Quantifying Emerald Ash Borer Induced Ash Mortality & Assessing Ash Reintroduction Strategies in the Duke Forest

Master's Project submitted in partial fulfillment of the requirements for
the Master of Forestry and Master of Environmental Management degrees in
the Nicholas School of the Environment at Duke University

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December 15th, 2023

Executive Summary

The Emerald Ash Borer (*Agrilus planipennis*), or EAB, is a non-native invasive pest responsible for the widespread loss of millions of ash trees (genus *Fraxinus*) in the United States. Initially detected near Detroit, Michigan, in 2002, this wood-boring beetle has since expanded its range to thirty-six states, including North Carolina. Observational evidence suggests *A. planipennis* began impacting ash trees in the Duke Forest as early as 2017. This project serves as the first organized survey of ash decline and mortality in the Duke Forest, quantifying the current extent of EAB damage. Additionally, plot samples from select hardwood covertypes were used to model ash regeneration strategies and inform reintroduction recommendations. Although damage from *A. planipennis* is ongoing and a strategy for landscape-level protection of mature ash has not been developed, the results from this project assist the Duke Forest in understanding current conditions and will inform future reintroduction efforts.

The research questions for this project are:

1) What is the extent of EAB-induced ash mortality in the Duke Forest?

2) What restoration strategies might the Duke Forest employ to reintroduce ash trees in the future?

The first question was answered by an ash tree survey, which sampled the top four forest covertypes with the highest predicted abundance of ash in the Durham and Korstian Divisions of the Duke Forest. Results from this survey quantified the average abundance of mature ash trees to be about 1.2 per acre. This aligns with previous Duke Forest inventories as well as literature about ash trees in the southeastern United States. The survey also quantified the extent of EAB induced ash mortality at 79%. Nearly eight out of every ten mature ash trees were dead by the summer of 2023, with more mortality expected to follow. Additionally, data on ash tree signs and symptoms of stress were collected as a measure of current ash tree health conditions.

The second question was answered by simulating ash reintroduction scenarios. Using field data collected from nested plots within the four covertypes of interest, the USFS Forest Vegetation Simulator (FVS) was used to model different levels of tree thinnings. To model site preparation for planted ash seedlings, four levels of thinnings were modeled: no thin, thin from below to 80 BA, thin from below to 40 BA, and clear cut. The results from the FVS cut list were combined with regeneration data collected in the field to simulate species competition among planted ash and non-ash species in REGEN-3. The report generated by REGEN-3 was then summarized into management recommendations per covertype for future ash reintroduction efforts.

It is unknown how the Emerald Ash Borer will persist in the Duke Forest in the long term. It is expected, however, that the Emerald Ash Borer will be a permanent resident that will cause near complete functional extinction of the Duke Forest's native ash species. While no management actions can reasonably be taken now to protect remaining ash trees on the landscape level, the Duke Forest has options for limited reintroductions where insecticide can be regularly applied to individual trees.

Finally, if landscape level solutions are found to control or eradicate the EAB, or to effectively protect ash trees, then this project has created a record of historic ash tree abundance and distribution in the Duke Forest. These results, along with the management recommendations, can guide ash tree reintroduction efforts.

Land Acknowledgement

Duke University does not yet have an official land acknowledgement; however, the Nicholas School of the Environment offers a land acknowledgement authored by Professors Ryan Emanuel and Malinda Lowery of the Lumbee tribe, provided here:

> “What is now Durham was originally the territory of several Native nations, including Tutelo and Saponi – speaking peoples. Many of their communities were displaced or killed through war, disease, and colonial expansion. Today, the Triangle is surrounded by contemporary Native nations, the descendants of Tutelo, Saponi, and other Indigenous peoples who survived early colonization. These nations include the Haliwa-Saponi, Sappony, and Occaneechi Band of Saponi. North Carolina’s Research Triangle is also home to a thriving urban Native American community who represent Native nations from across the United States. Together, these Indigenous nations and communities contribute to North Carolina’s ranking as the state with the largest Native American population east of Oklahoma.”

My research takes place within the Duke Forest, stolen lands taken from the original stewards of this landscape that were eventually bought by the Duke family and Duke University. I first want to honor the connection local Indigenous peoples have with this land, historically and at present, and to thank them for thousands of years of stewardship as well as their contemporary contributions to our understanding of the environment. This project explores what the presence of a non-native beetle might have on ecologically and culturally valuable ash trees, which is a story interconnected with the ongoing human history of this land.

Acknowledgements

This Master's Project would not be possible without the continued support from a wonderful team of friends, colleagues, mentors, and family. First, I would like to thank my advisor, Professor Nicki Cagle, for believing in, standing up for, and teaching me throughout my time at the Nicholas School. When I needed an ally the most, you were fighting in my corner. Second, I would like to thank the Duke Forest team – Sara Childs, Tom Craven, Zach Carnegie, Maggie Heraty, Craig Hughes, Blake Tedder, and Tim Barco – for proposing this Masters Project idea and granting me a summer internship to complete my data collection. Working in the Duke Forest was the *best* experience of my time at the Nicholas School and taught me necessary forestry and research skills that will benefit me for years to come. I am blessed to have met you all. I would also like to thank my academic team at the Nicholas School. Important feedback and input came from: Professor Dean Urban, Professor Jenn Swenson, Professor Alex Finkral, Professor Jeff Vincent, Professor Sari Palmroth, Professor Ram Oren, and Jenna Schreiber. I especially appreciate Collin Carol for imparting Forest Vegetation Simulator wisdom upon me and helping me troubleshoot. Thank you for seeing the ways in which this project Is exciting and valuable, and for sharing that enthusiasm with me. I owe Tara Keyser at the USFS Southern Research Station thanks for helping me use her tool, REGEN-3.

Additionally, I want to thank my dear classmates and friends who listened to my many project ideas (and laments), provided valuable feedback, and attended my practice and final MP presentations. Christine Martens, Meixin Wang, Ben Chase, Emma Childs, Eni Bihari, Jackie Fahrenholz, Adam Lohman, Grace Tregidgo, Jessica Brooks, Kate Perry, Mel Merritt, Cait Markus, Gabe Piacsek, Jordan Haber, Katie Kresja, Lydie Costes, Micah Ezekiel, Nat Blackford, Reed Carradine, Sam Nurmi, Schuyler Fica-Goetzel, Tasha Griffiths, Yuntian Bi, Parker White, Simon Sharp, Xiaoge Zhang, Samantha Trueman, Israel Golden, and Abby Martell. Thank you, Joell Stephenson, for being by my side through the trials and triumphs of this project. I appreciate your patience and understanding during this challenging and transformative undertaking. Finally, I would like to thank my family – Roman, Holly, and Michaela – for making my education and this project possible. Thank you for investing in me and supporting this journey. I love you all.

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Statement of Purpose

The Emerald Ash Borer (*Agrilus planipennis*), or EAB, is a non-native invasive pest introduced to the United States from its natural range in temperate northeastern Asia. Although small in size, these wood boring beetles have caused billions of dollars of damage and killed tens of millions of ash trees since the first outbreak near Detroit, Michigan in 2002. As of April 2023, the Animal and Plant Health Inspection Service (APHIS) reports known occurrences of *A. planipennis* in 36 states. By 2013, Emerald Ash Borer spread to North Carolina and was first detected in the Duke Forest four years later.

This project was proposed in response to the time-sensitive need to understand the soon-to-be historic distribution of ash trees and to understand the extent of Emerald Ash Borer-induced ash mortality in the Duke Forest. Additionally, this project serves to inform the Duke Forest staff about current research and considerations for future reintroductions.

In addition to meeting the needs of the dedicated Duke Forest staff, this project fulfills the graduation requirements of a 3S Masters Project for the Masters of Environmental Management and the SAF-accredited Masters of Forestry degrees.

Client: The Duke Forest

Established in 1931, the Duke Forest is a teaching and research laboratory spanning over 7,000 acres across six divisions in Durham, Orange, and Alamance counties (Lynch, 2006). The mission of the Duke Forest is to "facilitate research that addresses fundamental and applied questions across a variety of disciplines and to aid in the instruction of all students in their pursuit of knowledge, especially regarding the stewardship of our natural resources (Duke Forest, n.d.)." The Duke Forest also manages activities including nature-based recreation, citizen-science programs, and forest management for conservation and timber (Lynch, 2006).

The advent of the Emerald Ash Borer on the Duke Forest landscape has already resulted in additional costs and labor to the Duke Forest staff and may impact ongoing research projects. For example, dead and declining ash trees have been and continue to be removed along major roadways such as NC-751 and along pedestrian paths to reduce risk to people and traffic. No studies have yet been conducted to understand the extent of EAB dispersal and ash mortality within the Duke Forest.

The first known report of Emerald Ash Borer activity in the Duke Forest comes from former Duke Forest resource manager Judd Edeburn in the summer of 2017. Mr. Edeburn observed the characteristic D-shaped exit holes attributed to the Emerald Ash Borer in an ash tree exhibiting stress in the Duke Forest. Observing D-shaped exit holes at a height observable from the ground suggests the infestation has been underway for some time, as the EAB typically works from the top of the tree down (Cappaert et al., 2005).

In October 2022, Sara Childs and Tom Craven, the Executive Director and Forest Supervisor, respectively, reached out with this project idea and posited three questions paraphrased below:

1. What is the extent of ash mortality in the Duke Forest?
2. What are the expected forest composition shifts following the loss of ash trees?
3. What can we expect of the EAB in the long term and how should we manage, if at all?

Through investigative research in the field and in the literature, combined with considerations for time and resource limitations and Nicholas School Masters Project requirements, these questions were adjusted into a final form, as described in the Introduction below.

Introduction

The Emerald Ash Borer

The Emerald Ash Borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), or the EAB, is an iridescently green wood-boring beetle native to northeastern temperate Asia (Cappaert et al., 2005).

Figure 1. Photo of adult Emerald Ash Borer; "Plant Pest and Disease programs - Emerald Ash Borer"; USDA APHIS, Dec 12 2023, https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/emerald-ash-borer

Adults are usually between 12 and 15mm long and about 5 mm wide, with an elongated shape and a bright, metallic, uni- or bicolored emerald-green color (Volkovitsh et al., 2020). With the naked eye, one can observe six legs, a short head and torso, and two long elytra (hardened sheaths protecting the wings) constituting most of the body length (see fig. 1). Additional morphological traits distinguishing *A. planipennis* from other similar beetles throughout its native and non-native range can be observed under hand lens or microscope (Volkovitsh et al., 2020).

The native range of the Emerald Ash Borer includes northeastern China, Mongolia, Japan, the Korean peninsula, and eastern Russia (Herms & McCullough, 2014, USDA APHIS, 2023). Within its native range, the EAB generally only impacts weakened or stressed ash trees *(Fraxinus* spp.) as a secondary colonizer (Herms & McCullough, 2014). In the United States, however, the native species of ash trees do not exhibit the same level of EAB resistance and thus even healthy trees are infested to the point of mortality.

Introduction & Dispersal in the United States

The Emerald Ash Borer was first formally detected in the United States in the summer of 2002, near Detroit, Michigan (Cappaert et al., 2005; Kovacs et al., 2010). Before this confirmed outbreak, *A. planipennis* was likely present – but undetected – at low levels in the United States (Cappaert et al., 2005). The method of transport from its native habitat range in Asia is unknown but was likely through infested wood shipping material such as crates or pallets (Herms & McCullough, 2014). According to Kashian and Witter (2011), the EAB has spread both by short- and long-distance dispersal methods. Short-distance dispersal occurs through the natural, wave-like spread of the EAB as individual beetles fly to infest new areas while long-distance dispersal is human-mitigated and follows major roadways (Kashian & Witter, 2011). The movement of infested firewood or other wooden material is the likely cause of new introductions in uninfested areas. As of April, 2023, the Emerald Ash Borer has spread to thirty-six states (see fig. 2).

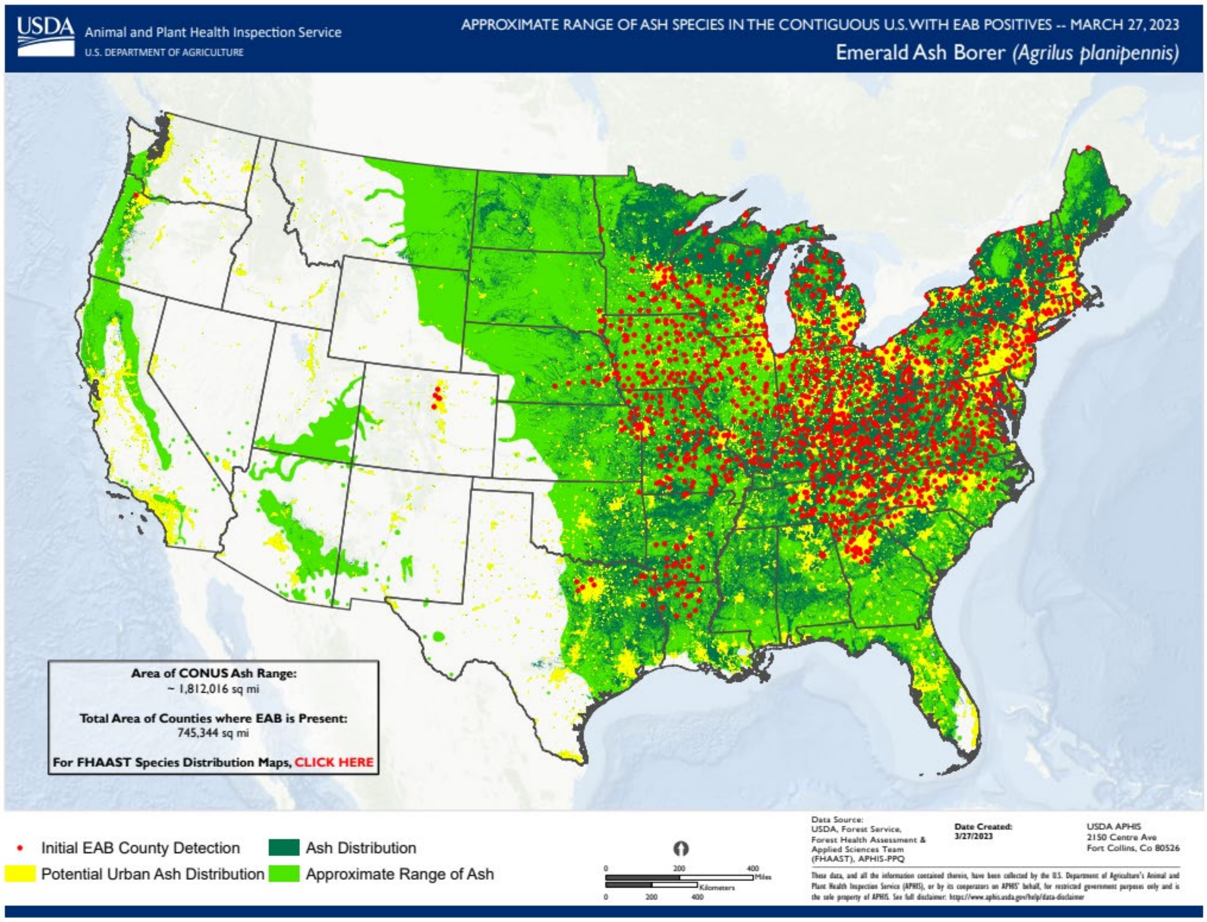

Figure 2. The approximate range of ash trees, including urban ash distribution, with the initial EAB detection location by county; “Plant Pest and Disease Programs – Emerald Ash Borer EAB Range Map”, USDA APHIS, Dec 12 2023, https://www.aphis.usda.gov/plant_health/plant_pest_info/emerald_ash_b/downloads/eab-ash-range-map.pdf

The Emerald Ash Borer was first officially detected in North Carolina in 2013 in four counties: Granville, Person, Vance, and Warren. Over the past ten years, the EAB has spread to at least seventy counties in North Carolina, mostly in the mountain and piedmont regions (see fig. 3).

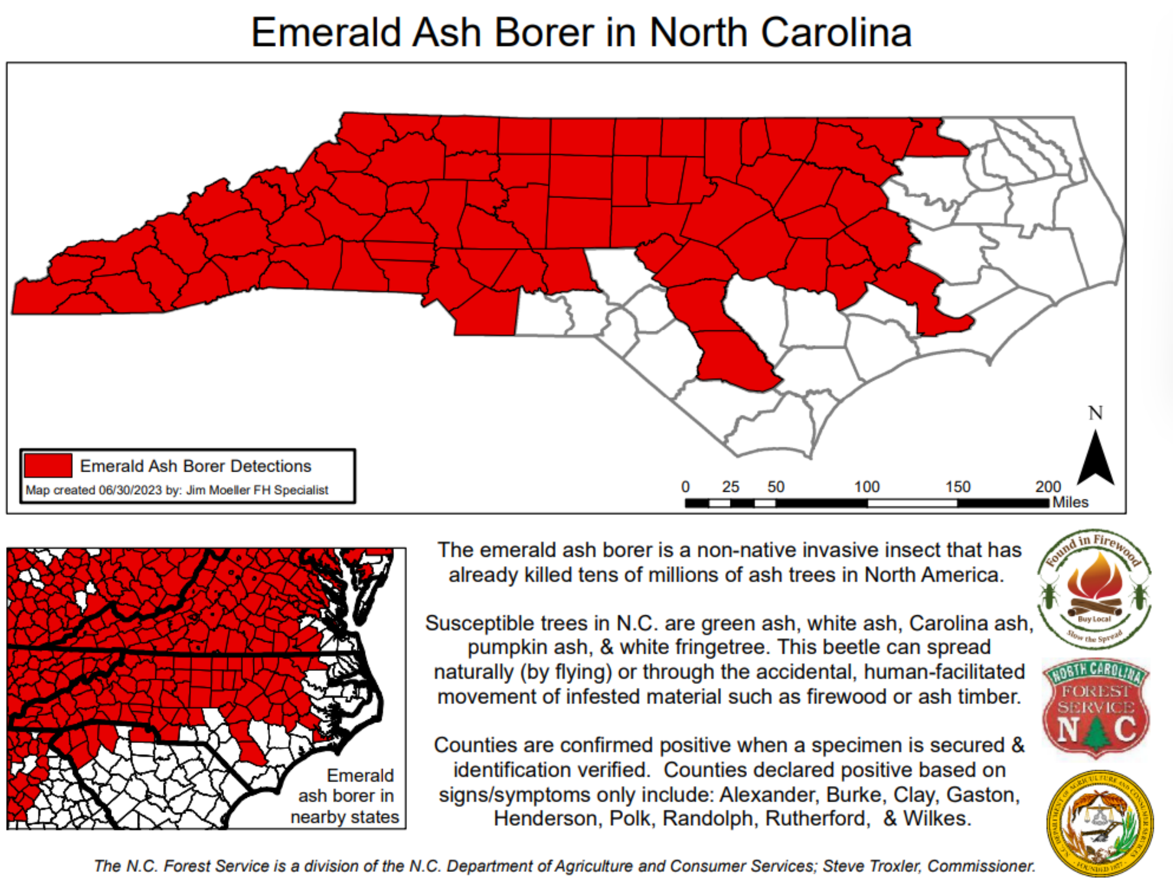

Figure 3. North Carolina counties with Emerald Ash Borer presence determined by positive specimen identification or by ash signs and symptoms. Current as of June 2023; "Forest Health EAB NC Tracking", NC Forest Service, Dec 12 2023, http://ncforestservice.gov/forest_health/pdf/Map_EAB_NCTracking.pdf

The first sign of the Emerald Ash Borer in the Duke Forest was reported by Judd Edeburn, former resource manager of the Duke Forest, in July of 2017. He identified the characteristic D-shaped exit holes of the Emerald Ash borer on a declining ash tree. Though unofficial, this serves as the first occurrence of the EAB in the Duke Forest. Over the next five years ash decline and mortality information was gathered only through informal observation, and this project is the first formal quantification of the extent of Emerald Ash Borer infestation and damage.

To understand how the EAB causes damage to, and ultimately kills, ash trees, it is necessary to understand the life cycle of this beetle.

Emerald Ash Borer Life Cycle

During their short adult lives, EAB females lay their eggs on the bark of ash trees in late spring or early summer (Bohannan et al., 2022). Shortly thereafter, larvae hatch from the eggs and bore through the bark into the cambium layer directly below (Bohannan et al., 2022). The larvae then feed on the xylem, phloem, and cambium layers of the tree, creating serpentine galleries backfilled with excrement known as frass. The xylem, phloem, and cambium layers are nutrient and water transport highways connecting the roots to the canopy. As EAB larvae eat through these layers, the transport of water, sugars, and nutrients are disrupted, which ultimately causes the infested ash tree to die. In forestry terms, the tree is "girdled" by the infestation, resulting in mortality in as little as one to three years under heavy infestation conditions (Poland & McCullough, 2006).

The larvae will next develop into pupae in chambers bored into the host tree (Bohannan et al., 2022). After overwintering, the pupae will mature and then emerge as adult beetles that will mate and repeat the lifecycle (Bohannan et al., 2022). Across both its native and introduced range, the Emerald Ash Borer can mature in 1-year (univoltine) or 2-year (semivoltine) cycles. The shorter univoltine cycle is typical of warmer climates, such as the conditions in the southeastern United States, and results in faster EAB development (Bohannan et al., 2022). The increasingly warm summers and mild winters of climate change may be exacerbating these impacts.

Adult Emerald Ash Borers generally live for about 3 weeks, during which time they will feed on the leaves of ash trees, and occasionally the Fringetree (*Chionanthus virginicus* Linnaeus) (Bohannan et al., 2022; Cipollini, 2015). The defoliation of ash trees from adult EABs are typically within the normal acceptable range of stress – the real damage causing decline and mortality is due to the larval stage.

Fraxinus, the Ash Genus

The Emerald Ash Borer can be hosted by all native ash trees in the United States (Cipollini, 2015). In North Carolina, particularly the Duke Forest, the two most common species of ash trees are White Ash (*Fraxinus americana* Linnaeus) and Green Ash (*Fraxinus pennsylvanica* Marsh). White Ash is generally found in upland sites, while Green Ash is found in more lowland or bottomland sites (Schlesinger, n.d.; Kennedy, n.d.). The species range, however, can greatly overlap. Species level identifications were not made during the data collection and analysis was conducted at the genus level for ash trees.

White Ash demands high soil fertility and soil moisture and grows from near sea level up to over 3,000 ft (Schlesinger, n.d.). The associated species that are commonly found with White Ash are listed in Appendix A. Similarly, Green Ash prefers fertile and moist soils, although when compared to White Ash, this species performs well on clayey, silty, or sandy sites subject to periodic inundation (Kennedy, n.d.). Green Ash is also known to survive better in acidic soil conditions. Thus, the habitat range of Green Ash is greater than that of White Ash. The associated species with Green Ash is also listed in Appendix A.

Both White and Green Ash are dioecious, meaning male and female reproductive structures are found on separate individuals (Schlesinger, n.d.; Kennedy, n.d.). The age to reproductive age for White and Green Ash is slow – for White Ash the minimum seed-bearing age is 20 years, and for Green Ash, flowering starts when the tree is between 8 and 10 cm in DBH, or diameter at breast height (Schlesinger, n.d.; Kennedy, n.d.). Once reproductive maturity is reached, female trees typically flower annually.

Successful White Ash seed germination occurs after stratification under moist conditions for 2 to 3 months (Schlesinger, n.d.). Even under laboratory conditions, successful germination occurs with a little over half of the seeds, on average (Schlesinger, n.d.). For Green Ash, a similar moist stratification must occur otherwise the seeds enter dormancy for up to several years (Kennedy, n.d.). Most ash seeds germinate two to three years after seed rain, and under laboratory conditions seeds can retain the ability to germinate up to 7 years (Klooster et al., 2014).

As mature individuals of both White and Green Ash disappear from the landscape due to the EAB, time is of the essence if wild seed collecting is desired. Seed collection timing and techniques are described in

Dr. Kathleen Knight's book, *Methods for Collecting Ash (*Fraxinus *Spp.) Seeds*. After the current seedbank sprouts and joins the class of young ash regen, it can be expected that the seed source for ash trees will entirely or almost entirely disappear. This is due to the long age to reproductive maturity and the EAB's ability to attack trees as small as 1 in (2.5 cm) in diameter at breast height, often abbreviated DBH (Klooster et al, 2014; Knight et al., 2014). In other words, the EAB will be able to attack the next generation of ash trees *before* they reach seed-bearing maturity. Thus, if the Duke Forest is interested in collecting wild ash seed stock, this must be done with immediacy.

Though ash trees are typically found at low abundances in hardwood forests of the southeastern United States, they are ecologically, culturally, and economically valued. At the time of EAB introduction, ash trees represented 7% of the sawtimber industry, with stumpage valued at an estimated $25 billion for the eastern United States (Cappaert et al., 2005). The loss of ash trees is the loss of a commercial species, which are used for lumber, pulp, and specialty products. Famously, ash is prized as the wood of choice in the manufacturing of baseball bats (Gansner & Widmann, 1990).

Ash trees are also a valued cultural resource for many Indigenous groups. For example, Black Ash (*Fraxinus nigra*) is annually harvested for making artistic and utilitarian baskets by several groups (D'Amato et al., 2023). Additionally, ash trees provide a suite of ecological benefits. First, ash trees are heavy seed-producing trees that feed a variety of birds, small mammals, and insects. The bark of young trees is a food source for animals such as beavers and porcupines (Schlesinger, n.d.; Kennedy, n.d.). Ash trees also provide browse, canopy cover, and protection for game species such as white-tailed deer and moose (Schlesinger, n.d.; Kennedy, n.d.). The impact of losing ash species in their respective forest communities is a subject of research across the United States.

Ash Decline & Mortality

When Emerald Ash Borers infest an ash tree, there are common signs and symptoms that can be observed. These signs and symptoms are often used in absence of a physical EAB specimen to confirm the presence of the pest.

Often, the first observable symptom is canopy dieback (Knight et al., 2014). Because the leaves are cut off from nutrient and water supply from the roots due to the formation of EAB larval galleries, the foliage begins to die. Sometimes, canopy dieback can be heavier on one side of an ash, corresponding to an area of infestation being greater on a particular side of the tree. In Knight et al. (2014) "Monitoring Ash (*Fraxinus* spp.) Decline and Emerald Ash Borer (*Agrilus planipennis*) Symptoms," a standardized way to assess the level of ash decline is defined on a scale of 1 (full health) to 5 (mortality) according to the level of canopy dieback. The Ash Decline Index (ADI) and Ash Mortality Index (AMI) are two measurements of ash tree health and decline that are especially useful in

Figure 4. Common signs and symptoms of EAB infestation observable in ash trees. Canopy dieback (a), bark splits (b), D-shaped exit holes (c), serpentine galleries (d), and basal sprouts (e). Photos by author in Duke Forest.

tracking infestations over time (Knight et al., 2014). This Master's Project serves as the first record of ADI and AMI for the Duke Forest, which may be useful if further studies are conducted.

Other common signs and symptoms observable on ash trees are D-shaped exit holes, increased woodpecker activity, serpentine galleries, bark splits, epicormic sprouting, and basal sprouting (see fig. 4). Many of these signs are only observable under heavy infestation levels, which is why it is believed that the EAB has been in the United States for years before the first major outbreak (Cappaert et al., 2005; Flower et al., 2013). This suggests that the original introduction of the EAB in the Duke Forest likely occurred before 2017, when the first ash tree sign of infestation was observed.

Under low to moderate levels of infestation, ash tree mortality usually takes up to 5 years. However, under high levels of infestation or ash tree stress, mortality can occur as quickly as 1-3 years after initial attack (Poland & McCullough, 2006).

Management Response to the Emerald Ash Borer

In the early days of Emerald Ash Borer infestations, the federal government oversaw quarantine restrictions in an effort to abate the spread of the pest. *Agrilus planipennis* spreads across the United States through both short- and long-distance dispersal methods (Kashian & Witter, 2011). The EAB is a flying beetle that can move tree to tree within a forest, allowing populations to slowly spread from highly infested areas to new unaffected trees. People often mitigate long-distance dispersal, through the transportation of firewood, logs, and woody materials (Kashian & Witter, 2011). Unfortunately, quarantine restrictions and public awareness campaigns were not enough to halt the spread of the pest. In January 2021, the federal quarantine on the EAB was lifted (APHIS, 2023).

One solution that currently exists that can protect ash trees is pesticide. Through different modes of application, including pouring liquid pesticide around the base of the tree or injection directly to the trunk, ash trees can effectively be protected from the EAB (Herms et al., 2009). Generally, these pesticides are taken up by the tree and larvae are poisoned when they attempt to feed (Herms et al., 2009). This method, though effective, has a few drawbacks.

First, many landowners and community members may have concerns about extensive use of pesticides in the forest, and some negative effects to other plants and animals may not be yet realized. Second, as soon as a tree stops receiving pesticide treatment, it becomes susceptible to EAB infestation. Thus, individual trees must be treated according to the longevity of the pesticide. Third, the ability to scale this method to the landscape level is not feasible for large landowners such as the Duke Forest. Individually treating mature ash trees across the hardwood forests in the Duke Forest year after year is not within the time and resource budget of the small team of permanent staff. Unfortunately, the time frame for an effort to locate and treat ash trees in the Duke Forest has also passed, as EAB induced mortality has already begun.

Though outside of the scope for this Master's Project, landscape-level solutions are worth researching further. For example, parasitoid wasps have been released in multiple states as biocontrols for the EAB (Bohannon et al., 2022). Parasitoid wasps attack EAB larvae, helping to control the pest population levels (Bohannon et al., 2022). Though introductions of parasitoid wasps have faced considerable challenges in the southeast, populations of biocontrols have been successfully established in the northeast (Bohannon et al., 2022).

Researchers are also interested in exploring hybridization between North American and Asiatic species of ash trees. Asiatic species from the EAB's native range coevolved with the beetle, and trees from those species how higher tolerance, resistance, and recovery (Perry et al., 2022). To date, hybridization tests have resulted in limited success, and reintroducing hybrids is not a viable option.

Finally, there is interest in ”lingering ash.” Lingering ash are defined as mature individuals (above 10 inches in DBH) that survive in infested areas where greater than 95% mortality has occurred and the EAB has been present for several years (Knight et al., 2014). Though the genetics behind lingering ash are not understood, surviving individuals have been observed in areas otherwise hit hard by the EAB. If the Duke Forest encounters lingering ash within its boundaries, that tree may be of interest to those researching surviving individuals.

Research Question

In October 2022, Sara Childs and Tom Craven responded to my request for potential project ideas with the Duke Forest. Among the ideas, exploring the Emerald Ash Borer and ash mortality in the Duke Forest excited me the most. The Duke Forest was most interested in understanding the extent of EAB-induced ash mortality, predicting potential compositional shifts due to the loss of ash across the landscape, and any management recommendations.

Due to the scarcity of regeneration observed in mature hardwood stands within the Duke Forest and the low abundance of ash creating only minor canopy gaps, the subject of compositional shifts was abandoned. Instead, I wanted to focus my efforts on collecting data on the abundance and distribution of mature ash trees prior to their disappearance from the landscape. Information about ash tree health through recording signs and symptoms was also of interest. The lack of landscape-level solutions to protect current ash trees or to reintroduce resistant trees narrowed my scope to the establishment and management of limited reintroduction sites. Examples of limited reintroduction might be a small ash tree research plot or an ash stand grown for public demonstration. Thus, the two research questions developed for this project are as follows:

1) What is the extent of EAB-induced ash mortality in the Duke Forest?
2) What restoration strategies might the Duke Forest employ to reintroduce ash trees in the future?

The first question was answered through an ash tree survey. After my research areas were identified, I tree mapped my survey sites for all mature ash trees above 10 inches in DBH. I recorded health information, including the common signs and symptoms described in the Introduction, from which ADI and AMI can be computed.

The second question was answered by modeling limited ash reintroduction scenarios. After collecting forest characteristic information where ash trees were surveyed, I used the USFS Forest Vegetation Simulator (FVS) and REGEN-3 tools to model different thinning prescriptions and regeneration competition for planted ash trees. Results from the FVS and REGEN-3 modeling were summarized and translated into management recommendations for use by the Duke Forest should a future reintroduction be of interest.

Methods

This Masters Project is divided into five steps: covertype selection, stand selection, ash tree mapping, plot sampling, and reintroduction modeling.

In May 2023, multiple scouting visits in the Duke Forest were conducted in randomly selected hardwood stands to gain perspective on the abundance and distribution of ash trees. These scouting events showed that ash trees represented only a small proportion of canopy space – in my estimate, less than 3%. Ash trees also were not uniformly distributed, and instead tended to grow in clusters.

Another important observation during my scouting visits was that regeneration in the Duke Forest hardwood stands was sparse. Most hardwood stands in the Duke Forest are overmature, with well-established closed canopies that only open with natural mortality or disasters. As such, the forest floor receives too little sunlight for abundant regeneration. It was at this stage that studying forest compositional shifts and ash regeneration were abandoned as research questions. Instead, the focus was shifted towards documenting and understanding the dead and declining mature ash trees in the Duke Forest.

Step 1. Covertype Selection

After several scouting trips, I determined that random sampling in the Duke Forest would not capture enough ash trees for a meaningful study or effective use of my time. Instead, I needed to be directed in my search for ash trees. First, I consulted the literature to determine if any environmental variables might limit ash distribution in the Duke Forest and help me narrow my search. Information for White and Green Ash was primarily collected from species descriptions produced by the USFS Southern Research Station (Kennedy, n.d.; Schlesinger, n.d).

No environmental variables such as elevation, habitat range, slope, aspect, or soil conditions suggested that particular areas of the Duke Forest would not contain ash. Instead, management history and covertype might be better indicators. As such, I summarized the primary species associates for White and Green Ash (see Appendix A). These were compared to the covertype codes the Duke Forest uses to classify its forest types (see Appendix B). Primary associates of Green and White Ash were mostly hardwood species that correspond to Duke Forest cover type codes of M, P, A, B, C, E, or F.

To focus my ash tree survey to fewer covertypes, I used two past forest inventory datasets – the 2010 Forest Inventory and the 2019 Continuous Forest Inventory – to determine which Duke Forest covertypes contained the highest abundance of ash. At this stage, the following criteria were applied to limit the scope of the project to an efficient degree and to remove inappropriate cover types:

> *<u>Hardwood and Mixed Stands only</u>:* The Duke Forest manages its pine stands for harvest, unlike its hardwood and mixed stands (with exceptions). Ash that is found in, for example, a managed loblolly stand is incidental and unwanted natural seed in or stump sprout. Thus, I am interested in hardwood and mixed stands where ash is allowed to grow to canopy height and will not be cut down during the next harvest cycle.
>
> > Response: filter out L, S, V, M, and P single cover types

<u>Mature Stands only</u>: I am interested in hardwood or mixed stands where ash has reached canopy-level maturity. Information on White Ash suggests that it takes 40 years for a tree to reach a DBH of 25 cm (10 inches) and height of 21 m (69 ft).

Response: filter out -0 to -3 age classes

<u>Durham and Korstian Divisions only</u>: To limit the amount of travel and optimize the feasibility of this project, only stands from the Durham and Korstian Divisions (which make up approximately 63% of Duke Forests total acreage) will be sampled. Thus, the CFI and Forest Inventory datasets were filtered for these two divisions (Oosting Natural Area was retained).

Response: filter out divisions other than Durham and Korstian

<u>Mature Ash only</u>: Because I am interested in surveying canopy level trees, only mature ash (defined as >10" in DBH) with tree measurement information were filtered for minimum DBH. 7" was selected to account for growth, allow for some early stage reproductive individuals, and to cast a slightly wider net for mature ash, given its rarity.

Response: filter out ash trees below 7" DBH

After these limiting criteria were applied, the top four covertypes with the highest abundance of ash were selected as areas of interest. Covertypes with low sample sizes (defined here as less than 7 plots) were excluded, even if they had a greater proportion of plots with ash.

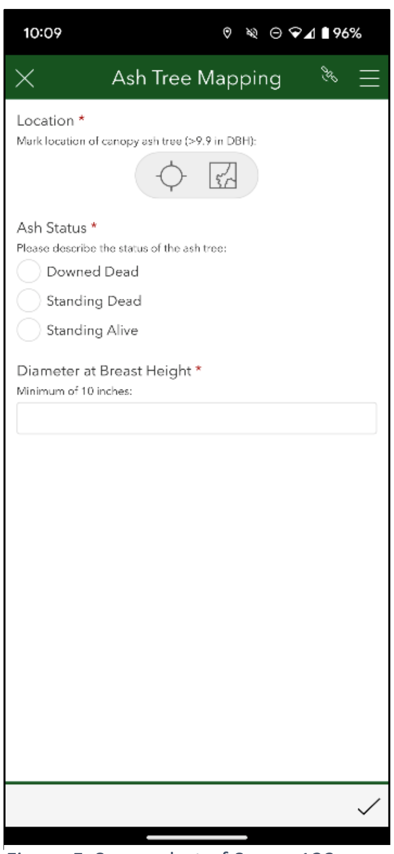

Figure 5. Screenshot of Survey123 project.

Step 2. Stand Selection

After selecting which covertypes to include in my study, I generated a list of stands within those covertypes. For each of the selected covertypes, I summed the acreage and calculated the percent area each covertype represented within the Durham and Korstian Divisions using the 2019 covertype layer in ArcGIS Pro. The area covered by these four covertypes is greater than what can be surveyed by myself within my time frame, so I decided to conduct a 10% survey of the total area for each covertype.

Using a random number generator, I selected stands (greater than 4 acres in size to increase efficiency) until the minimum 10% acreage was achieved. Once stands were selected, I created a Survey123 project to collect data in Step 3.

Step 3. Ash Tree Mapping

From mid-June to late-August, I visited each of the selected stands and tree mapped every mature ash tree I encountered. Mature trees were defined as 10 inches in DBH, dead or alive. I conducted my tree mapping using Avenza to track my transect lines, ensuring no trees were missed. For every ash tree I found, I marked its location using a Survey123 project I created (see fig. 5). In this project, I collected the geographic location, DBH, and whether it was alive, standing dead, or downed dead. After recording an ash tree in Survey123, trees were marked with tree chalk to avoid double counting.

Step 4. Plot Sampling

In order to collect more information about the forests within each of the covertypes, I decided to return to 20% of the ash trees surveyed in each covertype and conduct a nested plot sample. To meet this threshold, I selected *at least* 20% of ash trees in each stand. After consulting the user guides for Forest Vegetation Simulator (FVS) and REGEN-3, I designed a sampling protocol sheet for collecting data (see Appendix G).

At the plot level, the following information was collected: stand name, plot number, coordinates, invasive species (species list), basal area (living ash, dead ash, non-ash, and total), and ground cover (percentage native, non-native, and bare ground). The date, start time, and end time were also noted. The largest plot was a 5th acre circle with a 52.7 foot radius. All trees 18 inches or greater in DBH were recorded in this plot. Species, DBH (in), height (ft), and crown height (ft) were recorded for each tree. The second largest plot was a 10th acre circle with a radius of 37.2 feet. All trees greater than 6 inches in DBH were recorded. Again, species, DBH (in), height (ft), and crown height (ft) were collected.

The medium plot was a 100th acre circle, measured by a pre-measured 11.8 foot dog chain. All trees greater than 1.5 inches and less than 6 inches in DBH were measured. Tree species, DBH (in), and height (ft) were recorded. Finally, four 1000th acre subplots were created. Each was collected 16 ft from the plot center in each cardinal direction. Seedlings were counted by species as small, medium, or large. Small seedlings are less than 2 feet in height and at least 6 inches tall. Medium seedlings are 2 feet or taller, with a maximum height of 3.9 feet. Large reproduction is at least 4 feet in height but less than 1.5 inches in DBH.

With the exception of ash trees, only living trees were recorded. Additionally, all ash trees found in the large plots had additional information collected about them. Each tree was recorded as dead or alive, rated on the Ash Decline Index as defined in Knight et al., 2014. Additionally, signs and symptoms of EAB infestation were recorded for each ash tree, with protocol described in the aforementioned paper. Exit holes, woodpecker activity, epicormic sprouting, basal sprouting, and bark splits were recorded as present or absent for each ash tree.

The tools necessary for this step were: clipboard, printed sampling sheets, phone with plot points, writing utensil, range finder, DBH tape, distance tape or pre-measured dog chain, clinometer or hypsometer, 10 factor prism, and tree chalk.

Step 5. Modeling Ash Reintroduction with FVS and REGEN-3

The data collected from the nested sampling plots were digitized and formatted for input into the USFS Forest Vegetation Simulator, or FVS. Plots taken in each covertype were analyzed together. Four levels of tree removal were simulated in FVS: no thin, thin from below to 80 BA, thin from below to 40 BA, and clear cut. A modification was added to preferentially cut red maple and yellow poplar in these scenarios.

The number of trees cut per acre by species were scaled down to a 100th acre area. Normal rounding was used to convert these values to the average number of trees cut per species for a 100th acre area. The average stem size of each species that was cut was averaged from the cut list output file from FVS.

In REGEN-3, the North Cumberland Plateau – submesic conditions (NCum_sm) Knowledge Base was selected based on recommendations from Tom Craven and Tara Keyser. Species observed in the Duke Forest that were not found in the NCum_sm Knowledge Base were given values from a most closely related species. For example, the regen probabilities and ranking values for Fraxinus spp. were derived from the values associated with White Ash.

Small, medium, and large regeneration recorded in the field were used to populate the species found in each plot. Data from each covertype were run separately. In each plot, four medium ash seedlings were "planted in" to represent a reintroduction at 400 trees per acre. For each simulation where cutting occurred, the average size and quantity per species were added to each plot. For example, if FVS modeled two red maple stems cut in each 100th acre area with an average size of 2.8 during a reduction to 40 BA, then each REGEN-3 plot had two 2.8 inch stump sprout entries for red maple. REGEN-3 reports were generated for each covertype.

Results

The results of this Masters Project come from two analysis: the ash tree survey and the ash tree reintroduction modeling. The former describes ash tree abundance and health while the later generates management recommendations from simulations based on field measurements.

Step 1. Covertype Selection

After applying criteria filters to the 2019 Continuous Forest Inventory dataset, out of 2,291 trees measured in the 10th acre plots, 36 were ash (1.57%). After applying criteria filters to the 2010 Forest Inventory (CFI), of the 13,161 trees measured, 287 were ash (2.18%). The 100th acre and 1000th acre plots of the CFI showed higher proportion of ash – 4.00% and 7.48% respectively – which still reinforced a limited abundance of ash in the Duke Forest.

Subsequent to combining the number of plots with and without ash for both inventory datasets, I selected the top four covertypes with the highest proportion of plots with ash: A, C, CE, and E (see table 1). Again, covertypes with high proportion of plots containing ash but a low samples size (n < 7) were excluded. Detailed results from both inventories can be seen separately in Appendix C and D.

Table 1. Combined 2010 and 2019 forest inventory datasets, showing number of total plots, plots containing mature ash, and proportion of plots containing ash by covertype. Conditional color formatting displays the range of proportions from red (min: 0%) to green (max: 33%). The four selected covertypes for which to narrow my ash tree survey are highlighted in blue.

No.	Covertype	Total # Plots	# Plots with Ash	% with Ash
1	AB-U	1	0	0%
2	AC-U	33	2	6%
3	AE-U	4	0	0%
4	A-U	8	2	25%
5	CE-U	11	2	18%
6	C-U	55	11	20%
7	E-U	7	2	29%
8	LA-U	18	2	11%
9	LC-U	10	1	10%
10	LE-U	21	0	0%
11	PE-U	3	1	33%
12	SA-U	1	0	0%
13	SC-U	4	0	0%
14	SE-U	5	1	20%
		181	24	

The top four covertypes containing the highest proportion of plots with ash were A-U (Yellow Poplar-Sweetgum, uneven age), **C-U** (White, Black, Red Oak, uneven age), **CE-U** (White, Black, Red Oak – Mixed Hardwood Species, uneven age), and **E-U** (Mixed Hardwood Species, uneven age). Again, PE-U and SE-U were also not selected because 1) they represent a small proportion of Durham and Korstian covertype area and 2) there were only 6 or less inventory plots.

Step 2. Stand Selection

I calculated the total area of each covertype in the Durham and Korstian divisions and represented each as a percent of the combined area (see table 2).

Table 2. Area in acres per covertype in the Durham and Korstian Divisions of the Duke Forest, with areas represented as percent of total area. The acres needed to tree map each covertype at least 10% of the total area calculated in the column to the right.

Covertype	Acreage	% of Combined Area	Tree Mapping Area (at 10%)
A-U	147.3	14.9%	14.7 acres
CE-U	74.1	7.5%	7.4 acres
C-U	652.2	65.8%	65.2 acres
E-U	117.5	11.9%	11.8 acres
Totals	991.1	100%	99.0 acres

By selected stands using a random number generator, I selected 13 stands that covered 10% of each covertype's area (see Appendix E). A map of the selected stands can be found in Appendix F.

Step 3. Ash Tree Survey

After surveying a total of 117 acres across the four covertypes of interest for mature ash trees, I found 139 individuals. Averaged out across the landscape, this is about 1.2 mature ash trees per acre. This further confirms that ash trees represent a small percentage of the coverall canopy cover in the Duke Forest. The exact locations of all ash trees I found were uploaded from Survey123 into a layer file in ArcGIS Online. This data layer was downloaded and saved (see Data Storage section) for future access and use.

Step 4. Plot Sampling

This step was completed between August 8th and September 4th, 2023, over a total of more than 45 hours (including travel times). Of the 33 original plots I selected, 30 were successful. Three plots were removed due to overlapping plots (all within the A-U covertype).

Figure 6. Visual representation of the extent of ash mortality in the Duke Forest in the summer of 2023. Nearly 8 out of every 10 ash trees was dead, and the remaining trees showed clear signs and symptoms of EAB infestation

From the ash tree health assessment portion of the plot sampling, two major results were realized. First, 79% of all ash trees encountered during these plot samples were fully dead (see fig. 6). All dead ash trees had clear signs and symptoms of EAB infestation. No mature ash trees that were still alive were symptom free – all living ash trees I encountered showed evidence of decline and will likely be dead within a few years.

In addition to a mortality assessment (from which AMI can be derived), I collected presence/absence data on the most common signs and symptoms of EAB infestation (see fig.7). This was done for both living and dead trees. The most common signs and symptoms to see were D-shaped exit holes, bark splits, and epicormic sprouts. Increased woodpecker activity and basal sprouts were found on a minority of ash trees. Almost all ash trees displayed a combination of signs and symptoms.

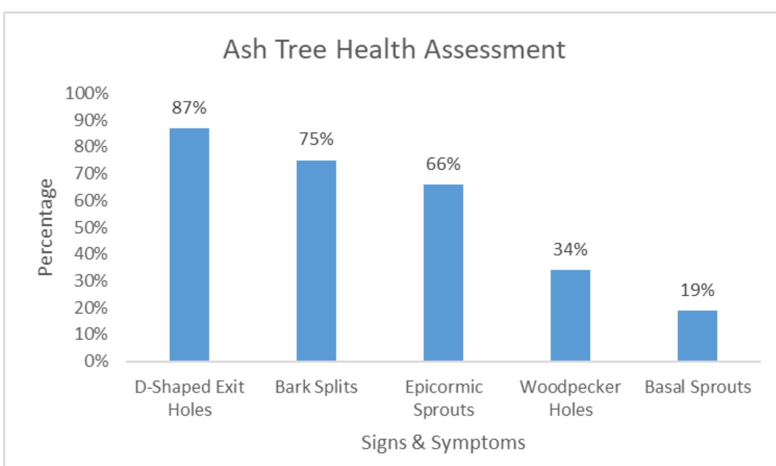

Figure 7. Percentage of mature ash trees encountered during plot sample collection displaying the most common signs and symptoms of EAB infestation.

This ash tree health assessment, on its own, serves as a snapshot of the genus *Fraxinus* in the Duke Forest as of Summer 2023. If subsequent ash tree health surveys are conducted using the same plots, it could be possible to extrapolate the rate of decline and even the approximate infestation year or different areas of the Duke Forest.

Step 5. Modeling Ash Reintroduction with FVS and REGEN-3

The REGEN-3 reports for each covertype were analyzed to determine if covertype composition and/or thinning regime played a role in planted ash survival. Predicted declines in ash survivorship were translated into additional management actions that can be taken to ensure successful ash reintroduction.

Instructions for accessing the full reports generated for each covertype from REGEN-3 (and intermediate reports from FVS) are in the Data Storage section.

<u>A-U Covertype: Yellow Poplar-Sweetgum, Uneven Age</u>

In the A-U Covertype simulation, planted ash trees do not face enough competition among other regen to show a decline in survivorship.

However, planted ash regen does respond to thinnings. A benefit of an increase in relative abundance is realized with all cutting regimes compared to the no cut simulation.

The greatest threat to planted ash survival from competition among other regen comes from Sweetgum (*Liquidambar styraciflua* Linnaeus).

If reintroducing ash in a A-U covertype, thinning is recommended. No mechanical or chemical treatments of other regen or stump sprouts is likely needed, but keeping an eye out for competition from Sweetgum is recommended. All ash trees must be treated with pesticide for the duration of their lifespan in order to survive persistent levels of EAB.

<u>C-U Covertype: White, Black, Red Oak, Uneven Age</u>

In the C-U Covertype simulation, planted ash trees *does* face enough competition among other regen to show a decline in survivorship. Competition from other regen, namely red maple, is enough to affect ash survivorship in every thinning scenario. The worst cutting scenario in terms of ash survival and relative abundance is a thinning to 80 BA.

The greatest threat to ash survival is Red Maple (*Acer rubrum* Linnaeus), followed distantly by oak species (*Quercus* spp.). Red Maple presents a serious threat as competition in the regen class. Oak species present a minor amount of competition that, if Red Maple were eliminated, may not impact ash at all. Additionally, oak regen may be viewed as a positive gain, depending on the goals of the reintroduction site.

No matter what kind of thinning regime is chosen to help reintroduce ash in a C-U covertype, managing for Red Maple regen *and* stump sprouts is imperative. This can be done by revisiting the site and treating stump sprouts or removing regen mechanically or chemically. All ash trees must be treated with pesticide for the duration of their lifespan in order to survive persistent levels of EAB.

<u>CE-U Covertype: White, Black, Red Oak – Mixed Hardwood Species, Uneven Age</u>

In the CE-U Covertype simulation, planted ash trees do not face enough competition among other regen to show a decline in survivorship.

However, planted ash regen does respond to thinnings. A benefit of an increase in relative abundance is realized with all cutting regimes compared to the no cut simulation.

The greatest threat to planted ash survival from competition among other regen comes from American Hophornbeam (*Ostrya virginiana* (P. Mill.) K. Koch).

If reintroducing ash in a CE-U covertype, thinning is mildly recommended. No mechanical or chemical treatments of other regen or stump sprouts is likely needed, but keeping an eye out for competition from American Hornbeam is recommended. All ash trees must be treated with pesticide for the duration of their lifespan in order to survive persistent levels of EAB.

<u>E-U Covertype: Mixed Hardwood Species, Uneven Age</u>

In the E-U Covertype simulation, planted ash trees *does* face enough competition among other regen to show a decline in survivorship. Survivorship of planted ash is 100% in the no thin scenario. Once thinnings are introduced, the pressure from stump sprouts impacts the number of surviving ash trees per acre. The ash seedlings that do not survive are more likely to be the natural small regen observed in the field.

The greatest threat to planted ash survival from competition among other regen comes from American Hophornbeam (*Ostrya virginiana*) and Sweetgum (*Liquidambar styraciflua*).

If reintroducing ash in a E-U covertype, thinning is not recommended from the standpoint of competition from non-ash regen. If a thinning is conducted, it is recommended to return to these reintroduction sites to perform mechanical and/or chemical treatments of American Hophornbeam and Sweetgum stump sprouts. All ash trees must be treated with pesticide for the duration of their lifespan in order to survive persistent levels of EAB.

Discussion

Abundance of Ash. Through the covertype selection process using the 2010 Forest Inventory and 2019 Continuous Forest Inventory datasets, the abundance of mature ash trees in the Duke Forest was demonstrated to be between 1.5 and 3%. This aligns with what the literature suggests for ash abundance in the southeast. The average of about 1.2 mature ash trees per acre when scaled up to the total area of the covertypes sampled in Korstian and Durham creates an estimate of over 1,100 lost trees. Although the Duke Forest does not manage their hardwood stands for timber, they are valued on a periodic basis.

Unlike the northeast, where urban and wild populations of ash trees are much greater, the amount of detriment to the overall landscape the EAB can cause is limited. Thus, the volume of lost timber (and its value) does not have a strong impact on the Duke Forest. However, the ecological consequences of ash trees disappearing from the landscape is yet to be realized. The loss of food and habitat these trees provide to fauna may have bearing on wildlife populations.

Ash Mortality. The discovery that 79% of mature ash trees are already dead in 2023 paints a grim picture. We are past the point of preventative or reactive management to protect the ash. Emerald Ash

Borer infestations in the northeast experience near complete mortality, with rates above 85% mortality within 5 years of infestation (Kashian & Witter, 2011). In Garner, North Carolina, a stand of nealy all ash trees experienced near complete mortality within six years of discovering the EAB onsite (Bohannan et al., 2022). Therefore, complete or near complete mortality across the landscape will likely be realized in the next few years. It is not known how the EAB will persist in the southeast, but it will likely exist at low levels, attacking ash regen that grows big enough.

Implications for Piedmont. Due to time and resource limitations, my Master's Project sampled only from the four covertypes within the Duke Forest where the highest abundance of ash trees was predicted to be. This is a small sample of the many different habitat types ash trees are found in, representative of only a portion of forests in the Piedmont. Even at low abundances, however, ash trees dying across the Southeast will represent a staggering number of canopy-level tree loss that will affect timber markets and the ecosystem. If reintroduction methods become viable, it will be a tremendous effort to return ash to the full extent of its natural range.

Recommendations. For now, the EAB cannot be eradicated from the landscape. Most ash trees are severely stressed from infestation or already dead. The immediate course of action would be to continue to continue removing hazard trees from pedestrian trails and along roads. If ash seed gathering is desired, this must be conducted in the next few years before mature ash trees disappear. No other active management against the EAB or for the ash is needed.

Although no lingering ash trees were encountered during my surveys or plot sampling, the possibility of lingering ash trees persisting in the Duke Forest remains. Although a systematic search for these trees may not be a possibility for the Duke Forest staff and its resources, staff should keep an eye out for healthy ash trees during other management activities. In a few years, if a mature ash tree is found in healthy condition, it may be a candidate lingering ash – in which case it might be of interest to scientists researching rare survivors.

If the Duke Forest decides to reintroduce ash, it must be in a limited capacity with reasonable access to allow for the periodic application of pesticides. Without the protection of pesticide, any ash reintroduced into the Duke Forest will be infested and succumb to the EAB before it reaches reproductive maturity.

As research into parasitoid wasp biocontrols advances, the Duke Forest may be a good introduction site. Additionally, if other solutions - such as the creation of native-Asiatic hybrids or genetically modified ash trees – are successful, both limited and landscape-level ash reintroductions may become possible. Therefore, it is my recommendation for the Duke Forest to stay abreast of emerging solutions to protect ash trees from the EAB and to consider how a reintroduction will align with the mission and sustainability certification standards, should the opportunity arise.

Should a REGEN-3 knowledge base be created for the Piedmont region of North Carolina, repeating the FVS and REGEN-3 analysis would produce regeneration competition predictions and management recommendations that are more closely tailored to the region.

Limitations. While landscape-level solutions for protecting and/or reintroducing ash are not yet available, the limited reintroduction models for the four covertypes of interest and four levels of thinning provide basic management recommendations for planting ash in the future. Both FVS and REGEN-3 are tools that are limited to how they are programmed. For example, the current version of

REGEN-3 does not have a knowledge base (or species competition rankings) calibrated to the North Carolina Piedmont area. Instead, the knowledge base for submesic North Cumberlands conditions were used. Therefore, outputs might not be accurate to our landscape.

Finally, my analysis of ash survivorship in sixteen reintroduction scenarios focuses only on the interspecies competition that can be modeled in REGEN-3. The influence of water availability, sun exposure, deer browse, and other environmental factors cannot be modeled using these tools. Thus, the scope of these results should be considered when making management plans for the future reintroduction of ash.

Future Directions. This project marks the beginning of understanding the Emerald Ash Borer in the Duke Forest. We are in the early years of EAB induced ash decline and mortality. The long-term persistence of the EAB in the Duke Forest, and the southeast in general, is not yet fully understood. Further studies about the extent of EAB damage in other covertypes and over time can improve our understanding of this pest in the landscape. A repetition of gathering ash tree signs and symptoms data (including ADI and AMI) can build a long-term monitoring dataset that can be more informative.

Additionally, the Duke Forest may participate in future studies with biocontrols, hybrid trials, or other solutions-oriented projects once they emerge. A limited reintroduction is currently possible if the desire to create an ash demonstration plot or an ash research area is to be established. Whenever the solution for landscape level ash reintroduction occurs, the data gathered during this Masters Project can serve as a historic record of ash distribution and abundance to guide planting efforts.

Conclusion

The Emerald Ash Borer is here, and here to stay. The amount of damage this pest has caused is extensive, and ongoing. This project has created important records of what will soon be the historic abundance and distribution of ash trees in four major covertypes in the Duke Forest. With hope of a future reintroduction, this data can assist researcher and land managers in understanding where to reintroduce ash.

All data collection was completed in a manner that is replicable and accessible. Further data analysis is possible with the datasets produced by this project – and even more is possible if additional data is collected. Despite the pessimistic snapshot of EAB induced ash mortality this project provides, there is more research to be done to find a solution. Biocontrols, ash hybrids, pesticide, or another method might be developed to allow for the reintroduction of ash back into the landscape. When the solution for landscape level ash reintroduction is found, the results of this Master's Project can be used to guide those efforts.

Data Storage

All data has been sent to and saved within the Duke Forest server. Data was duplicated from my personal DukeBox drive, where this data was originally saved.

All papers referenced or used as background research during my Master's Project are collected and saved in the folder called "Papers." Archived intermediate steps are found in the "Archive" folder. Final products (such as this document, the 10-line abstract, and presentation) are not filed in any subfolders.

Step 1. Covertype Selection

The 2010 Forest Inventory and 2019 Continuous Forest Inventory datasets, as provided to me, are available in this folder. All intermediate steps and final tables are also within this folder, with additional notes in the Box note.

Step 2. Stand Selection

All spreadsheets and screenshots associated with this step are found in this folder. Stand selection maps are recreateable using the 2019 covertype map of the Duke Forest and the names of the stands.

Step 3: Ash Tree Survey

The ash tree survey was conducted using Survey123 and connected to my personal ArcGIS Online account. The screenshot of my Survey123 survey and the GIS layer files were and saved in this folder.

Step 4. Plot Sampling

All completed plot sampling sheets were scanned and saved in this folder. The digitized version can also be found in spreadsheets within this folder. Intermediate steps and calculations are included.

Step 5. Modeling Ash Reintroduction

All plot sampling data sheets, intermediate FVS inputs and outputs, and REGEN-3 reports are saved in this folder.

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Appendices

Appendix A

White Ash	Green Ash
White Pine (*Pinus strobus*)	Boxelder (*Acer negundo*)
Northern Red Oak (*Quercus rubra*)	Red Maple (*A. rubrum)*
White Oak (*Q. alba*)	Pecan (*Carya illinoensis*)
Sugar Maple (*Acer saccharum*)	Sugarberry (*Celtis laevigata*)
Red Maple (*A. rubrum)*	Sweetgum (*Liquidambar styraciflua*)
Yellow Birch (*Betula alleghaniensis*)	American Sycamore (*Platanus occidentalis*)
American Beech (*Fagus grandifolia*)	East Cottonwood (*Populus deltoides*)
Black Cherry (*Prunus serotina*)	Quaking Aspen (*P. tremuloides)*
American Basswood (*Tilia americana*)	Black Willow (*Salix nigra*)
Eastern Hemlock (*Tsuga canadensis*)	Willow Oak (*Quercus phellos)*
American Elm (*Ulmus americana*)	American Elm (*Ulmus americana*)
Yellow-Poplar (*Liriodendron tulipifera*)	

This is a compiled list of species associates for both White and Green Ash. In The USFS Southern Research Station reports the primary associates of White and Green Ash, sources listed below:
White Ash: https://www.srs.fs.usda.gov/pubs/misc/ag_654/volume_2/fraxinus/americana.htm
Green Ash: https://www.srs.fs.usda.gov/pubs/misc/ag_654/volume_2/fraxinus/pennsylvanica.htm

Appendix B

Cover Types		Hardwood Types		Age Classes			
L	Loblolly Pine	A	Yellow Poplar-Sweetgum	0	1-10 years	7	71-80 years
S	Shortleaf Pine	B	River Birch-Sycamore	1	11-20 “	8	81-90 “
V	Virginia Pine	C	White, Black, Red Oak	2	21-30 “	9	91-100 “
M	Misc. Pine Species	D	Post, Blackjack Oak	3	31-40 “	10	101-110 “
P	Mixed Pine Species	E	Mixed Hardwood Species	4	41-50 “	11	111-120 “
		F	Misc. Hardwood Species	5	51-60 “	12	121-190 “
				6	61-70 “	U	Uneven-aged

The Duke Forest uses this system to classify its forest stands into distinct covertypes with age classes. Covertypes can be combined into two letter codes. Provided by Duke Forest staff.

Appendix C

2010 Forest Inventory Dataset

No.	Covertype	Total # Plots	# Plots with Ash	% with Ash
1	AB-U	1	0	0%
2	AC-U	27	2	7%
3	AE-U	4	0	0%
4	A-U	6	1	17%
5	CE-U	9	0	0%
6	C-U	36	6	17%
7	E-U	6	2	33%
8	LA-U	17	2	12%
9	LC-U	10	1	10%
10	LE-U	16	0	0%
11	PE-U	2	1	50%
12	SA-U	1	0	0%
13	SC-U	2	0	0%
14	SE-U	4	0	0%
		141	15	

Results from the 2010 Forest Inventory dataset. The proportion of plots containing ash versus no ash were calculated using ash presence/absence. Conditional coloring shows the relative proportions from red (min: 0%) to green (max: 50%). Covertypes with the highest proportions are highlighted in yellow.

Appendix D

2019 Continuous Forest Inventory Dataset

No.	Covertype	Total # of Plots	# of Plots with Ash	% with Ash
1	AC-U	6	0	0%
2	A-U	2	1	50%
3	CE-U	2	2	100%
4	C-U	19	5	26%
5	E-U	1	0	0%
6	LA-U	1	0	0%
7	LE-U	5	0	0%
8	PE-U	1	0	0%
9	SC-U	2	0	0%
10	SE-U	1	1	100%
		40	9	

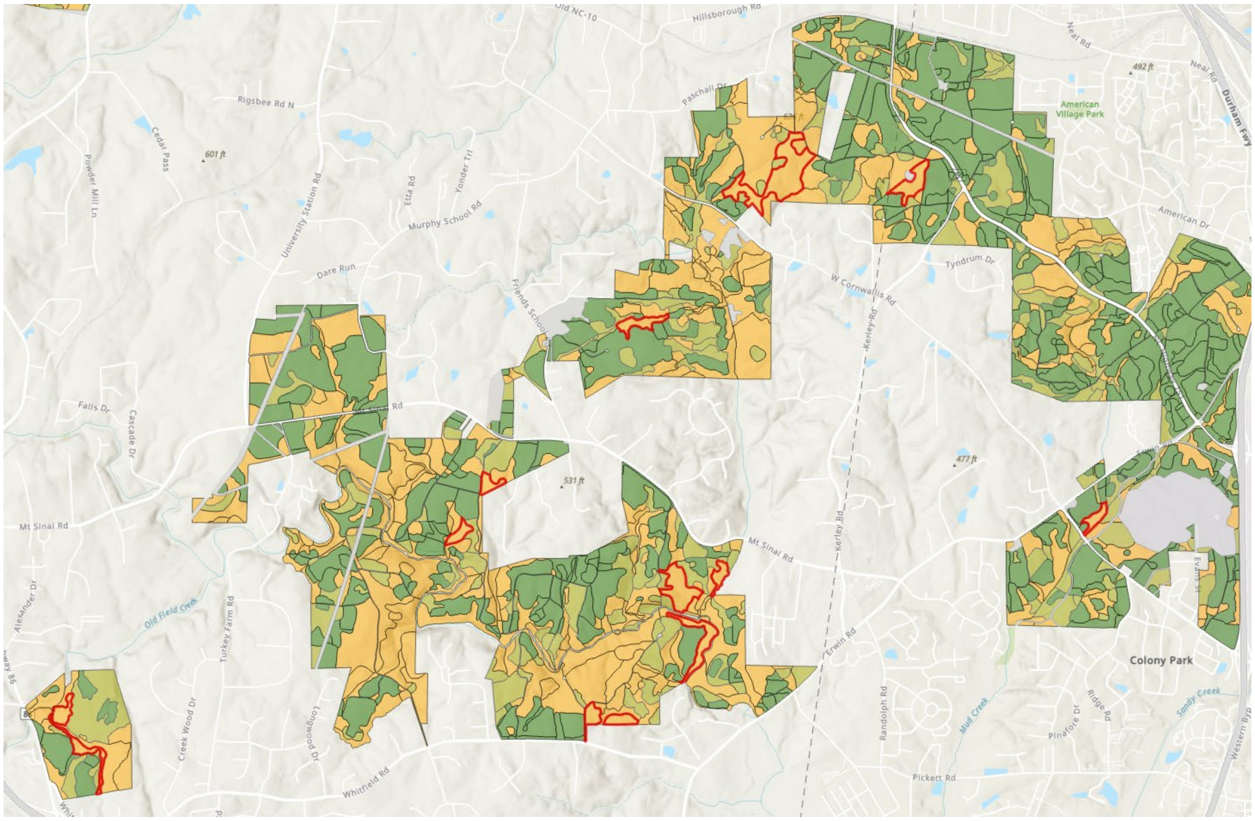
Results from the 2019 Continuous Forest Inventory dataset. The proportion of plots containing ash versus no ash were calculated using ash presence/absence. Conditional coloring shows the relative proportions from red (min: 0%) to green (max: 100%). Covertypes with the highest proportions are highlighted in yellow.

Appendix E

Covertype	Stand Name	Acreage	Division
A-U	oo36-11	6.54	Oosting
	du47-7	4.38	Durham
	ko37-18	4.45	Korstian
	Total acreage: 15.37 acres		
CE-U	ko40-20	4.74	Korstian
	ko12-9	4.32	Korstian
	Total acreage: 9.06		
C-U	du85-49	6.81	Durham
	ko-40-7	4.69	Korstian
	du75-1	35.41	Durham
	du67-1	9.95	Durham
	ko13-8	4.24	Korstian
	ko37-16	15.48	Korstian
	Total acreage: 76.58		
E-U	ko39-12	10.73	Korstian
	oo35-5	5.53	Oosting
	Total acreage: 16.26		

Selected stands for the 10% Ash Tree Survey of the four covertypes of interest in the Durham and Korstian Divisions of the Duke Forest. Stand name (according to Duke Forest record keeping), acreage, and division listed for each selected stand.

Appendix F



Screenshot of highlighted (outlined in red) stands that were randomly selected to be surveyed in ArcGIS Online project.

Appendix G

Ash Tree Sampling Protocol

Site Name: _____________ Plot Number: ___________ Coordinates: ______________ ______________

Date: __________ Start Time: ________ End Time: ________

Invasive Species present: ____

Living Ash BA: ________ Dead Ash BA: _________ Non-Ash BA: _________ Total BA: _________

Native Ground Cover (%)	Non-native Ground cover (%)	Bare Ground/Other (%)

Large Nested Plots (5th and 10th acre / 37.2 ft and 52.7 ft radius)

Ash Trees >6 inches DBH (10th acre) and >18 inches DBH (5th acre)

Tree #	Alive (Y/N)	Condition (1-5)	Height (ft)	Crown Height (ft)	Exit Holes (Y/N)	Wood Pecker (Y/N)	Epicormic Sprouting (Y/N)	Basal Sprouting (Y/N)	Bark Splits (Y?N)

Non-Ash Trees >18 inches DBH (5th acre plot)

Tree #	Species	Species Code	DBH (in)	Height (ft)	Crown Height (ft)

Non-Ash Trees >6 inches DBH (10th acre plot)

Tree #	Species	Species Code	DBH (in)	Height (ft)	Crown Height (ft)

Medium Plot (100th acre)

<u>All Trees</u> 0.1 to 1.5 DBH

Tree #	Species	Species Code	DBH (in)	Height (ft)

Small Plots (1000th acre, four times)

Small Seedlings (6 in min, <2 ft height)	Count

Medium Seedlings (≥2 ft and <4 ft in height)	Count

Large Seedlings ≥4 ft in height but <1.5 in DBH)	Count

Notes: