agent.

All *O. mellipes* larvae found in this study were on 74-78 mm diameter branches, collected from higher in the canopy. While no conclusion can be made about preferences with such a small sample size (n = 56), this may be due to ease of ovipositor insertion through thinner bark. Like many trees, *A. rubrum* exhibits thinner bark on younger, smaller diameter branches compared to the thicker plates developing as the stems age. Thicker bark in the main stem may be an obstacle for the wasps in late-stage infestations, as *O. mellipes* has a short ovipositor at 3.91 +/-.05 mm, and *A. rubrum* bark can be up to 20.26 cm thick (Wang et al. 2019, Schafer 2015). Recovery in small diameter branches may also be simply a result of where ALB is located. Early infestations of ALB start highest in the canopy, a tendency exhibited by many wood borers (e.g., EAB) to maximize the use of their available resources (Bean 2022).

Weather on the days of release seemed to have a drastic effect on the behavior of the wasps. On 20 July 2023, the temperature was 36°C, which is outside of the range of optimal temperatures for this wasp and visibly impacted their performance (Golec et al. 2017). It is possible it was shocking to them to come from a cooler laboratory setting to a hot and humid field, and it is undetermined how long of an adjustment period they may need. When vials were opened, the wasps attempted mating, likely due to the new stimuli. Both factors affected their time of release, so time recorded in this study is presented as time for 80% or 20 adults to leave the vials. To maximize the chances that the wasps get acclimated to the field, the next trials should take place in the morning, and preferably a day without storms in the forecast. A release when an immediate storm is in the forecast may inhibit the search and acquisition of adequate refuge. The likelihood of *O. mellipes* failing to locate the ALB larvae in the field is undoubtedly higher than in the laboratory due to prevailing winds, rain and other adverse weather, and distance. This presents another challenge in determining the ideal time of release in addition to the month of release. It is possible that success might be greater if weather at the time for release consists of no rain, no storm in the next 24 hours, no severe winds, and release be taken place in the mornings to avoid releasing into above 32°C temperatures. This would allow the wasps to better acclimate to the new conditions in the field.

Ontsira mellipes is being reared for potential use to manage ALB (Duan et al. 2016) but has the potential to target other cerambycids including *Elaphidion mucronatum* Say*, Monochamus caroliniensis* Olivier, and *M. notatus* Drury (Wang et al. 2019). Moreover, there may be additional cerambycids in Hollywood, SC that have not been evaluated as hosts, since original studies were done in mid-Atlantic and northeastern US (Wang et al. 2019). If an ALB biocontrol program using *O. mellipes* will be investigated further, potential cerambycids in the SC quarantine area should be included in the tests. Moreover, understanding how cerambycid populations will impact effectiveness of management using *O. mellipes* is crucial to this project. A future cerambycid survey may be useful in determining these populations in areas where ALB has infested.

The temperature of the environment has a positive relationship with the rate of development of *O. mellipes* from egg to adult, however after reaching 30°C, survival rate is nearly halved, and fecundity is significantly reduced. The ideal temperature for the fastest development and survival is 25°C (Golec et al. 2017). This might provide some insight into the low parasitization in the first trial which occurred at 35.5° C. Since 1,107 were released in the first trial, based on the findings in the lab study we can speculate that at least half of eggs laid did not make it to adulthood due to the temperature limitations. Future studies should attempt to repeat releases during weeks which do not exceed 30°C. This suggestion aligns with recommendations in the northern states to release the wasp during early spring and late fall.

In New Jersey (the farthest southern city included in the study), the estimated generations per year for *O. mellipes* were 3.7 (Golec et al. 2017). Because development is associated with temperature, it is also likely that in South Carolina the generations per year are higher due to the higher temperatures, however no studies have been done to determine this. Climate change may increase the number of generations as well as lower their survival rates during heat waves.

In previous studies, *O. mellipes* showed no significant difference in preference for *M. caroliniensis* (a cerambycid native to North America) over ALB. Thus, it may be beneficial to survey for *M. caroliniensis* in Hollywood, SC to sustain a population of *O. mellipes* where ALB populations are dwindling but control is still necessary (Wang et al. 2019). Of all species tested on ALB, *R. piceus* and *O. mellipes* had up to 100% parasitization rates in no choice laboratory assays, however *R. piceus* has never been reared successfully in the laboratory (Golec et al. 2016).

On 17 August 2023, an additional 568 females and 230 males were released at Royal Harbor Road with the intent to evaluate overwintering success through sampling in 2024. No trees were felled and inspected after this round of releases. A future study to determine parasitism rates on ALB may benefit from combining sentinel log methods from Chapter 2 and *O. mellipes* releases from Chapter 3. Desirable infested material at our study site is limited due to access to only 2 sites: the USDA Stono Facility and Royal Harbor Road. Trees at the USDA Stono Facility had been damaged from previous years and Royal Harbor Road has been depleted of prime infested material after many research projects being conducted there. A combination of sentinel log methods and wasp releases would allow for a consistent measure of parasitism over the course of the season.

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CHAPTER FOUR

Thesis Conclusions

Rearing ALB from infested trees yielded no recovery of native or naturalized parasitoids. In sentinel log trials, there was also no parasitism detected. For both procedures, this may be due to low sample size, considering the study only occurred over the course of 1.5 growing seasons. We suggest extending these surveys to align with longer periods of similar studies. In addition, if future native and naturalized parasitoid surveys take place, placing infested material into barrels and rearing them out may be more effective and have less contamination. Therefore, our data suggests that sites used within the SC ALB quarantine zone do not have sufficient populations of braconid or ichneumonid species which parasitize ALB to serve as biocontrol. This means that hopes for ALB biocontrol will likely include an active release program using an agent such as *Ontsira mellipes*.

Our study provided the initial evidence of *O. mellipes* successfully parasitizing ALB following field releases. Temperature has a drastic effect on fecundity, survival rate, development rate, body size, and behavior of *O. mellipes* and should be taken into consideration during releases. To maximize their potential as an effective control agent in South Carolina, releases must take place when there are no heat waves or storm events forecasted to attain the best results. They must also be released within the period between eggs hatch and larval tunnelling from June to August, otherwise *O. mellipes* will not be able to reach the larvae. This study should be replicated, however combining sentinel log trials and field releases of *O. mellipes.*While much work is left to be done in this realm, our study makes a notable contribution in progressing towards an effective biocontrol program for ALB.

LIST OF TABLES

Chapter 2 Tables

Table 2.1. Common trees and shrubs found in the Asian longhorned beetle quarantine zone in Charleston and Dorchester counties, South Carolina. * Species are considered invasive in the United States. Table adapted from Bean 2023 and Schmitt 2023.

Tree species	Common name
Pinus palustris Andropogon	longleaf pine
Pinus taeda L.	loblolly pine
Pinus echinata Mill.	shortleaf pine
Ulmus americana L.	American elm
Liquidambar styraciflua L.	sweetgum
Acer rubrum L.	*Acer rubrum*
Triadica sebifera L. Small *	Chinese tallowtree
Quercus phellos L.	willow oak
Quercus bicolor Willd.	water oak
Juniperus virginiana L.	eastern redcedar
Celtis laevigata Willd.	sugarberry
Quercus laurifolia Michx.	laurel oak

Morus rubra L.	mulberry
Ilex vomitoria Ait.	yaupon holly
Ligustrum sinense Lour.*	Chinese privet
Myrica cerifera L.	wax myrtle
Prunus caroliniana Ait.	cherry laurel
Sabal palmetto Walter	Sabal palmetto
Carolina palmetto Lodd. ex Schult.	Carolina palmetto

Table 2.2. Fate of larvae collected from infested material and evaluated for parasitization. LOC: Location; RH: Royal Harbor Rd, MR: McCombs Rd, GR: Gertrude Road; NP: Not Parasitized; NP*: Not Parasitized*, where * indicates that there was enough time (31 or more days) for parasitoids to develop and emerge, after which the larvae died or became compromised. EC: eggs contaminated.

TREE	LOC	DATE	NP	NP*	CONTAMINATED	PARASITIZED	eggs NP	EC
1	RH	2/10	4	0	6	0	0	0
2	RH	2/10	4	5	1	0	0	0
3	RH	2/11	0	0	0	0	0	0
4	RH	3/31	16	0	1	0	0	0
5	RH	3/31	8	0	3	0	0	0
6	RH	4/14	16	0	0	13 Tachinidae pupae	0	0
7	MR	5/24	0	0	0	3 Tachinidae pupae	0	0
8	RH	6/26	0	0	0	0	1	1
9	RH	6/26	0	1	0	0	1	1
10	RH	6/26	0	0	1	0	0	10
11	RH	6/26	1	1	0	0	1	6
12	RH	6/26	0	0	1	0	4	3
13	RH	6/26	0	0	0	0	18	0

14	RH	6/26	0	0	2	0	0	1
15	MR	6/26	1	1	0	0	4	3
16	RH	7/10	0	0	0	0	0	0
17	RH	7/10	0	1	0	0	1	13
18	RH	7/10	0	0	0	0	4	0
19	RH	7/10	0	0	0	0	3	1
20	RH	7/10	0	0	0	0	0	1
21	RH	7/10	0	0	1	0	0	0
22	GR	9/13	0	7	0	1 fly pupa	0	0
23	GR	9/14	0	7	0	0	0	0

Table 2.3. Four *Acer rubrum* sentinel log trials in 2023 using 165 Asian longhorned beetle larvae from the Newark, DE USDA laboratory.

Trial	Start	Fin	Days	Mass range	Inserted	Retrieved	% Parasitized
T1 22	9/10	10/9	29	-	54	48	0%
Trial 1	3/10	4/1	22	0.703-1.546g	54	50	0%
Trail 2	4/15	5/3	18	0.285-1.491g	54	52	0%
Trial 3	5/25	6/7	13	0.846-2.094g	50	43	0%
Trial 4	6/8	6/30	22	0.876-0.907g	3	3	0%

Table 2.4. Infested *Acer rubrum* material collections of 2023. Details tree ID, stem number, DBH, latitude and longitude, date, number extracted, mass range, and fly pupae presence.

Tree #	stems	DBH	Coordinates	Date	Extracted	Mass range	Fly pupae
1	3	12	32.778528, -80.145060	2/10	8	-	1
2	1	7.9	32.780433, -80.146408	2/10	10	-	0
3	1	9.8	32.781589, -80.146791	2/11	0	-	0
4	1	16	32.776233, -80.135796	3/31	18	.975-2.539 g	0
5	1	17	32.776233, -80.135796	3/31	12	1.726-2.572 g	0
6	1	14	32.779462, -80.143763	4/14	15	.341-1.921 g	13
7	3	17	32.779, -80.154733	5/24	0	-	3
8	1	18	32.777027, -80.136716	6/26	2 eggs	-	0
9	1	13	32.777027, -80.136716	6/26	1 larva 1 egg	.009 g	0
10	1	12	32.777027, -80.136716	6/26	10 eggs 2 larvae	.001-.012	0
11		6	32.777027, -80.136716	6/26	7 eggs 1 larva	.001-.014	0
12		10	32.777794, -80.137381	6/26	7 eggs 1 larva	0.016g	0
13		12	32.777794, -80.137381	6/27	18 eggs	-	0

14	1	26	32.777794, -80.137381	6/27	1 egg 2 larvae	.059-1.718g	0
15	1	18	32.780310, -80.146083	6/27	7 eggs 1 larva	.12g	0
16*	1	24	32.777236, -80.137332	7/10	10 eggs 9 larvae	.15-.35g	0
17*	-	16	32.777236, -80.137332	7/11	7 eggs 1 larva	.001g	0
18*	-	14	32.777236, -80.137332	7/11	4 eggs	-	0
19*	-	15	32.777236, -80.137332	7/11	7 eggs	-	0
20	-	14	32.777236, -80.137332	7/11	1 egg	-	0
21	-	6	32.777236, -80.137332	7/12	1 larva	.001g	0

*Trees used by Schmitt (2023); only eggs collected.

Table 2.5. Total number of non-parasitized (NP) and contaminated (CT) Asian longhorned beetle (ALB) larvae deployed in *Acer rubrum* sentinel log trials from May 2022 to June 2023.

	5/25/22	3/11/23	4/15/23	5/25/23	6/8/23	Total ALB larvae
TOTAL NP	25	45	46	46	3	165
TOTAL CT	29	5	8	8	0	50
PARASITIZED	0	0	0	0	0	0

Chapter 3 Tables

Table 3.1. *Ontsira mellipes* release Trial 1 on 17 August 2023. In total, 1224 *O. mellipes* adults were released and average time for wasps to exit vials * Indicates no data collected due to time constraints. Tree 10 was not felled due to time constraints.

Tree ID	Live ALB larvae retrieved	Tachinid pupae casings	Total *O. mellipes* released	ALB parasitized by *O. mellipes*	Time for 80% of wasps to exit vials (minutes:seconds)
1	5	0	91	0	7:50 m
2	1	0	130	0	4:20 m
3	38	1	136	0	4:06 m
4	5	15	140	1	8:48 m
5	1	1	134	0	3:25 m
6	3	0	143	1	3:31 m
7	2	0	115	0	6:48 m
8	1	0	107	0	*
9	0	0	110	0	*
10	-	-	118	-	*
Average			122.4		

Chapter 1 Figures

Figure 1.1. Current map of progress of the Asian longhorned beetle eradication program (USDA APHIS 2024). The United States Department of Agriculture's map of eradicated (green) and active (red) Asian longhorned beetle infestations. Map does not include Toronto, Canada in the eradicated list. Map retrieved on 3.15.2024 from https://www.aphis.usda.gov/plant_health/plant_pest_info/asian_lhb/downloads/albmaps/alb-program-progress-map.pdf

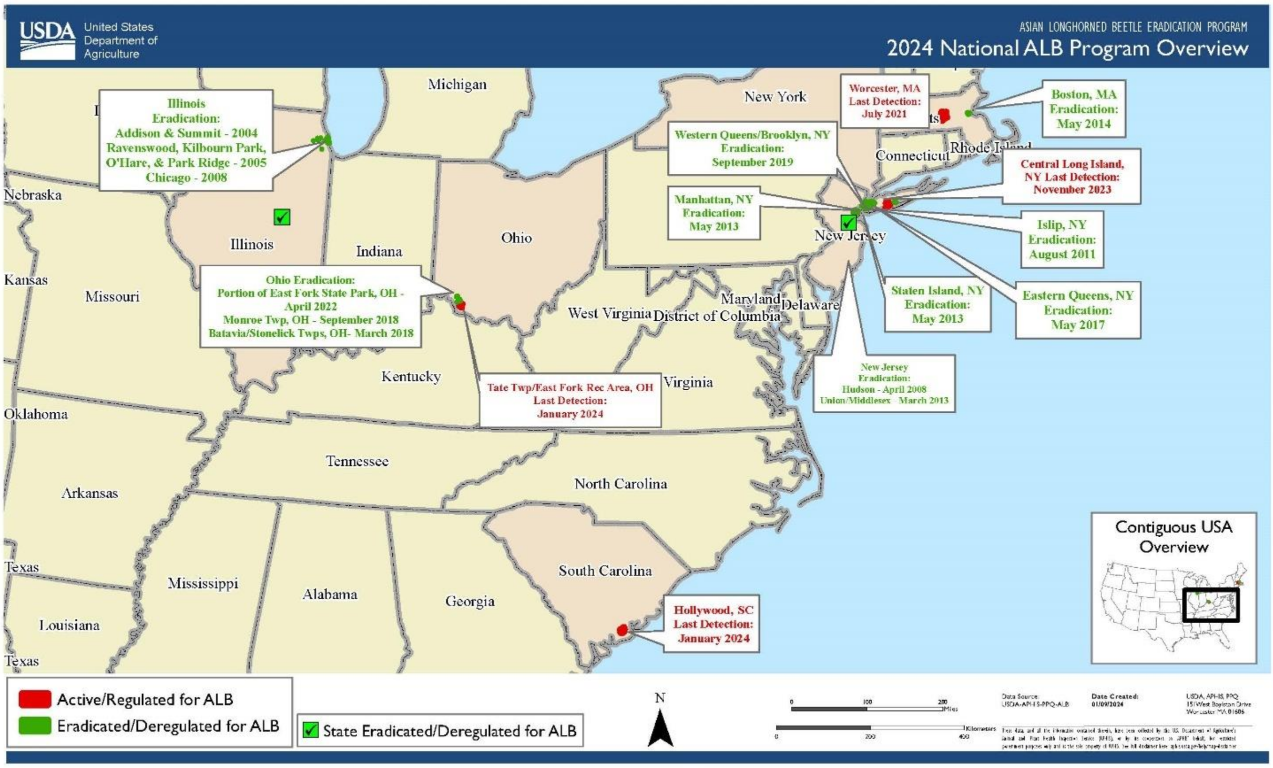

Figure 1.2. Life cycle of Asian longhorned beetle: egg, larva, pupa, and adult. In order: egg in egg niche, 3 larvae at different instars, 2 pupae with the second coming into the eclosion phase, and an adult female beetle. Eggs retrieved on 15 March 2024 from http://www.ontario.ca/page/asian-long-horned-beetle. Larva retrieved on 15 March 2024 from https://www.uvm.edu/albeetle/biology/larvae.htm. Asian long-horned beetle | ontario.ca. Larva, pupa, and adult images retrieved on 15 March 2024 from Asian Longhorn Beetle Pictures—AZ Animals. (n.d.). A-Z Animals. Asian Longhorned Beetle Larvae.

Photograph by Michael Bohne

Figure 1.3. Asian longhorned beetle egg niche and frass on *Acer rubrum* stem indicating larval development. Photo by D. Coyle, Clemson University.

Figure 1.4. Asian longhorned beetle egg niches and one exit site on *Acer rubrum*.

Photo by D. Coyle, Clemson University.

Figure 1.5. Three Asian longhorned beetle egg niches from at most 1 year prior, with resulting weeping and frass production on *Acer rubrum*, indicating live larvae present. Photo by D. Coyle, Clemson University.

Figure 1.6. Asian longhorned beetle frass production and warped bark on *Acer rubrum* as a result of gallery devleopment. Photo by D. Coyle, Clemson University.

Figure 1.7. *Ontsira mellipes* life stages: egg, larva, pupa, and adult. Adapted from Golec et al. (2016).

Figure 1.8. Dead *Acer rubrum* tree due to Asian longhorned beetle infestation. Pictured: egg niches, exit sites, cracked bark, galleries, and orange paint marking infestation.

Photo by M. Lupu, Clemson University.

Figure 1.9. Tachinid fly adults found in *Acer rubrum* infested material collections. Retrieved from tree #6. Photo taken 8 May 2023 by M. Lupu, Clemson University.

Chapter 2 Figures

Figure 2.1. Ideal *Acer rubrum* tree damage for collections. Pictured are darkened egg niches with weeping, indicating live eggs or larvae present. Photo taken 16 August 2023 by M. Lupu, Clemson University.

Figure 2.2. Sentinel log production. Grooves carved for larval insertion (A), bark flap secured with staple (B), larvae left for 24 hours inside the Stono USDA facility for frass development (C), installation of logs in the field using galvanized steel mesh cages and rope on (D) Photo taken 10 March 2023. Log photos taken 10 September 2023 by M. Lupu, Clemson University.

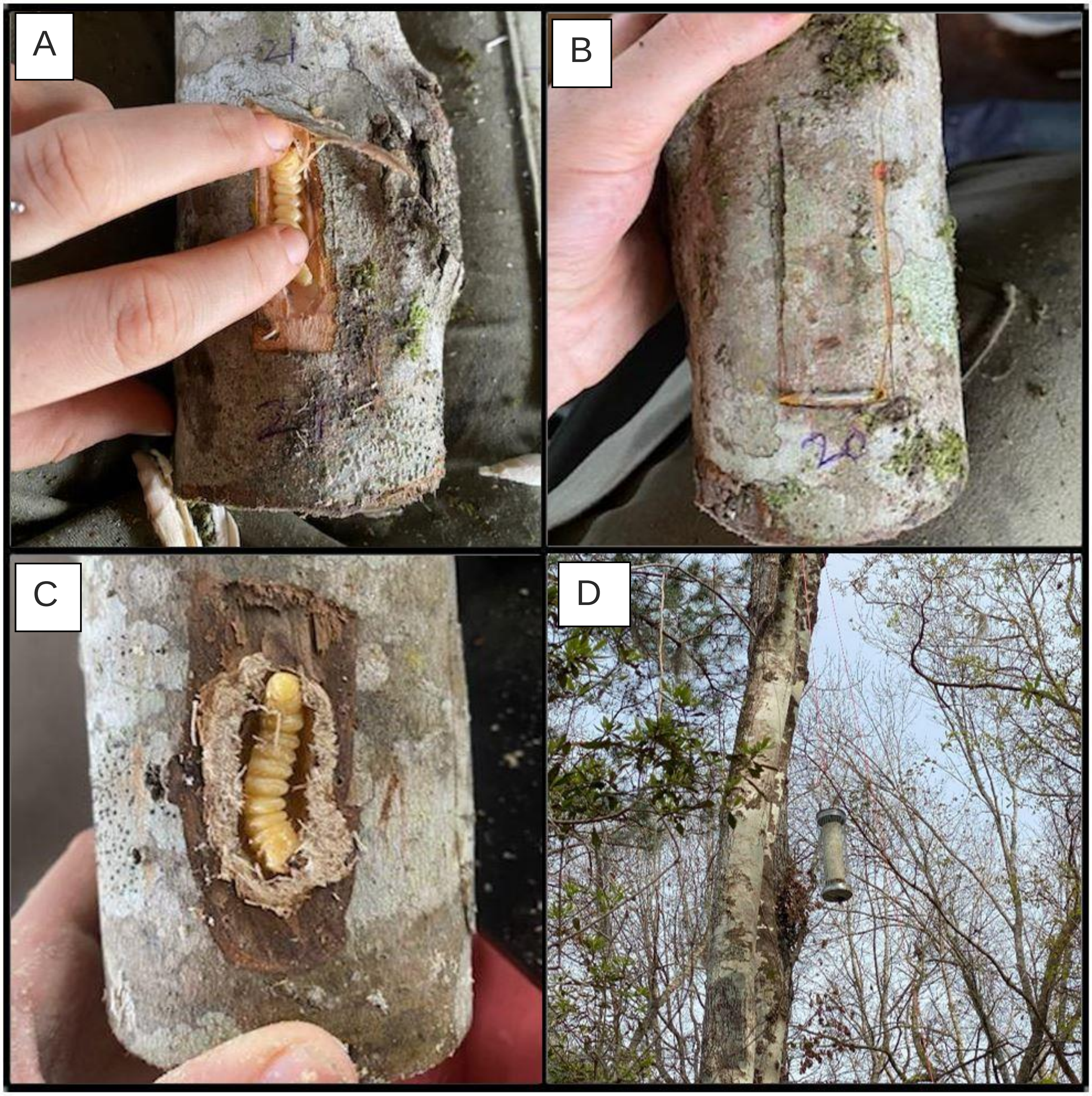

Figure 2.3. Tachinid fly pupa to the left of a dead Asian longhorned beetle larva. From the infested material collections. Photo taken 11 March 2023 by M. Lupu, Clemson University.

Figure 2.4. Contamination of Asian longhorned beetle larva by mites. On dead Asian longhorned beetle larva in artificial diet. From the Sentinel log trials. Photo taken 11 February 2023 by M. Lupu, Clemson University.

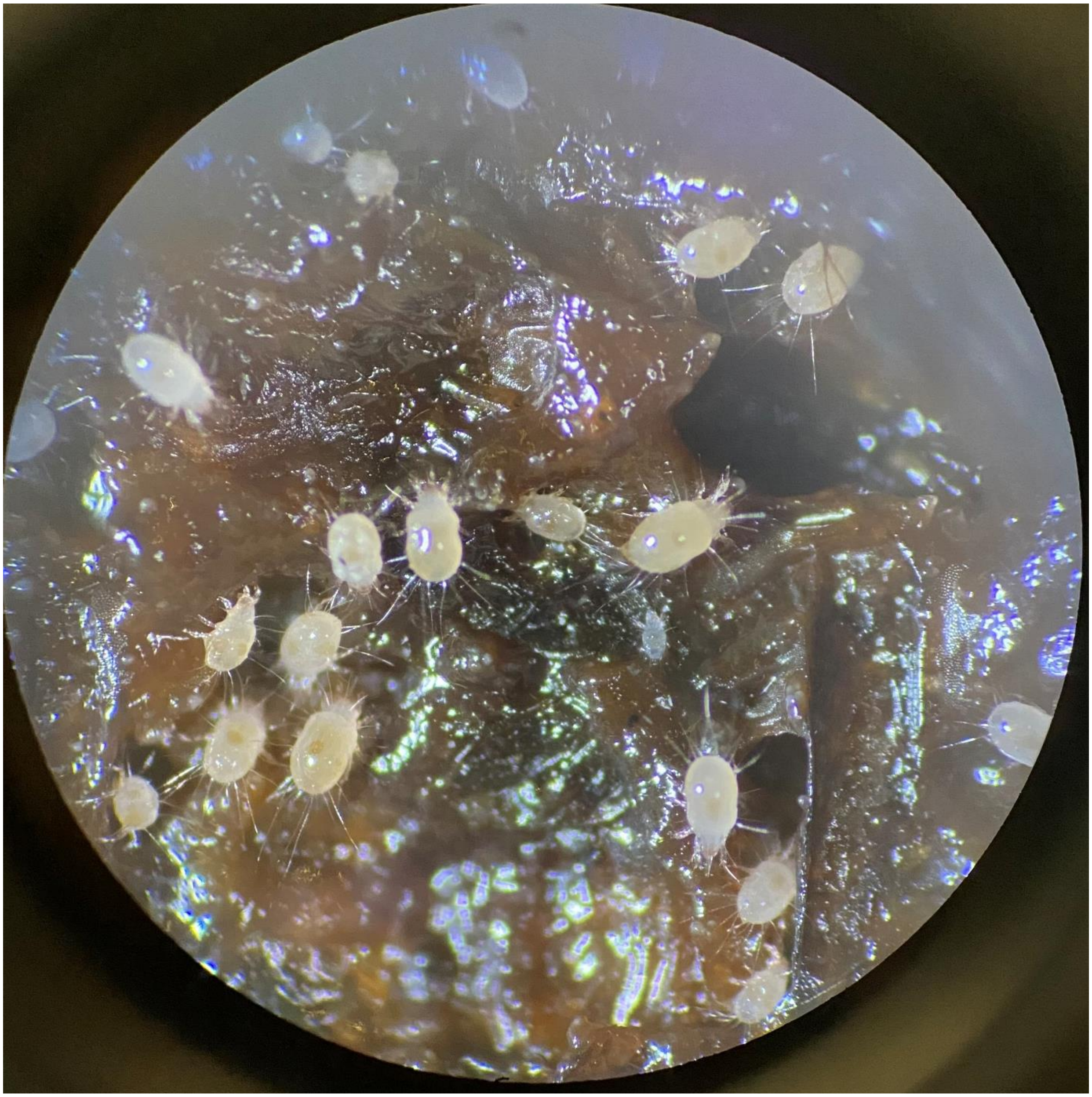

Figure 2.5. Asian longhorned beetle larva and egg predated on by the eastern eyed click beetle in infested material collections. Photo by M. Lupu, Clemson University.

Figure 2.6. Asian longhorned beetle eggs collected from infested material.

CHAPTER 3 FIGURES

Figure 3.1. Release of *Ontsira mellipes* adults (Trial 1, tree #2). Photos by M. Lupu, Clemson University.

Figure 3.2. Collection from Trial 1: 7 *Ontsira mellipes* larvae on one Asian longhorned beetle larva from *Acer rubrum* tree #4, on a 76mm diameter branch. Photo by M. Lupu, Clemson University.