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Phenology of Asian Longhorned Beetle (Anoplophora Glabripennis, Coleoptera: Cerambycidae) in South Carolina, U.S.A.

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PHENOLOGY OF ASIAN LONGHORNED BEETLE (*ANOPLOPHORA GLABRIPENNIS*, COLEOPTERA: CERAMBYCIDAE) IN SOUTH CAROLINA, U.S.A.

A Thesis
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the Graduate School of
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by
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ABSTRACT

A new population of the Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky, hereafter ALB), an invasive species in the United States since 1996, was discovered in Charleston County, South Carolina in 2020. This population is the furthest south ALB has been found in North America and provides an opportunity to examine the phenology of this beetle in a novel climate. I collected 1009 eggs, larvae, pupae, and adult beetles from the federal quarantine zone in South Carolina and used larval head capsule widths to determine the rate of development and voltinism of ALB in South Carolina's coastal climate. With this method I was able to determine that ALB in South Carolina appears to have a synchronous univoltine life cycle, in contrast to populations in the northern U.S. that typically develop in 1-3 years. Using this field data, I was also able to validate the accuracy of an ALB phenology model that had not previously been tested in a subtropical climate, and use the model to predict development rates for nine locations of varying latitudes around the U.S. The model predicted that several locations along the southern edge of the U.S. will have similar or even faster development than South Carolina, which could be concerning for management programs tasked with the eradication of ALB in the South, as well as northern programs that may see an increase in development rate as climate change increases temperatures.

DEDICATION

To my friends, without whom this project would never have been finished.

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CHAPTER ONE

OVERVIEW OF THE HISTORY AND IMPACT OF THE INVASIVE ASIAN LONGHORNED BEETLE IN SOUTH CAROLINA

Background and Relevance of Invasive Species

Invasive species are organisms that are not native to an ecosystem, and can cause damage to the environment, human health, or the economy (USDA Forest Service 2013, Hayes et al. 2021, Mayfield et al. 2021). Widely regarded as one of the most serious threats to native ecosystems around the world, invasive species negatively impact native species directly via predation, herbivory, infection, or parasitism, and indirectly by outperforming or displacing competing species (Mayfield et al. 2021). Invasive species are typically introduced to ecosystems through global trade networks (Westphal et al 2008, Hulme 2009, Krishnankutty et al. 2020). Many invasive species have been proven to decrease native biodiversity (McNeely 2001), and in rare cases have led to near extinctions of species like the American chestnut (*Castanea dentata* Marsh.) (Hepting 1974). Invasive species also have major economic costs. Lost profit from agricultural products, recreational value, and labor costs from management, research, and eradication efforts cost billions of dollars every year (Mayfield et al. 2021) The economic impacts of invasive insects have cost the U.S. alone an estimated $1.22 trillion dollars from 1960-2020 (Fantle-Lepczyk et al. 2022).

All ecosystems are impacted by invasive species, including forests. North American forests have been impacted by many invasive species, including invasive insects. Introduced in 1869, spongy moth (*Lymantria dispar* L.) larval feeding can damage a wide variety of native trees and this species is capable of widespread defoliation (Davidson et al. 1998, Tobin et al.

2012, Liebhold et al. 2021). Others, like the emerald ash borer (*Agrilus planipennis* Fairmaire) feed on the phloem of ash trees, causing damage to the phloem and rapid death of the tree (Herms and McCullough 2014). Wood-boring species can be easily transported to new locations inside firewood, and in solid wood packing material like crates and boxes (Lovett et al. 2016, Dodds et al. 2017, Biebl and Querner 2021).

The effects of climate change have also compounded the threat of invasive species in many areas. Insects are impacted by climate change, as their development, reproduction, and survival are all dependent on environmental factors (Keena and Moore 2010). As climate change shifts temperature and precipitation patterns, many areas where species were excluded by low temperatures will become suitable habitat for invasive species that could not previously survive there, and many invasive species will become more impactful due to the increased development rate and speed of spread brought on by a warmer climate (Finch et al. 2021). Being able to predict the distribution of invasive species is essential for management programs for effective surveys and timely responses to new threats (Finch et al. 2021).

Overview and Applications of Modelling

Because the rate at which insects develop has a direct connection to climate and temperature, phenology models are incredibly important parts of invasive insect management as they allow researchers and managers to predict timing of important life stages and development rate (Nietschke et al. 2007, Flint 2012, Zhang and DeAngelis 2020). Phenology models study the connection between weather and the timing of repeating events in a species’ life cycle, such as cycles of growth and reproduction (Nietschke et al. 2007, Flint 2012). This information is crucial to assess the potential risk of an infestation (Flint 2012, USDA Forest Service 2013) and to support and guide management efforts that require precise timing, such as applications of insecticide that target specific life stages (Poland et al. 2006). Modelling is also important because it can reduce the need for field collected data. Instead of management programs spending valuable time, labor, and money collecting specimens at every new location, potentially for multiple years before they can determine a pest's phenology, predictive models can give them an informed idea of how an insect will respond to a location's climate and other environmental factors (Nietschke et al. 2007, Flint 2012, USDA Forest Service 2013, Trotter and Keena 2016, Venette 2021). Finally, modelling allows managers to predict how an infestation's phenology will structure the future distribution of a species under changing conditions - most notably, climate change.

Many models utilize mathematics to predict insect phenology by creating formulas that project the distribution of life stages or development events within a population on the mean and variance in the development rate of the insect over its entire lifespan, usually based on laboratory data, and then make predictions for untested temperatures (Rebaudo and Rabhi 2018, Zhang and DeAngelis 2020). For insects, these models utilize degree-days, a unit of measurement that signifies the amount of heat that builds during a 24-hour span for each 1° above an insect's lower temperature threshold. This is a common method for predicting timing of insect development stages and is a more accurate measure than assuming life stages will be out at the same time every year or in every location (Flint 2012, Rebaudo and Rabhi 2018).

While these methods employ a wide variety of different methods and formulas, each with their own benefits and challenges, mathematical models are often relatively inflexible; the simplistic nature of using a single curve to map the complex range of factors that impact development rate does not leave much room for nuance or variation, and often leads to less specific and lower accuracy predictions (Rebaudo and Rabhi 2018). Although these models can still be useful tools, especially for insects that do not require complex or highly specific predictions (Rebaudo and Rabhi 2018), the development of software technology has led to the creation of new, more complex methods of modelling (Trotter and Keena 2016, Zhang and DeAngelis 2020).

One of these methods is an agent-based approach that works by simulating an individual insect's development from a newly laid egg to an adult laying eggs of its own, then repeating the process for hundreds or thousands of insects for hundreds or thousands of generations (Trotter and Keena 2016). Agent-based models use computer simulations rather than simple mathematic formulas, which allow for much greater complexity and detail. These models can input separate degree day requirements for each life stage and instar, as well as consider upper and lower critical temperatures, insects with variable instar requirements, and dormancy periods (often during winter) that postpone pupation (Trotter and Keena 2016). Although much more complicated to create than a simpler mathematical model, and impossible to do without a computer repeating tens of thousands of individual calculations, these models can account for far more complexity than a simple curve (Trotter and Keena 2016, Zhang and DeAngelis 2020). Despite their usefulness, however, models are a simplification of a complex system, and therefore need to be tested and validated in as many different conditions as possible to ensure their predictions are reflective of field conditions.

Models are an important tool in invasive species management efforts. Being able to predict how invasive species will act in various situations and locations gives management programs the knowledge to help optimize preventative measures, survey for new infestations, conceptualize the population size and potential for growth of already established populations, and prioritize eradication of invasives in high-risk locations (Nietschke et al. 2007, Flint 2012, USDA Forest Service 2013, Reeves et al. 2021, Venette et al. 2021). The most common models used in invasive species management focus on predicting probability of introduction, habitat suitability, speed and pathways of spread and, especially in the case of invasive insects, phenology models that predict development and the timing of life stages (Flint 2012, Zhang and DeAngelis 2020, Reeves et al. 2021).

History of the Asian Longhorned Beetle

The Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky, hereafter ALB) has had a decades-long history as an invasive species around the globe. ALB is endemic to China and the Korean peninsula, and since the 1980s has been harmful even in its native range (Hu et al. 2009). In China, damage caused by ALB costs the country the equivalent of $1.5 billion yearly (Hu et al. 2009). ALB likely became a major pest in its native range after 1980 because of the Three-North Shelter Forest Program that was implemented in the late 1970s to combat desertification (Wang et al. 2010). Over the course of 40 years (from 1978-2018), more than 27 million hectares were planted with trees, and another 17 million hectares were aerially seeded with trees (Cao et al. 2020). Unfortunately, a large portion of the planted trees were North American *Populus* species, many of which are highly susceptible to infestation by ALB (Haack et al. 2010). This poor-quality infested wood was then used for solid wood packaging material, such as crates, pallets, and dunnage, and the use of this wood in international trade likely contributed to ALB's spread to Europe and North America (Coyle et al. 2021, Greenwood et al. 2023). While ALB typically moves from its native range to new environments via wood packing materials such as pallets, crates, or dunnage (Fleming et al. 2003, Haack et al. 2010), its movement within landscapes may be driven by human-mediated transport such as the movement of infested firewood (Carter et al. 2010, Javal et al. 2019). The native range of ALB extends from 21 to 51° N latitude (Lingafelter and Hoebeke 2002), although a population was also established in Helsinki, Finland, at 60° N latitude, indicating that it can survive at temperatures colder than what it experiences in its native range (Trotter and Keena 2016).

ALB has become invasive in at least ten European and two North American countries. The first ALB in North America was found in 1992, and the first established population was found in 1996 in New York (Haack et al. 1996, Haack et al. 1997, Haack et al. 2010). In 2001, Austria was the first European country to experience an ALB infestation, followed by France in 2003, Germany in 2004, Italy in 2007, Belgium in 2008, the Netherlands in 2010, Switzerland in 2011, the United Kingdom in 2012, Finland in 2015, and Montenegro in 2015 (Javal et al. 2019). Canada discovered its first outbreak in 2003 on the border of Toronto (Turgeon et al. 2015). ALB infestations in the U.S. have been found in several states: Illinois, Massachusetts, Ohio, New Jersey, and South Carolina (Haack et al. 2010, Coyle et al. 2021). Of the thirteen populations established in the U.S, all but four have been successfully eradicated (USDA APHIS 2023).

ALB eggs are roughly the size, shape, and color of a grain of rice (Figure 1.1a), and larvae are legless, white, and grow to be 30-50mm long. Larvae have large, red-brown mandibles and a patterned pronotal shield (Cavey et al. 1998, Haack et al. 2010, Figure 1.1b). Pupae have the appearance of a translucent white or cream version of the adult, with their tarsi tucked underneath the thorax, and antennae curled underneath and around the front two pairs of legs (Figure 1.1c). Adult beetles are large (17-40mm) and shiny black in color with 10-20 distinctive white or yellow patches on their elytra (Haack et al. 2010). All six tarsi, and the base of each antennal segment, are powder blue or blue grey (Yan and Qin 1992). Antennae have 11 segments on both males and females, though the length differs, with female antennae approximately 1.3 times, and male antennae approximately 2.5 times, their body length (Yan and Qin 1992, Figure 1.1d).

Adult females chew oviposition pits in the bark of a host tree and may lay a single egg underneath the bark in each pit. A single female can lay up to 170 eggs under laboratory conditions but usually lays an average of 50-75 in the field (Keena 2002). Once the eggs hatch, the larvae spend their first three instars chewing a gallery in the cambium before tunneling into the heartwood, where they will continue to feed until pupation (Haack et al. 2010). After pupating, adults chew a circular exit hole 10-15mm in diameter and seek out mates (Haack et al. 2010). Mark-recapture studies have found that adults can fly at least 1,000m in a single season to find new host trees (Nowak et al. 2001, Smith et al. 2001) and estimates suggest they have the potential of flying more than 2000m per season (Smith et al. 2004). However, adults are more likely to remain within 600m of their emergence point, and often reinfest the same tree for multiple generations (Smith et al. 2001, Smith et al. 2004, Xu and Teale 2021).

ALB can use host trees from at least fourteen genera: *Acer*, *Betula*, *Elaeagnus, Fraxinus, Hedysarum*, *Hippophae*, *Koelreuteria*, *Platanus, Populus, Robinia*, *Salix, Sophora, Tilia*, and *Ulmus* (Hu et al. 2009). In North America, it infests almost exclusively *Acer*, *Betula*, *Fraxinus, Platanus, Populus, Salix,* and *Ulmus* (Ludwig et al. 2002, Haack et al. 2006, Turgeon et al. 2022), with strong preference given to *Acer* species (Haack et al. 2006, Dodds et al. 2014, Meng et al. 2015, Coyle et al. 2021).

ALB relies on a wide variety of semiochemicals, including kairomones and pheromones to find host trees and mates. The volatiles that ALB uses to locate host trees tend to be complex combinations, with research efforts finding a wide array of differing results on the subject. Recent research suggests a diverse combination of terpenes, aldehydes, acetates, and alcohols are all involved in the beetle's ability to locate suitable hosts (Xu and Teale 2021). Additionally, trees that have previously been infested are more likely to be re-infested, likely due to compounds that are only emitted from ALB infested trees (Xu and Teale 2021). Since ALB in North America prefers *Acer* species, there is likely also a specific chemical, or combination of chemicals, produced by maples that allows ALB to find its preferred host (Xu and Teale 2021).

Pheromones, the chemicals produced by organisms to find or attract a mate, are often used in invasive insect management, as they can often make surveying and mass-capture of insects possible. The interactions between behaviors and chemicals used by ALB for mate location, unfortunately, are still poorly understood, despite significant research on the subject (Graves et al. 2016, Mitchell et al. 2017, Xu et al. 2020). Current work suggests mate finding involves a four-step process: first, males and females are attracted to host trees with long-range semiochemicals and visual cues; second, either the beetles are attracted towards volatiles released by other ALB, including a trail pheromone left by females and used by males to find them; third, once males are near the females, the males produce a short-range pheromone that attracts the females; and fourth, males find females using visual cues and by using their antennae to detect female contact pheromones (Xu and Teale 2021). Overall, the complicated and diverse nature of the semiochemicals and pheromones used by ALB have precluded the development of an effective trapping method.

Asian Longhorned Beetle Impacts and Management

Adults feed only a small amount on green twigs, petioles, and the midrib of leaves (personal communication, Melody Keena, USFS) and cause little damage, but larvae can cause extensive damage to host trees by feeding on vascular tissue and boring tunnels that weaken a tree's structural integrity (Figure 1.2). This can lead to limb loss, canopy topping, and eventually the death of the tree (Haack et al. 2010). As well as a threat to human safety and property from tree breakage, ALB infestations can incur enormous costs from tree removals and replacements, and result in a mass elimination of host trees in the area (Nowak et al. 2001). If left unmanaged, it is estimated that 12-61% of a city's tree population could be at risk, costing anywhere from $72 million-$2.3 billion per city (Nowak et al. 2001). In Canada, loss of saleable maple wood could cost up to $1.6 billion, and the loss of edible maple products adds on an additional $358 million a year (Pedlar et al. 2020). The risks and costs increase with the speed of ALB population growth, and research suggests that faster larval development could increase the damage and cost caused by an infestation (Nowak et al. 2001, Pedlar et al. 2020).

Many management practices have been evaluated to determine an effective method of controlling ALB infestations, including pheromone trapping, insecticides, and tree removals. As previously described, trapping has been largely unused as a management option due to ALB's complicated mate-finding processes (Xu and Teale 2021). Some insecticides (e.g., imidacloprid) have proven effective against both adults feeding on twigs and foliage and larvae feeding in the vascular tissue of the tree (Poland et al. 2006, Ugine et al. 2011). Systemic insecticides may only target certain life stages, often the first few instars before the larvae tunnel deeper into the heartwood, and therefore need to be applied with more precise timing (Poland et al. 2006). While often effective when used in tandem with other management methods, insecticides are not typically used at a large scale or as a stand-alone practice due to the cost of purchasing and applying the chemicals and the need for frequent reapplications to maintain lethal levels of the chemical in trees (Wang et al. 2002, Hu et al. 2009).

The most common management practice used to eliminate ALB is felling and destroying infested trees (Smith and Wu 2008, Hu et al. 2009, Haack et al. 2010, Turgeon et al. 2022). Although this tactic is also costly and may remove large quantities of host tree species from forests and residential areas, it has proven to be a successful method of controlling and eradicating multiple ALB populations (Hu et al. 2009). This method is being applied in South Carolina; however, large areas of swamps and wetlands make it difficult or impossible to utilize heavy machinery like wood-chippers (Coyle et al. 2021). As such, alternate methods of control are being evaluated (Ratcliff 2022).

The Asian Longhorned Beetle Infestation in South Carolina

Although within the latitudes of ALB's native range, the population of ALB found in 2020 in Charleston and Dorchester Counties, South Carolina (32° N) is the furthest south ALB is known to have established in North America. Although it is uncertain how this population reached South Carolina, genetically it matches the population previously found in Ohio, suggesting that the ALB population in South Carolina was either brought to the area on infested material from Ohio or was imported from a population in China with similar genetics to those in Ohio (Coyle et al. 2021). The infested area in South Carolina has a novel ecology and climate compared to other infestations in North America, providing a unique opportunity to examine this ALB population's development rate and timing of important life stages, such as when eggs or pupae are present, and adult emergence (Coyle et al. 2021).

The Importance of Phenology for the Asian Longhorned Beetle

The duration of each ALB instar is predominantly determined by latitude and climatic conditions (Keena 2002, Keena 2006, Keena and Moore 2010, Meng et al. 2015). The native range of ALB reaches from the North American equivalent of southern Canada to southern Mexico. ALB requires 1264.2 accumulated degree-days (DD) to reach adulthood (Hu et al. 2009) and can survive and develop in locations where temperatures routinely drop lower than -10°C (Trotter and Keena 2016), although in laboratory settings using ALB from populations in Chicago, Illinois, and Queens, New York, the lower threshold for development has been estimated between -3°C and 14°C, and the upper threshold for development is estimated between 35°C and 40°C (Keena 2006, Keena and Moore 2010).

On average, ALB throughout their native and nonnative range reach adulthood in one to three years but can take as long as 10 years to reach adulthood in colder climates, with development time partially dependent on the length and severity of the winter (Trotter and Keena 2016). Extreme cold brings greater risks to survival, however, since the larvae are less likely to develop properly (Keena and Moore 2010) and are more likely to succumb to environmental risks including predation and plant defenses (Trotter and Keena 2016). In the northeastern U.S., adults are generally active starting in May and continuing until November (Haack et al. 1997, Haack et al. 2010), although egg-laying typically does not begin until July (Haack et al. 1997, Lingafelter and Hoebeke 2002, Sánchez and Keena 2013). ALB are capable of overwintering as eggs, larvae, or pupae (Yan and Qin 1992), but typically overwinter as larvae, and if the larvae reach a size and instar capable of pupation late in the year, they may delay pupation to remain in larval form until spring (Trotter and Keena 2016).

The U.S. latitude spans from 25 to 48° N, well within the bounds of which ALB can survive. The eastern half of the country, however, is the main section of the country at risk for invasion by ALB due to the widespread presence of host trees (Kappel et al. 2017). The northeastern U.S. and Appalachian Mountains, especially, have a high percentage of host tree basal area, but cooler temperatures mean that beetle populations will take longer to reach maturity. In contrast, the southeastern U.S. has a lower percentage of host tree basal area, but higher temperatures mean the area is more at risk for increased rate of beetle development (Kappel et al. 2017). The southern portion of South Carolina, Georgia, Alabama, and Mississippi, the eastern half of Texas, and all of Louisiana and Florida, are predicted to have accelerated ALB growth compared to the rest of the country, with phenology modeling suggesting that beetles in this area could fully develop in less than a year (Kappel et al. 2017). The population of ALB established in South Carolina therefore provides an opportunity to substantiate this prediction and determine the phenology of this invasive beetle in the southeastern U.S.

Knowing the detailed phenology of an invasive species is vital for management programs tasked with prediction, monitoring, and eradication. All these responsibilities require a thorough knowledge of the species' development rate and the timing of life stages throughout the year (Oswalt et al. 2021). Monitoring is necessary for effective invasive management, as it gives programs insight into the amount of damage that has been caused, the size and spread rate of the population, and areas at risk to be invaded in the future (Oswalt et al. 2021). For a species like ALB that spends most of its life cycle hidden inside their host trees, phenology is especially important. Without accurate timing on when specific life stages are present in the ecosystem, surveyors seeking out signs of damage at the wrong time of year may conclude that the organism is not present, leading to an inaccurate report on the severity of the infestation (Oswalt et al. 2021). Eradication efforts that require careful timing, such as the use of parasitoid wasps that only attack a certain size of larva, or insecticides that must be applied before larvae tunnel into the heartwood, are also highly reliant on precise timing to be effective (Poland et al. 2006). Although these time-sensitive methods have not been used to eradicate many of the populations of ALB in northern areas, the southern U.S. presents a uniquely challenging combination of warmer temperatures and conditions not conducive for heavy machinery, that has the potential to increase ALB's threat to the area. The compounding effects of faster beetle development and less-efficient control methods puts the South at risk for faster-spreading, more-damaging, harder-to-control infestations of ALB, and creates an urgent need for accurate phenology information so that management programs can effectively detect, monitor, and eradicate new outbreaks.

Thesis Objectives

1. My first objective was to determine the rate of development, life cycle synchronicity, and timing of life stage presence of Asian longhorned beetle in South Carolina.
2. My second objective was to test an available Asian longhorned beetle phenology model (Trotter and Keena 2016), ensure its accuracy in South Carolina, and use the updated model to predict ALB development rates throughout the U.S.

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CHAPTER TWO

DETERMINING PHENOLOGY AND VOLTINISM OF INVASIVE ASIAN LONGHORNED BEETLE IN SOUTH CAROLINA

Introduction

Invasive species can have a significant impact on the environment, economy, and human health and safety (Liebhold et al. 1995, Holmes et al. 2009, Lovett et al. 2016, Diagne et al. 2021). Often a consequence of international trade (Krishnankutty et al. 2020), invasive insects such as the spongy moth (*Lymantria dispar* L.), hemlock woolly adelgid (*Adelges tsugae* Annand), and emerald ash borer (*Agrilus planipennis* Fairmaire) are serious threats to eastern North American forests (Davidson et al. 1999, Herms and McCullough 2014, Lovett et al. 2016, Ellison et al. 2018). Invasive species cause widespread harm to ecosystems by killing or outcompeting native species (Liebhold 1995, Moser et al. 2009) and can generate significant economic and ecological losses both directly by their presence, and indirectly by the cost of management and eradication (Holmes et al. 2009). Wood-boring invasives (e.g., emerald ash borer) are especially harmful, causing major damage to ecosystems and costing billions of dollars in tree removals and replacements (Herms and McCullough 2014).

The Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky, hereafter ALB) is a polyphagous wood-boring beetle native to China and the Koreas, and invasive in ten European countries, the U.S., and Canada (Cavey et al. 1998, Hu et al. 2009, Dodds and Orwig 2011, Turgeon et al. 2015, Javal et al. 2019). The first established population of ALB in North America was found in 1996 in New York (Haack et al. 1996, Haack et al. 1997, Haack et al. 2010). Since that first detection, additional populations have been found in Toronto, Canada, and several U.S.

states, including Illinois, Massachusetts, Ohio, New York, New Jersey, and South Carolina (Coyle et al. 2021, USDA APHIS 2023). ALB is typically introduced to new landscapes via wood packing materials such as pallets, crates, or dunnage (Fleming et al. 2003, Haack et al. 2010), and human-mediated transport such as moving firewood has contributed to localized spread (Carter et al. 2010, Javal et al. 2019).

ALB can feed on and develop in members of at least 14 tree genera, but in North America infestations are almost exclusively limited to *Acer*, *Betula*, *Fraxinus*, *Platanus*, *Populus*, *Salix*, and *Ulmus* (Ludwig et al. 2002, Haack et al. 2006, Turgeon et al. 2022), with preference given to *Acer* species (Dodds et al. 2014, Coyle et al. 2021). Trees become infested when adult females chew oviposition pits in the bark, underneath which they may insert a single egg into the cambium. A single female can lay up to 170 eggs under laboratory conditions but usually lays an average of 50-75 in the field (Keena 2002). Once the eggs hatch, larvae spend roughly three instars feeding in the cambium before tunneling into the heartwood, where they will continue to feed until pupation (Haack et al. 2010). After pupating, adults chew a circular exit hole 10-15mm in diameter and exit the tree. Free living adults feed on the thin bark of twigs and petioles and may disperse to other host trees to find mates or suitable oviposition sites (Haack et al. 2010). Adults usually stay within 600m of their emergence point, and often reinfest the same tree in which they developed (Smith et al. 2001, Smith et al. 2004, Xu and Teale 2021).

ALB was first discovered in South Carolina in 2020, the furthest south a population has established to date in the United States (Coyle et al. 2021). Although it is uncertain how this population reached South Carolina, genetic analyses indicate the population is related to the population found in Ohio, suggesting it was either brought to the area from Ohio on infested woody material (e.g., firewood) or arrived via cargo from the same region of China as the Ohio population (Coyle et al. 2021). The infested area in South Carolina has a novel ecology and climate compared to other infestations in North America and provides a unique opportunity to examine this population's phenology (Coyle et al. 2021). Although several laboratory studies have investigated the development rate of ALB under different temperature conditions (Keena 2002, Keena 2006, Keena and Moore 2010, Sánchez and Keena 2013), little information exists on the phenology of field-collected ALB; most of what does exist was collected in more northern latitudes (Roden et al. 2008, Feng et al. 2014, Liu et al. 2016). The information provided by laboratory studies has been used to build a predictive model and maps of growth rate and risk for the United States (Trotter and Keena 2016, Kappel et al. 2017). These predictions indicate that while northern populations of ALB in the United States develop in an average of 2-3 years, ALB in South Carolina could reach adulthood in as little as 8 months (Kappel et al. 2017). Verification of the accuracy of this prediction is vital to understanding how this invasive species will develop in warmer climates.

Survey and management efficacy is dependent on having accurate knowledge of ALB phenology. The four fundamental elements of invasive species management (prevention, detection, control, and restoration) all necessitate knowledge of the species' growth rate and timing of life stages to be effective (Flint 2012). Awareness of when specific life stages are present during the year can improve the accuracy of surveys (Flint 2012, Oswalt et al. 2021) and can increase the effectiveness of control methods that require carefully timed applications, such as some insecticides (Poland et al. 2006, Ugine et al. 2011), and alternative management methods applied in locations unsuitable for the heavy machinery used in typical ALB control (Ratcliff 2022). Because of the unknown impacts of this new climate on ALB development, having accurate phenological information is necessary for both detection and management of

ALB. As such, our objectives were to determine (1) how long it takes for ALB to complete a generation in South Carolina, (2) if ALB has a synchronous life cycle, and (3) what time of year each instar and life stage are present.

Methods

Site Description

The South Carolina federal ALB quarantine zone is currently a 76 mi^2 area covering sections of Charleston and Dorchester counties (USDA APHIS 2023, Figure 2.1). Located in the coastal marsh ecoregion of South Carolina, this quarantine zone is characterized by pine and hardwood forests, with a humid, subtropical climate that features hot summers and mild winters (Griffith et al. 2002, SC DNR 2005, Griffin and Mogil 2021). The North Charleston Weather Station (KHB29 Charleston) recorded an annual high of 36˚C (98˚F) and low of -8˚C (18˚F) in 2022, with a total of 127.3 cm (50.1 in) precipitation. 2021 had an annual high of 35˚C (95˚F) and low of -3˚C (26˚F), with a total of 149.1 cm (58.7 in) of precipitation (climate data acquired from the National Oceanic and Atmospheric Administration's (NOAA) annual climate report from the National Weather Service Forecast Office in Charleston, SC: https://www.weather.gov/wrh/Climate?wfo=chs, accessed 4 October 2022).

Tree Selection

To track the timing of ALB life stages and their development, I collected live beetles monthly from infested trees from August 2021 through July of 2023. I exclusively collected red maple trees (*Acer rubrum* L.) since they make up the majority of ALB's host trees in this area (Coyle et al. 2021). I preferentially selected highly infested red maple trees (between 15-50cm diameter at breast height) to maximize the quantity of ALB collected for the quantity of wood that had to be processed. Signs of heavy infestation vary among different stages of the ALB life cycle. Eggs and neonates can only be identified via the egg site chewed by their mothers, but after a few weeks of growth, bleeding sap and frass caused by the larval feeding is exuded from the egg site, providing additional visual cues. As the larvae grow larger and tunnel into the heartwood, bleeding sap production declines, frass and sawdust discharge increases, and the bark covering the galleries begins to dry and crack (Figure 1.2, Chapter 1). In heavily infested trees, external signs of stress such as epicormic sprouting can appear. I collected between three and eleven trees each month, with an average of 4.4 trees collected per month. Selected trees were cut down, and visibly infested sections were transported to the field laboratory at the USDA APHIS Facility on the College of Charleston’s Stono Plantation (32°44'39.9"N, 80°10'56.0"W).

Life Stage Collection

Eggs were recorded on a presence/absence basis during the first five months of sampling (August - December 2021) but were collected and counted during the remainder of the study (January 2022 - July 2023). Eggs and early instars were collected by peeling the bark down to the cambium layer with a draw knife, chisel, or pocketknife to locate egg sites and galleries in the vascular cambium. I started the peeling at the egg site, exposing the entirety of the visible gallery, and if a larva was not present on the surface, identified the location of the tunnel where the larva chewed deeper into the heartwood. Tunneling larvae and pupae were collected by splitting the wood using an electric log splitter (Central Machinery, Calabasas, CA) and pulling out specimens with forceps. Logs with visible signs of ALB damage were split into approximately one-inch sections, which helped ensure no section of wood large enough to hold undetected larvae remained. Collected ALB specimens were preserved using 95% ethanol and transported to the Forest Health and Invasive Species Laboratory at Clemson University in Clemson, SC (34˚40’12” N, 82˚49’48” W). Timing of flying adults was collected via visual observations of adult ALB in the field by pooling the observations made by both USDA APHIS and Clemson University personnel.

Larval Head Capsule Measurements

In soft-bodied holometabolous species such as coleopterans, larval body size can vary as individuals grow, but the size of the head capsule for each instar is set when it sclerotizes after a molt. Because of this fixed size, head capsule measurements through time tend to have a multi-modal distribution, with modes associated with specific instars, as described by Dyar’s Rule (Logan et al. 1998, Calvo and Molina 2008, Delbac et al. 2010). I measured head capsule width to determine larval age and the number of instars before pupating (Panzavolta 2007, Calvo and Molina 2008, Delbac et al. 2010). Head capsule width was measured by taking digital images of the ventral side of the head with a microscope and camera system (Motik K-400 Stereomicroscope with Canon DSLR Camera) with inclusion of a digital scale bar. Images were then imported to ImageJ (Schneider et al. 2012) and the head capsule was measured (in mm) at its widest point.

HCAP Program

HCAP is a statistical program created in 1998 by a team of researchers seeking to determine the instar distribution of the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) using head capsule widths (Logan et al. 1998). The program was developed using

MATLAB (Math Works 1993) and uses a combination of user input and iterative mathematics to determine the statistical modes in the head capsule measurement data to identify and calculate the size ranges associated with each instar. The program starts by producing a histogram of input head capsule widths, then asks the user to provide a set of initial estimates of the locations of size-breaks between instars. Once the user has chosen where they believe the instar peaks fall, the program uses the user-provided breaks to start an iterative process of fitting lines using multiple polynomial models to the histogram, and then graphs individual curves where the program calculates each instar distribution lies. The final output of the program is a histogram with three lines; a line fitted to the user's original guess, a line fitted to the best fit line, and a parabola for each distinct instar showing the possible range of head capsule sizes. I used this program with slight alterations, namely updating the code to work with the current version of MATLAB (Math Works 2021), and modifications that generate graphs that display the monthly head capsule widths on top of the combined head capsule widths of both years to better display the changes in distribution over time. I used the pre-established parameter of 30 boxes in my histogram and chose to select five instars as my estimation because of the appearance of the graph and because any instars past the fifth do not greatly increase in size (Keena and Moore 2010), making any differentiation based on size difficult.

Results

Instar Determination

I collected a total of 148 eggs, 819 larvae, 38 pupae, and 1 un-emerged adult over the two years of sampling (Table 1). The number of larvae collected varied each month, ranging from 0 (May 2022) to 139 (December 2022). The HCAP program determined that first instar head capsules range in size from 0 - 1.19mm; second instar ranges from 1.20 - 1.64mm; third from 1.65 - 2.20mm; fourth from 2.21 - 4.06; and fifth from 4.07 - 5.97mm (Figure 2.3). Using this calculation, I collected 28 first, 75 second, 46 third, 148 fourth, and 521 fifth instars. First to third instars were present during and immediately after time periods when eggs were found, from June until November of both years, but were only found in relatively large numbers in June, July, and August (Figure 2.4). Fourth and fifth instars comprised the largest proportion of the population for most of the time in which larvae were present. Fewer small larvae than large were collected, with fifth instars making up more than half of the larvae collected (Figure 2.5). A small percentage of larvae reached fourth or fifth instar as early as August, and most of the population reached fourth or fifth instar by September (Figure 2.4).

Head Capsule Widths and Life Cycle

Eggs were present from June through November (Figure 2.5) and comprised the largest portion of the population in June of both years (Figure 2.5). Larvae were present year-round, except for May 2022 where the only specimen collected was a single adult that had not yet chewed a tunnel from its pupal chamber to exit the tree. From December through March of both years, larvae were the only life stage present (Figure 2.6). Pupae were only collected in April in 2022 but were collected April-June in 2023. No un-emerged adults were collected in 2023. Adults were present in May and June in 2022, and May through July in 2023.

Larval head capsule width increased in size from June to December, and then maintained size until April (Figure 2.5). The average larval head capsule width was smallest in June in both 2021 and 2022 (average head capsule width of 1.52mm ± 0.4 and 1.09mm ± 0.01 respectively), and largest in January 2022 (4.82mm ± 0.05) and December 2023 (5.01mm ± 0.03) (Figure 2.2).

November in both years had the greatest size difference between the largest and smallest larval head capsules (Figure 2.2, Figure 2.5). Over the course of both years of sampling, first instars were present June - September; second instars were present July, August, and November; third instars were present July, August, October, and November; fourth instars were present August-April, as well as June; and fifth instars were present September - May (Figure 2.1). The range in sizes of head capsules were almost entirely identical between the two years of sampling (Figure 2.5). A considerable difference in head capsule size ranges is evident only in August for each year (Figure 2.5).

Discussion

Based on these results, ALB in South Carolina appears to have a synchronous univoltine life cycle. Eggs laid in June, July, and August can reach their full size as fifth instars and are large enough to pupate the following April. This is a considerable increase in development rate compared to populations in the northern U.S. but is slower than the eight months predicted by previous modelling, which may be due to the larvae maintaining (or requiring) a pupal hold during winter months despite the warmer temperatures (Trotter and Keena 2016, Kappel et al. 2017). The majority of ALB larval growth occurs within the first three months after egg hatch (Figure 2.2). Larval growth rate slows down after they reach the fourth instar, which most did by September. By October most are in the fifth instar, although slight growth continues until December (Figure 2.4). Larvae spend December through March growing only slightly or maintaining size until they pupate in April. Flight season appears to be from May until June. Over both years, only one larva was collected in May (Figure 2.5), which coincides with when pupae that were present in April would be emerging and the adult flight season beginning (Figure 2.5, Figure 2.6).

The data show that ALB can develop from an egg to a fifth instar in as little as five months in South Carolina and can complete development to emerge as adults the following spring. So far, these populations of ALB appear to retain a pupal hold period with little or no growth throughout the winter months and are emerging as adults at approximately the same time in the spring. It is unknown if a warmer location with milder winters will cause a pupal hold (Keena and Moore 2010, Torson et al. 2021). Without the pupal hold slowing larvae to a yearly life cycle, multiple generations per year may be possible.

This rapid development rate becomes more concerning when seeing the variation in larval size collected in November of 2021 with eggs and first instars present during a time when I expected to find fourth and fifth instars. This variation could be caused by several scenarios: 1) adults from the 2021 flight season were ovipositing much later in the year than usual; 2) eggs laid during the 2020 flight season grew unusually slowly, and took a year and a half to develop into adults, therefore laying their eggs several months later than their generational cohort; 3) eggs laid during the 2021 flight season grew unusually fast, and were able to gain weight fast enough to pupate before the weather cooled down and the pupal hold went into effect.

This last possibility is the most concerning option, since it would indicate that in an ideal climate ALB would be capable of having a six-month life cycle. This would be a major concern for management and eradication efforts, since the slow growth and spread of ALB is one of the major reasons it has been successfully controlled in so many of the locations it has invaded (Haack et al. 2010). Climate change providing warmer habitat for ALB may also contribute to the creation and perpetuation of fast-growing populations of ALB. So far, these small larvae do not appear to be persisting in the population after November, as there were no first, second, or third instars collected in December of either year (Figure 2.4), but more research is needed to examine this anomaly.

Phenology is an important tool for forest pests in North America, but in many cases, it is overlooked in favor of research studying immediate eradication or management concerns. Tactics like pheromone trapping, insecticide applications, host species risk, biocontrol, and modelling distribution and spread often take precedence over investigating underlying biological mechanisms of a species, such as phenology and voltinism. However, without this information, many of these eradication methods are hampered by inexact targeting of specific life stages. Phenology research is even more important for wood-boring beetles like ALB and emerald ash borer because much of their life history is obscured while their larvae are burrowed inside trees (Herms and McCullough 2014, Haack et al. 2010). While some phenological milestones may be easy to monitor, such as flight seasons, especially of species like spongy moth that are easy to trap (Liebhold et al. 2021), more subtle changes such as timing of ALB or emerald ash borer’s specific instars cannot be determined just by observation. Phenological data is necessary to give management programs precise timing on an invasive species’ life stages so they can precisely target eradication efforts, especially with increasing temperatures from climate change causing shifts in insect ranges and development rates.

Modelling is also an essential tool to give management programs the phenology information without requiring labor-intensive field work at every new location. The ability to anticipate how an invasive insect will respond to local climates before a population is established gives management programs a significant advantage, allowing them to locate areas at high risk for invasion and negative impacts, time surveying for new detections, and have a management plan in place for a rapid response to new invasions. A phenology model has been created for ALB but has only been tested using data from the beetle's native range, European infestations, and populations from the northern U.S. (Trotter and Keena 2016). More research is needed to test whether this model will continue to make reliable predictions in the southern U.S.

Study Limitations

As with all studies, there are limitations on the interpretation of the data resulting from methods and data availability. In this case there are two primary limitations. First, the shift in visual cues as the larvae grow could also have caused a sampling bias. For example, if I looked for trees with signs of large larvae because that was the most prevalent damage type to be found that month, I could have failed to notice or collect trees that had signs of smaller instars. Likewise, if I expected to see signs of newly hatched larvae, I may have unintentionally passed over trees with signs of older larvae. Unfortunately, this method of choosing trees was necessary to find the number of larvae needed to represent larval development as the year progressed. Additionally, it is possible that only one adult oviposited on a single tree, which would consequently lead to all the larvae in that tree being roughly the same age and size.

A second limitation on the interpretation is imposed by using the HCAP analyses to identify putative instars in the head capsule size data. The HCAP program was a very useful tool in my determination of instars, but there were some areas where it was limited by my sample size and study organism. The HCAP program was originally designed for instar determination of mountain pine beetle (Logan et al. 1998). With mountain pine beetle, it is relatively easy to collect thousands or tens of thousands of larvae, whereas I was only able to collect fewer than a thousand larvae over the course of two years. The lower sample size made selections of the instar bound more difficult and made for much more variable results. The addition of ALB's supernumerary instars also made using the program more complicated, as although I chose to select five instars, it is possible that the variability of size in the fifth instar is because it is also including instars past fifth that may or may not lead to a change in the larvae's size. Despite these issues the HCAP program was able to give at least a rough estimate of instar size and timing.

Conclusions

The data described here show that ALB populations in South Carolina show a pattern in sizes and life-stages consistent with a synchronous, univoltine life cycle. This univoltine life cycle seems to be imposed by the hold in development that occurs in winter prior to pupation. The loss of this pupal hold period could lead to faster development with some potential for a multivoltine cycle. This has significant implications for management, as faster development and speed of spread could make controlling invasions of this species much more difficult.

These data also provide key information for management options such as insecticides or biocontrol agents that are only effective against first through third instars that have not tunneled into the heartwood are also impacted by the rate of growth. If first through third instars are only present three to four months of the year, it greatly reduces the amount of time that these management options can be applied successfully. Careful timing will be necessary to manage this species using these methods. Overall, more research is needed to confirm the possibility of ALB reaching adulthood in less than a year, and predicting whether other areas of the United States will be at risk for a rapidly growing population.

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CHAPTER THREE

VALIDATING A VARIABLE-INSTAR, CLIMATE-BASED PHENOLOGY MODEL USING FIELD DATA FROM A NOVEL CLIMATE AND PREDICTING ASIAN LONGHORNED BEETLE DEVELOPMENT RATE IN NINE LOCATIONS WITH VARYING LATITUDES

Introduction

Invasive species are widely regarded as one of the greatest threats to the health and sustainability of natural ecosystems around the world (Liebhold et al. 1995, Holmes et al. 2009, Lovett et al. 2016, Diagne et al. 2021). Capable of dealing major damage to native ecosystems, and costing billions of dollars in lost profits and management costs every year (Liebhold et al. 1995, Holmes et al. 2009, Moser et al. 2009, Mayfield et al. 2021), effective management of invasive species is necessary to protect and conserve these ecosystems (Flint 2012, Mayfield et al. 2021). The four main elements of invasive species management are prevention, detection, control/management, and restoration, and each requires accurate knowledge of the target species' biology to function effectively, but hands-on field research is rarely suitable for the rapid response and quick control of a situation necessary to prevent invasives from spreading (Flint 2012). One way that management programs gain the information they need without having to spend time and money researching is by using models.

Predictive modelling is a valuable tool for invasive species management, as it can equip managers with information both before an invasive is introduced and after the infestation is established (Flint 2012). Models have been used to predict potential distribution and spread of ALB throughout its invasive and native range (Lu and Russell 2005, Schatz et al. 2013, Gourley and Lou 2014, Schatz et al. 2016, Trotter et al. 2018, Huang et al. 2020, Zhang et al. 2022), but have not been used to predict ALB phenology with comparable frequency (Faccoli et al. 2014, Trotter and Keena 2016). This often leaves management agencies without precise phenology information, which can render surveying and management methods that require strict timing ineffective (Finch 2012, Flint 2012, Venette et al. 2021).

The Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky, hereafter ALB) is a wood-boring beetle invasive throughout Europe and North America (Cavey et al. 1998, Hu et al. 2009, Dodds and Orwig 2011). Native to China and Korea, breeding populations of ALB have been found in ten European countries, one Canadian province, and six U.S. states (Haack et al. 2010, Coyle et al. 2021, USDA APHIS 2023a). ALB is typically introduced to new locations in solid wood packing material (e.g., crates and wooden pallets) and is also conveyed within countries through human movement of firewood (Fleming et al. 2003, Haack et al. 2010, Carter et al. 2010, Javal et al. 2019).

ALB was first discovered in North America in 1996 in New York, and of the thirteen total populations that have been established in the U.S., all but four have been completely eradicated (USDA APHIS 2023b). ALB is a polyphagous wood-boring beetle that can use over fourteen genera of trees as hosts, with a strong preference for *Acer* in North America (Ludwig et al. 2002, Haack et al. 2006, Dodds et al. 2014, Coyle et al. 2021, Turgeon et al. 2022). After hatching from eggs laid under the bark of their host trees, ALB’s first three instars are spent feeding on the cambium layer of the tree, after which the larva tunnels deeper into the sapwood and heartwood of the tree, where it feeds until pupation. After pupating, the adult beetle will chew a 10-15mm diameter exit hole and leave to feed and find a mate (Haack et al. 2010).

Prior to 2020, the range that ALB’s invasive populations inhabit tended to be high in latitude, stretching from Finland (60˚N) at the northern edge of its range down to Ohio, U.S.

(39˚N). This range was extended further south in 2020 with the discovery of an established population of ALB in Hollywood, South Carolina, U.S. (32˚N), but ALB still has potential to travel even further south given that its native range extends to the Hainan province of China (19˚N) (Zhang et al. 2022), the North American equivalent of southern Mexico.

Despite ALB's extensive native range, little field research has been conducted on the beetle's phenology, and past work has focused on the northern regions of this range (Roden et al. 2008, Feng et al. 2014, Liu et al. 2016); thus, the population in South Carolina provides an opportunity to examine ALB phenology at a lower latitude. Several laboratory studies have been conducted to determine ALB's development rate under various temperatures (Keena 2002, Keena 2006, Keena and Moore 2010, Sánchez and Keena 2013) and data from this research was used to create a predictive model and risk map for the U.S. (Trotter and Keena 2016, Kappel et al. 2017). These studies predicted that while most ALB in the northern regions generally develop in 2-3 years, ALB in the southeastern U.S. had the potential to reach adulthood in as few as 8 months (Trotter and Keena 2016, Kappel et al. 2017). This would be an abrupt increase in development rate compared to other infestations in the U.S. and would have major implications for the management programs involved in eradication efforts. As the current model has been validated only using data from northern regions, the South Carolina population provides a unique opportunity to evaluate the model and validate it with field data from a lower-latitude population. Therefore, my objectives were to 1) assess the model's predictive accuracy of ALB's development rate and generation times for South Carolina using field-collected validation data, and 2) use the updated model to make predictions about ALB's development rate/generation times for eight at-risk locations in the southern U.S.

Methods

The ALB phenology model (Trotter and Keena 2016, hereon referred to as simply "the phenology model") is an agent-based life-table model created in MATLAB (Mathworks 2013b) designed to predict the development rate of ALB based on climate in various locations around the world. Agent-based models utilize computer simulations that can study the interactions between many complex variables. This model works by using field research performed in ALB's native range (Yan and Qin 1992, Yang et al. 2000, Smith et al. 2004, Haack et al. 2006) and laboratory research of ALB's heating degree day requirements (Keena 2006, Keena and Moore 2010, Sánchez and Keena 2013), to simulate beetle development at various temperatures. Using this information, the model runs an individual beetle through its full development, from egg to adult, and calculates how long the process will take. By adding variation between individual beetles to simulate real-world differences in habitat and growth rate and increasing the number of beetles and number of generations of beetles produced, the model can produce a histogram of predicted beetle development times for a given location (Figure 3.1).

I ran the model with 400 beetles for 400 years, using all the recommended parameters included in the program: eggs were started on Julian date 230; flight season was ended on Julian date 345; a pupal hold was included; and the recalculated heating degree day parameters were used. For more information on the exact parameters used in this model, see Trotter and Keena (2016).

To test the model's accuracy in South Carolina, I updated the code to run in MATLAB 2021 (Mathworks 2021) and added temperature data from Charleston, SC (32˚N). I validated the accuracy of the model using field data collected from Hollywood, SC, as detailed in Chapter 2, and after demonstrating the model's continued accuracy in the southern U.S., added eight more locations: Chicago, IL (41˚N), Salt Lake City, UT (40˚N), Bowling Green, KY (37˚N), Norfolk, VA (36˚N), Albuquerque, NM (35˚N), Atlanta, GA (33˚N), Baton Rouge, LA (30˚N), and Tampa, FL (28˚N). These locations were chosen based on the variety of latitudes, areas with high percent host tree basal area (Kappel et al. 2017), areas with high risk to new invasions (e.g., near a large port), and areas with continuous temperature records from January 1, 2003 - January 1, 2023. Temperature data for each location was obtained from NOAA's climate data archives (National Centers for Environmental Information (NCEI) n.d.).

Results

The model predicted that ALB in Chicago (Figure 3.1a) and Salt Lake City (Figure 3.1b) could complete development in approximately 1-3 years; beetles in Bowling Green (Figure 3.1c), Norfolk (Figure 3.1d), and Albuquerque (Figure 3.1e) could complete development in approximately 1-2 years; beetles in Atlanta (Figure 3.1f), Charleston (Figure 3.1g), and Baton Rouge (Figure 3.1h) could complete development in approximately 0.5-1.5 years, and beetles in Tampa (Figure 3.1i) could take only four months to one year to fully develop. Charleston and Baton Rouge have the most clustered results, with most beetles in each location (58.1% and 61.0%, respectively; Table 3.1) predicted to complete development in exactly one year (Figure 3.1). The next highest cluster is in Chicago, where 43.1% of beetles are predicted to develop in exactly two years, followed by Atlanta, with 35.0% of beetles predicted to develop in exactly one year (Table 3.1).

Overall, ALB in cities at lower latitudes (with warmer climates) are predicted to take less time to develop (Table 3.1), with the exception of Bowling Green, Norfolk, and Albuquerque, which all take approximately the same amount of time to develop (Table 3.1, Figure 3.1c,d,e).

Tampa, with the lowest latitude, has the shortest predicted development time of the cities, with the longest predicted development period (13 months; Table 3.1) shorter than beetles in Baton Rouge, the next most southern city, by nearly seven months (Table 3.1). Tampa also has the most continuous development completion predicted, with every month from four to thirteen observing beetles completing development, whereas the rest of the cities have adult beetles predicted to finish development in clumps around the six month, one year, one and a half year, two year, and three year marks (Figure 3.1, Table 3.1).

Discussion

The type of phenology model used here to make these predictions is a relatively new type of tool for determining the phenology of invasive insects. Many management agencies and research projects still utilize simple degree-day models to predict flight seasons and general development patterns (Smith et al. 2004, Faccoli et al. 2014), but these models do not always make highly precise predictions and are not able to compute more nuanced or complex factors, such as variation in degree day requirements between instars, or the timing of diapause periods (Nielsen et al. 2016, Rebaudo and Rabhi 2018). The agent-based life-table model evaluated and used here, however, is a much more complex method that can capture more nuances of ALB development by creating a step-by-step simulation of beetle development under varying temperature conditions (Trotter and Keena 2016). This method of modelling has been successfully utilized to determine phenology of lepidopterans and heteropterans (Chen et al. 2011, Nealis and Règniére 2014, Nielsen et al. 2016), and the increased accuracy allows researchers and managers to survey and apply management methods more effectively.

This phenology model was able to reasonably predict the development rate of ALB in South Carolina, but there are still questions left unanswered. Most ALB in Charleston are predicted to take around one year to develop to adulthood. This is consistent with field data collected in the Hollywood, SC area, which also showed a consistent one-year life cycle (Chapter 2). The similarity in the model output and field-collected data indicate that the model, despite being originally designed and validated using laboratory data from ALB's native range in China and from northern U.S. populations, is making reliable predictions in a more southern climate.

The model also predicts a small percentage of the population will develop in as few as four to five months, a considerable jump in development rate compared to northern populations (Table 3.1). The possibility of ALB developing in half a year is alarming, since no other infestations in the U.S. have been found to develop in less than one year (Trotter and Keena 2016). The potential for increased reproduction rates and resulting population spread could make management of this species a much greater challenge, especially in the southeastern U.S. where management agencies are already facing more difficult conditions due to the large quantity of swamps and wetlands where heavy machinery used in most eradication operations cannot be utilized (Ratcliff 2022). It is unknown, however, whether generations that take less than a year to develop will be able to persist in the population or will be re-synchronized to a yearly cycle by the pupation hold that ALB undergoes in cold weather.

Although the agreement between field observations and the model output is far less clear-cut when comparing them with the field data, there is some evidence to support the possibility of one or both situations occurring. The presence of eggs in November 2021 and October 2022 (Chapter 2) does match the timing of an egg laid in May or June taking five to six months, or a year and six months, to develop to adulthood and lay eggs of its own. It is impossible to say if either of these scenarios is truly what is occurring without more research being done, especially because these unusually timed eggs do not seem to persist into the population, as there were no first instars present during or immediately after the months these eggs were found (Table 2.1). More research, especially in the months of October and November, is needed to determine if the model's prediction of ALB developing in half a year is possible. To dispel the uncertainty around these predictions, continued evaluation of the model's accuracy at a wider variety of latitudes and ecosystems would be ideal, but because the South Carolina population is the furthest south ALB has been found in North America it is currently the only available opportunity for collecting field data at lower latitudes.

Overall, the latitude of the cities is a major determinant in how quickly beetles are predicted to develop. Although this pattern breaks slightly with the three cities most similar in latitude (Bowling Green, Norfolk, and Albuquerque), it holds true for the northernmost and southernmost locations (Figure 3.1). Tampa, the location furthest south, is an even more abrupt decrease in development time compared to the rest of the cities. Although Florida has a lower basal area of host trees at risk than the rest of the Southeast (Kappel et al. 2017), it also has the potential for very fast beetle growth, with the longest predicted development only 13 months. Not only is this fast, but the continuous timing of development, with beetles maturing to adulthood every month, also has the potential to morph into a continuous year-round emergence of adults. This rate of growth and constant presence of adults would almost certainly lead to faster reproduction, population growth, and spread of beetles, and even in an area with relatively few host trees would make it much more difficult to manage and eradicate the infestation.

ALB development in warmer areas also raises the question of whether the pupal hold ALB has maintained is obligate. Research is limited on this topic, and what exists does not reach a strong consensus. One study indicated that ALB required a period of chilling for larvae to pupate, but research was not able to determine the minimum dormancy time a larvae needed before it was able to pupate and was not able to determine the maximum temperature at which ALB would enter into this dormancy period (Torson et al. 2021). Another study indicated that ALB were capable of pupating without this chilling period when reared at a continuous 20˚C, but chilling was required at higher rearing temperatures (Keena and Moore 2010). More research is needed on this topic to determine whether this dormant period is obligate for ALB and, if not, what temperature threshold is required to bypass it, as this information is critical to understanding how development will change in warmer locations.

These predictions also become potentially more concerning when taking climate change into account, as states like South Carolina that have both an abundance of host trees and an already accelerated beetle development rate compared to northern populations experience increased temperatures. For management programs tasked with quarantining, controlling, and eradicating infestations, faster spread will mean more difficult, labor-intensive, and costly work. This increased risk makes it especially important for ALB management programs like the USDA APHIS to be able to predict where new populations of ALB will occur, and how fast they will be able to grow in those locations now and in the future. The global use of solid wood packing material for shipping leaves ports at risk for introductions of wood-boring beetles, and despite phytosanitary treatment of wood and inspection of material at entry ports, the constant influx of nonnative beetles means that new populations of invasive species will always be a concern (Haack 2006, ISPM 15 2017, Wu et al. 2017).

ALB is not the only invasive species that can benefit from this type of phenology model. Management programs of other forest pests will also be improved by the introduction of agent-based phenology models. Even emerald ash borer (*Agrilus planipennis* Fairmaire), one of the most devastating invasive insects in North America (Herms and McCullough 2014, Klooster et al. 2018), has had surprisingly little research done on the phenology of the species until recently (Lyons and Jones 2005, Lyons et al. 2006, Bohannon et al. 2022), and phenology modelling of this species is similarly limited to simple degree day models (Discua Duarte 2013). Without specific, location-tailored phenology knowledge, management programs must contend with imprecise targeting of life stages, and subsequently less effective control of pest species negatively impacting North America's forests. This model will help improve others around the world, as it provides evidence for the accuracy and versatility of agent-based phenology models. It is vital for management agencies to have accurate phenological information of invasive species in as wide a range of conditions as possible so they can prepare for and effectively manage new and current infestations.

Conclusions

Based on validation data from the South Carolina ALB population, the phenology model has continued to make reasonable predictions in the southern U.S. ALB between 40-41˚N (Chicago and Salt Lake City) are predicted to develop in 1-3 years; ALB from 35-37˚N (Bowling Green, Norfolk, and Albuquerque) in 1-2 years; ALB between 30-33˚N (Atlanta, Charleston, and Baton Rouge), are predicted to have a predominantly univoltine life cycle, with a small percentage developing in 4-5 months and 1 year and 8-9 months; and ALB in Tampa (28˚N) are predicted to develop in one year or less. This has major implication for management programs in the southern U.S., as faster development may lead to faster population growth and more difficult eradication of beetle populations. Additionally, as climate change increases temperatures, both new and already established populations could see an increase in development rate. Since the beetle’s native range extends much further south than South Carolina, they are capable of surviving warmer temperatures than they are currently, leaving all the southern U.S. and most of Mexico at risk for new infestations.

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CHAPTER FOUR
THESIS CONCLUSIONS

1. **ALB in South Carolina have a synchronous univoltine life cycle, which appears to be caused by the pause in development that delays pupation during the winter months.** Eggs are laid June through November, larvae are present June through April, pupae are present April through June, and adults are present May through July. This population of ALB is significantly faster development than the one-to-three-year development rate that has been recorded in ALB populations in the northern U.S. This univoltine cycle appears to be caused by the pupation hold that halts development during the winter months, as all larvae slow or stop developing around November and December. There may be a partial generation occurring in less than one year, but field sampling was inconclusive.
2. **The ALB phenology model predicts that most beetles in South Carolina will develop in one year.** The two locations in the southern U.S. with similar latitudes to the infestation in South Carolina (Atlanta, GA and Baton Rouge, LA) had similar predictions to the South Carolina population when evaluated by the model, but the location furthest south (Tampa, FL) was predicted to have an even more accelerated development rate, with all beetles developing in under a year. Although it is possible that this prediction is incorrect, and an ALB infestation would be synchronized to a univoltine cycle due to the presence of a pupation hold, validation of this model is needed to confirm these predictions. If these predictions hold to be true, however, there is potential for ALB to develop much faster than they are in the northern U.S., leading to faster growing populations and more difficult eradication for management agencies.

3. **Although the mid-range locations analyzed by the model are more southern in latitude compared to previously established ALB populations, their latitude and climate still resulted in their predictions of ALB development rate to be considerably different from both the northern and southern locations.** ALB in the three mid-latitude locations (Albuquerque, NM, Bowling Green, KY, and Norfolk, VA), were all predicted to complete development in 1-2 years. Although ecological differences between the locations could cause deviation from this prediction, the similarities in climate mean that these three locations will likely have similar patterns of development if infestations occur.

APPENDICES

Appendix A

Tables

Chapter Two Tables:

Table 2.1: Counts of Asian longhorned beetle life stages and instars for each month over two years of sampling. Instar counts were calculated using the HCAP program (Logan et al. 1998).

	Eggs	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Pupae	Un-emerged Adults	Totals
August 2021	5	0	1	4	51	0	0	0	**61**
September 2021	0	1	0	0	14	1	0	0	**16**
October 2021	0	0	0	0	16	15	0	0	**31**
November 2021	2	0	5	1	8	4	0	0	**20**
December 2021	0	0	0	0	0	22	0	0	**22**
January 2022	0	0	0	0	4	79	0	0	**83**
February 2022	0	0	0	0	3	32	0	0	**35**
March 2022	0	0	0	0	3	18	0	0	**21**
April 2022	0	0	0	0	2	42	24	0	**68**
May 2022	0	0	0	0	0	0	0	1	**1**
June 2022	36	6	0	0	1	0	0	0	**43**
July 2022	27	0	5	3	0	0	1	0	**36**
August 2022	17	6	21	20	5	0	0	0	**69**
September 2022	0	0	0	0	11	3	0	0	**14**
October 2022	1	0	0	1	5	10	0	0	**17**
November 2022	0	0	0	1	13	24	0	0	**38**
December 2022	0	0	0	0	1	138	0	0	**139**
January 2023	0	0	0	0	3	15	0	0	**18**
February 2023	0	0	0	0	1	50	0	0	**51**

March 2023	0	0	0	0	4	33	0	0	**37**
April 2023	0	0	0	0	3	34	10	0	**47**
May 2023	0	0	0	0	0	1	1	0	**2**
June 2023	19	3	0	0	0	0	1	0	**23**
July 2023	46	12	43	16	0	0	0	0	**117**
Totals	**153**	**28**	**75**	**46**	**148**	**521**	**37**	**1**	**1009**

Chapter Three Tables:

Table 3.1. Percent of ALB population predicted to complete development during each month for nine cities in North America. * indicates <0.01%.

Month	Chicago	Salt Lake City	Bowling Green	Norfolk	Albuquerque	Atlanta	Charleston	Baton Rouge	Tampa
1	0%	0%	0%	0%	0%	0%	0%	0%	0%
2	0%	0%	0%	0%	0%	0%	0%	0%	0%
3	0%	0%	0%	0%	0%	0%	*	0%	0%
4	0%	0%	0%	*	*	0.20%	0.39%	0.22%	0.95%
5	0%	0%	0%	0.01%	0.01%	0.55%	4.76%	5.31%	4.96%
6	0%	0%	0%	*	0%	0%	0%	0%	11.81%
7	0%	0%	0%	0.01%	0%	0%	0%	0.01%	13.18%
8	0%	0%	0%	*	0%	0%	0.05%	0.34%	8.00%
9	0%	0%	0%	0%	0%	0.06%	2.15%	3.90%	14.02%
10	0%	0%	0.05%	0.23%	0.06%	3.71%	7.37%	6.87%	11.96%
11	0%	0.04%	3.75%	5.22%	3.08%	14.09%	10.92%	9.68%	10.92%
12	0.24%	6.01%	23.14%	24.87%	23.69%	34.95%	58.10%	60.98%	23.18%
13	5.62%	14.52%	21.91%	22.29%	13.68%	12.73%	5.36%	3.57%	1.02%
14	12.89%	13.54%	8.39%	8.85%	14.77%	15.76%	2.43%	1.23%	0%
15	0.05%	0.13%	2.87%	10.17%	0.97%	4.08%	4.38%	5.64%	0%
16	*	0%	0.01%	0.01%	0.03%	*	0%	0%	0%
17	0%	0%	*	0%	*	0%	0%	0%	0%
18	0%	0%	0%	0%	0.01%	0%	0%	*	0%
19	0%	0%	0%	0%	*	0.84%	2.39%	1.95%	0%
20	0%	0%	0.01%	0.15%	0.01%	4.22%	1.37%	0.27%	0%
21	0.02%	1.38%	9.29%	10.40%	7.97%	3.91%	0.36%	*	0%
22	7.22%	14.79%	8.58%	6.37%	11.64%	4.70%	0%	0%	0%
23	13.47%	14.83%	5.79%	6.88%	3.97%	0.18%	0%	0%	0%
24	43.10%	24.79%	16.19%	4.54%	19.90%	0%	0%	0%	0%

25	2.42%	1.50%	*	0%	0.24%	0%	0%	0%	0%
26	5.19%	4.24%	0%	0%	0%	0%	0%	0%	0%
27	0%	0%	0%	0%	0%	0%	0%	0%	0%
28	0%	0%	0%	0%	0%	0%	0%	0%	0%
29	0%	0%	0%	0%	0%	0%	0%	0%	0%
30	0%	0%	0%	0%	0%	0%	0%	0%	0%
31	0%	0%	0%	0%	0%	0%	0%	0%	0%
32	0%	0%	0%	0%	0%	0%	0%	0%	0%
33	0.06%	1.30%	0%	0%	0%	0%	0%	0%	0%
34	5.91%	2.93%	0%	0%	0%	0%	0%	0%	0%
35	2.37%	0%	0%	0%	0%	0%	0%	0%	0%
36	1.43%	0%	0%	0%	0%	0%	0%	0%	0%
Totals	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**

Appendix B

Figures

Chapter One Figures:

Figure 1.1: Life stages of Asian longhorned beetle. A: An ALB egg, laid just underneath the bark. B: Two second instar and one fourth instar larvae side by side. C: A pupated ALB. D: An adult female ALB. Photos by L.R. Schmitt, Clemson University.

Figure 1.2: Signs of damage made by various life stages of Asian longhorned beetle. A: An ALB oviposition site, chewed into the bark by an adult female. Each pit will have a single egg laid underneath the bark. B: Three oviposition pits bleeding sap, caused by larval feeding in the cambium. C: A gallery made by a first to early third instar ALB. D: A gallery made by a third or later instar ALB, with a tunnel (arrow) leading deeper into the heartwood of the tree. E: Tunnels and pupal chambers dug into the heartwood of the tree by a pre-pupa larvae (left arrow) and a pupal stage ALB (right arrow). F: ALB exit holes, made by adults chewing a tunnel out of the tree after pupation. Photos by L.R. Schmitt, Clemson University.

Chapter Two Figures:

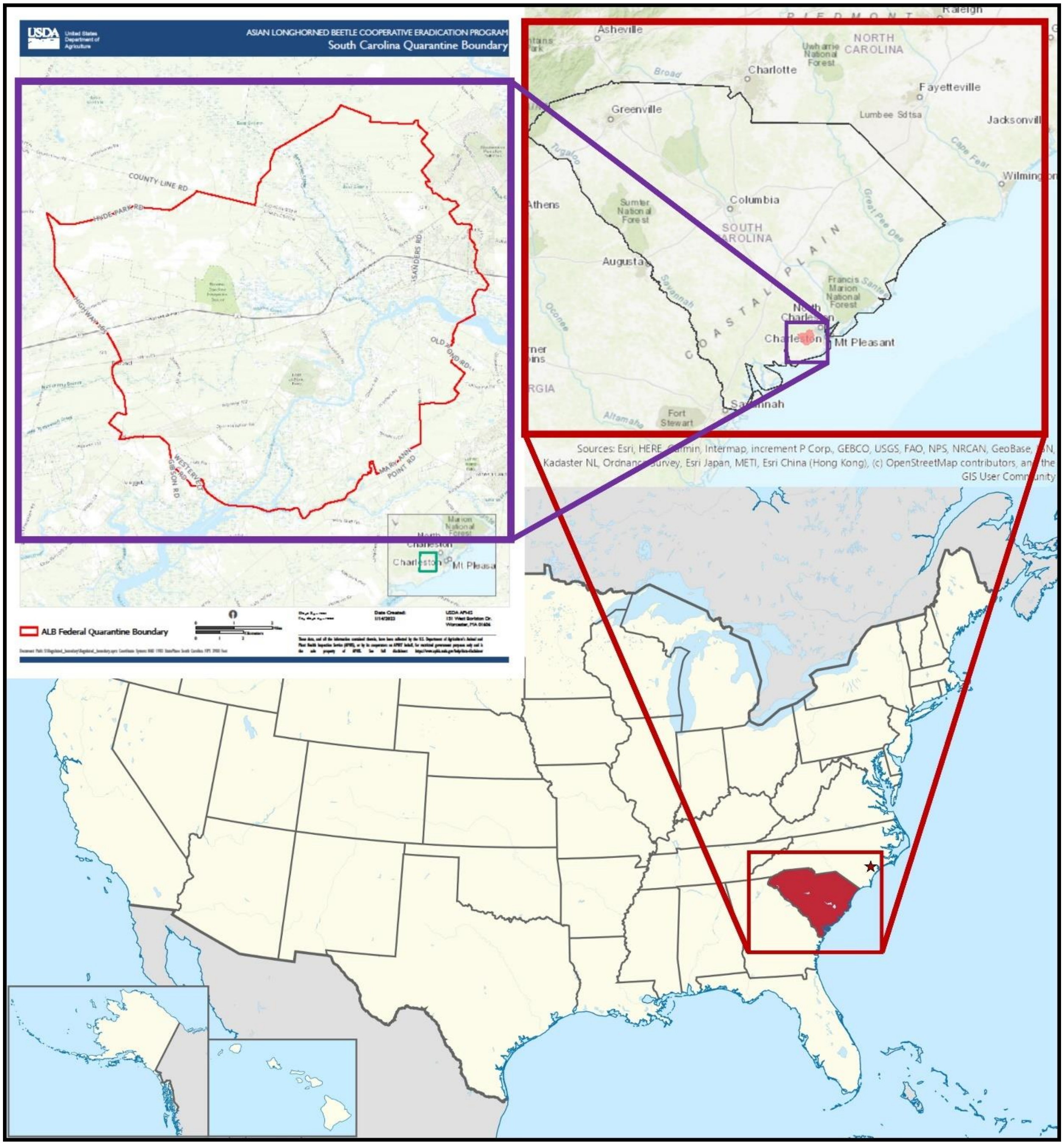

Figure 2.1: Map of the federal Asian longhorned beetle quarantine in Charleston County, South Carolina (APHIS Plant Protection and Quarantine (PPQ) n.d, TUBS 2011, USDA APHIS 2023).

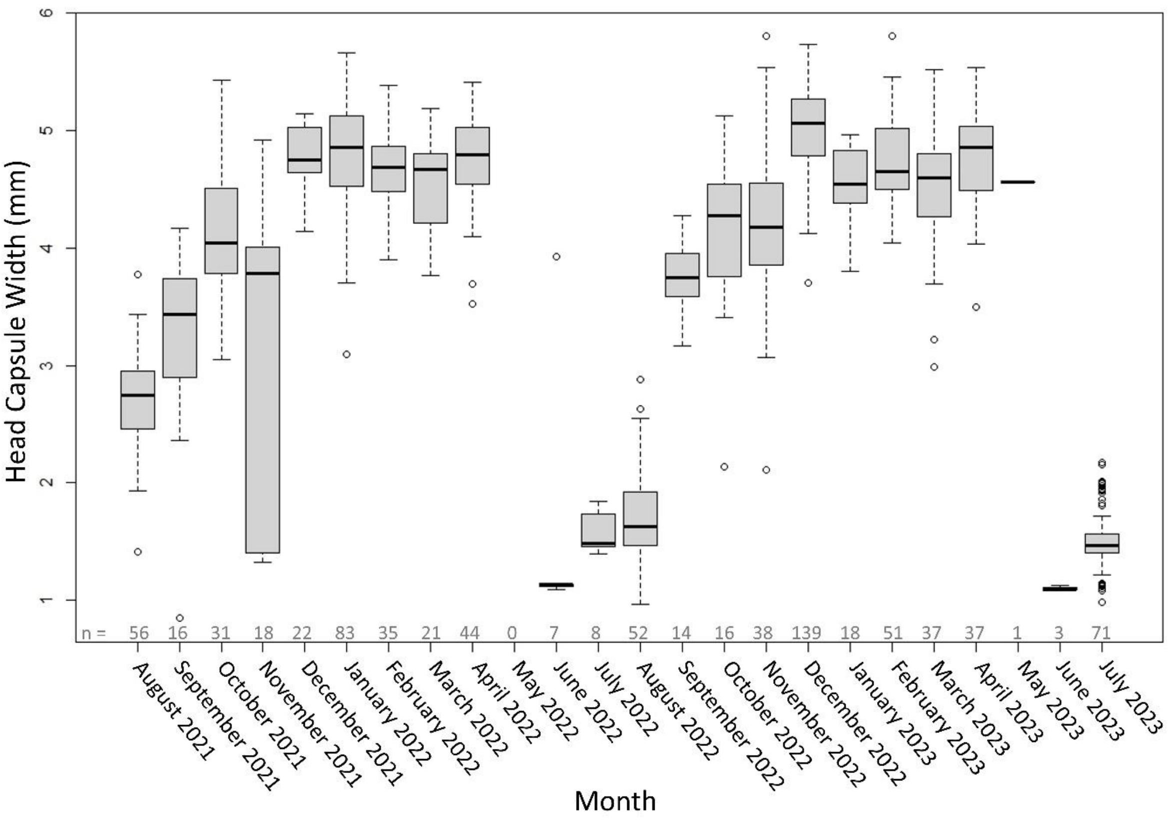

Figure 2.2: Boxplots of head capsule width (mm) of Asian longhorned beetle larvae every month for two years. Whiskers indicate values within 1.5x the interquartile range in either direction.

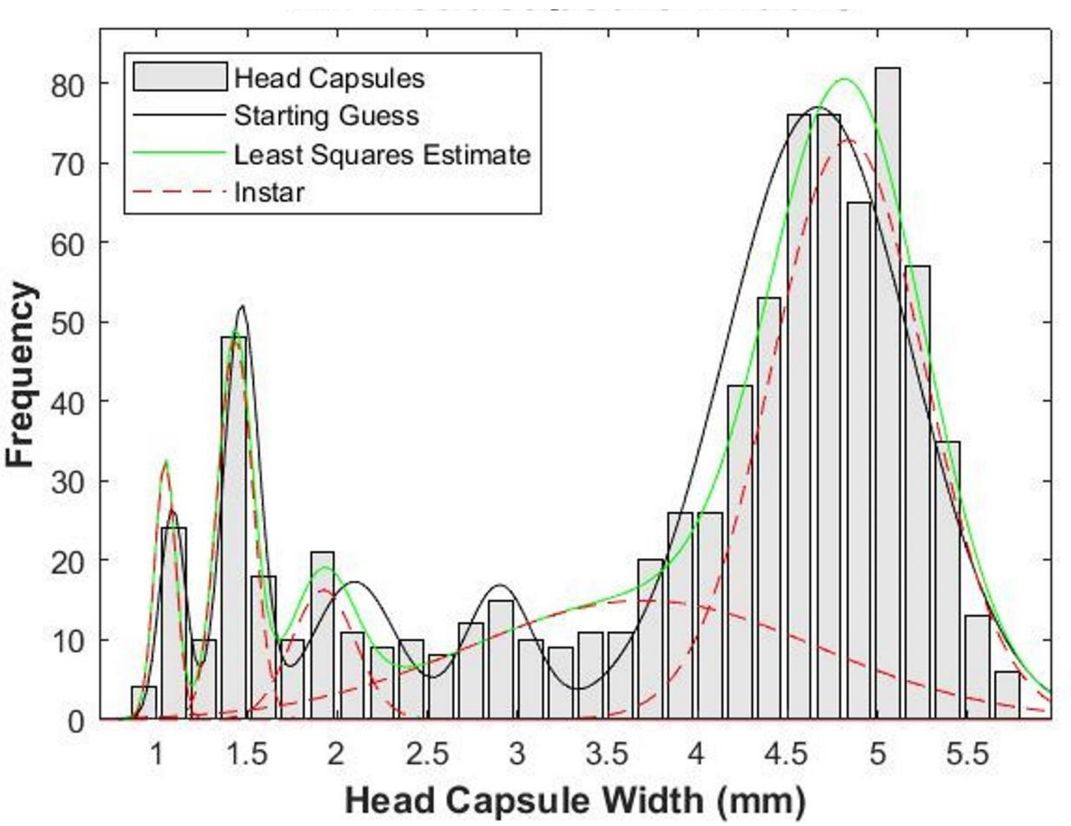

Figure 2.3: Instar curves created by using the HCAP program (Logan et al. 1998) based on head capsule width. Black line indicates curves based on my estimation of breaks between instars; green line indicates curves based on the best fit as calculated by the program; red lines indicate the distribution of each individual instar.

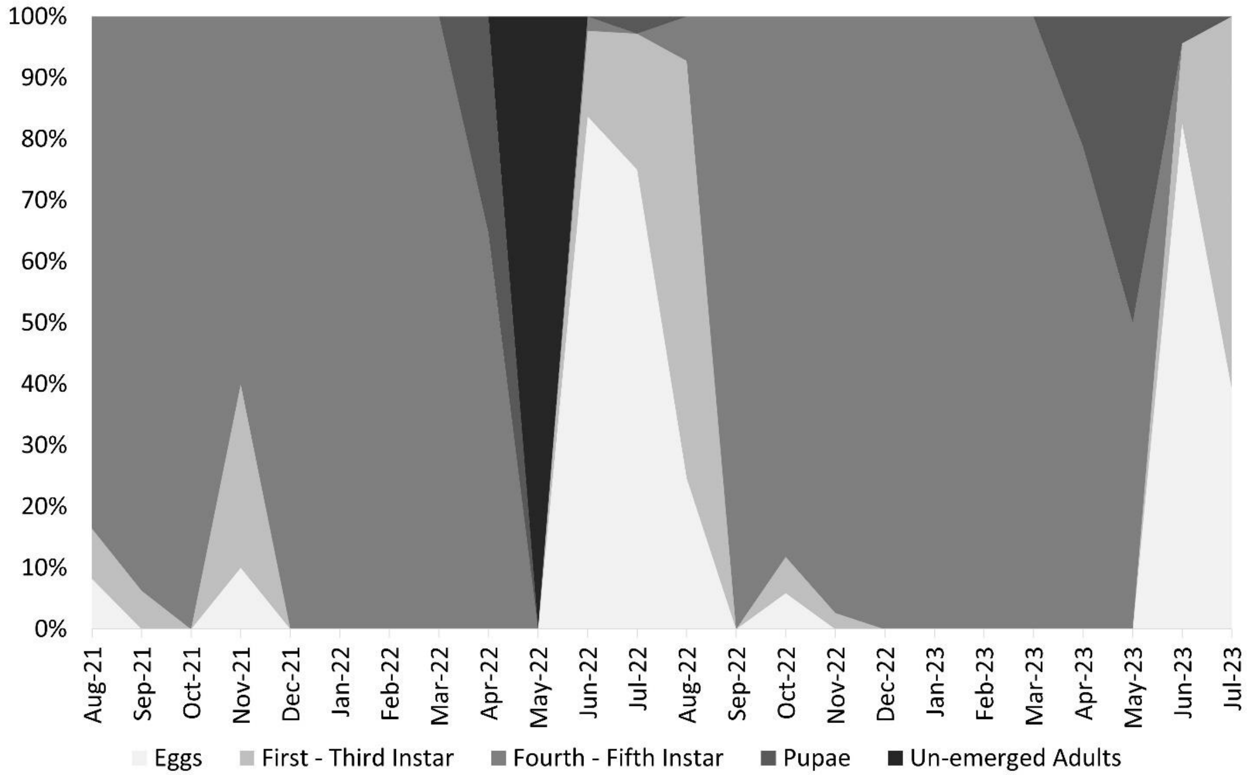

Figure 2.4: Proportions of ALB life stages (eggs, pupae, and un-emerged adults) and larvae split between first-third instars and fourth-fifth instars every month for two years.

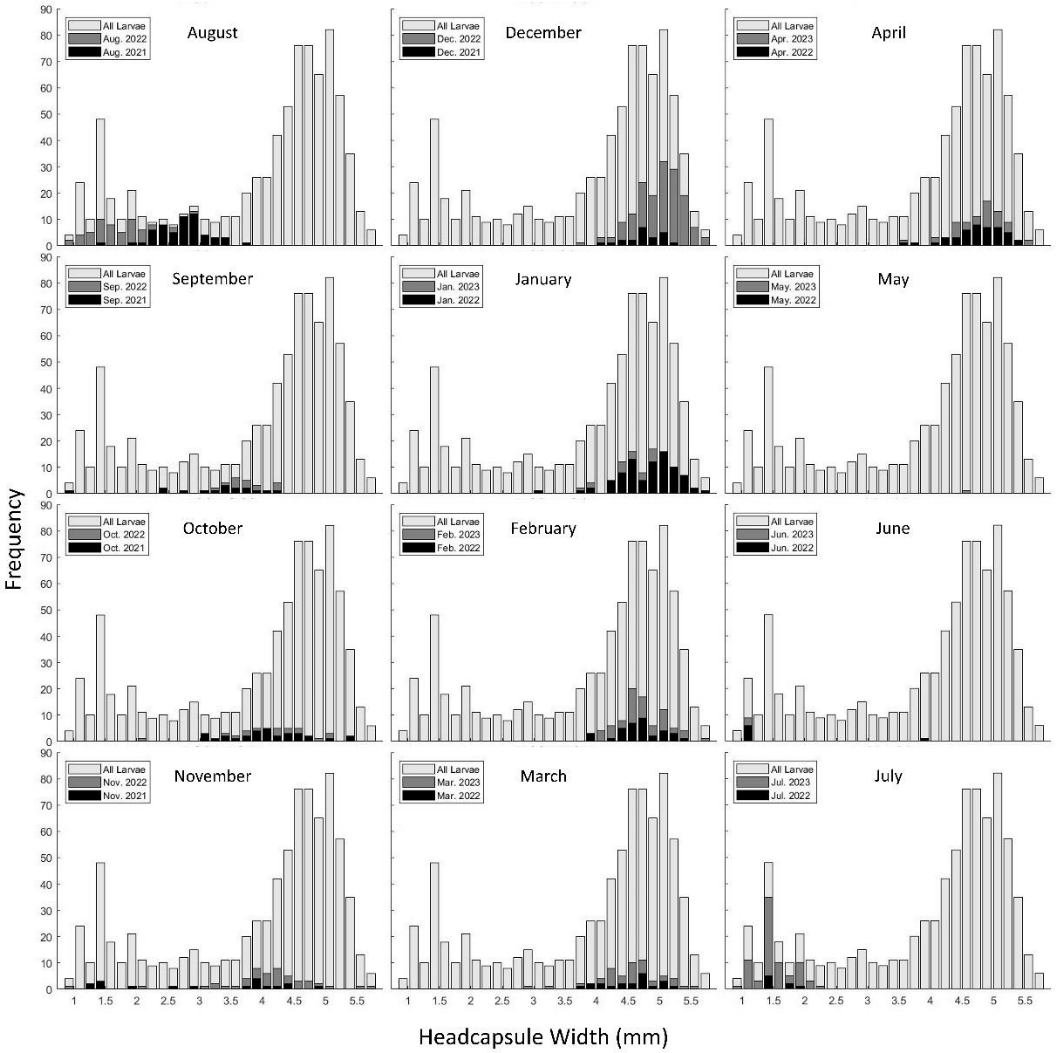

Figure 2.5: Frequency distribution of Asian longhorned beetle head capsule widths for each month over the course of two years.

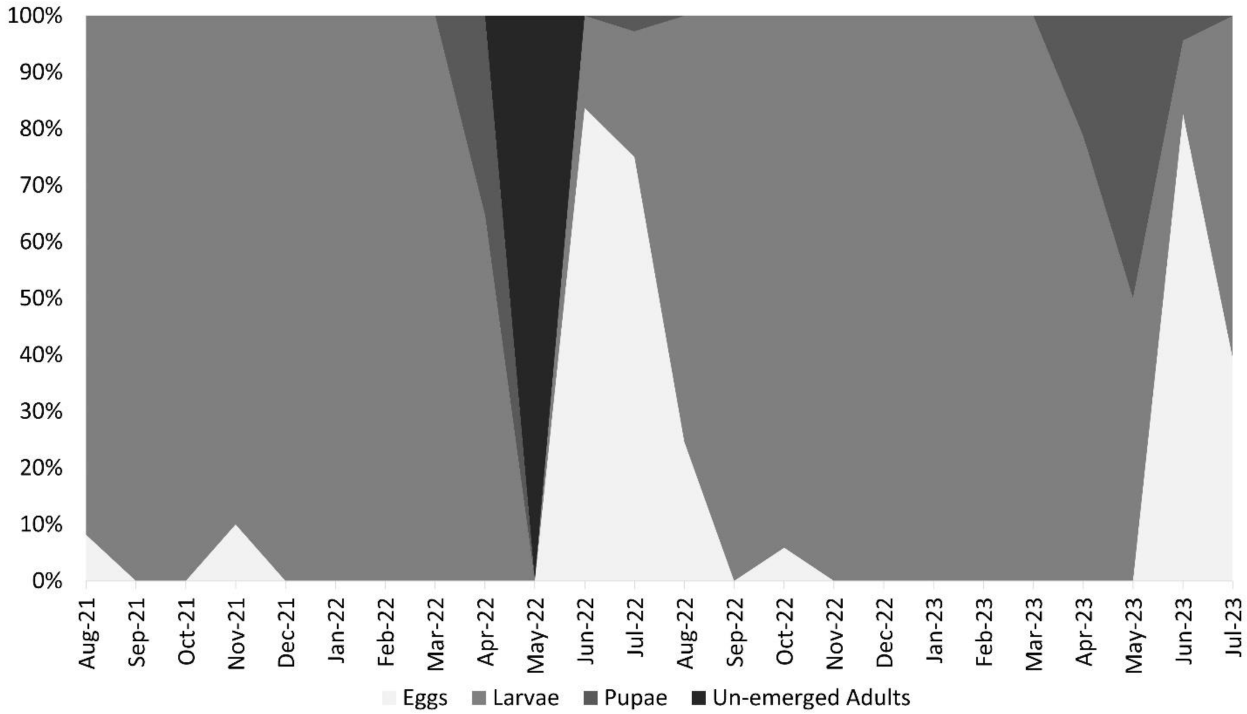

Figure 2.6: Proportions of ALB life stages (egg, larvae, pupae, and un-emerged adults) every month for two years.

Chapter Three Figures:

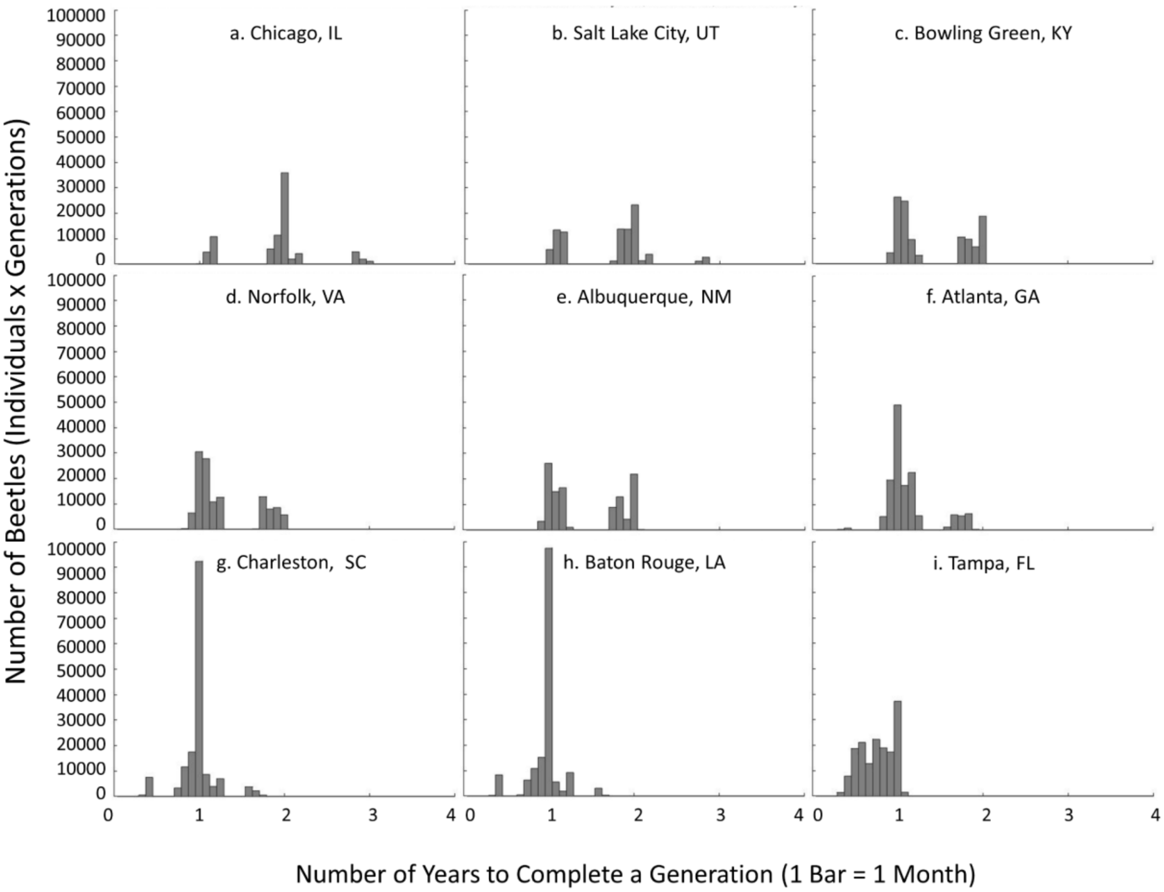

Figure 3.1. Model outputs displaying histograms of the number of years ALB populations take to develop in nine locations throughout the U.S. Locations are ordered from highest latitude to lowest.