Overstory Effects of Emerald Ash Borers (EAB) on a Riparian Forest

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I. Abstract

Agrilus planipennis (Emerald Ash Borers) (EAB) are an invasive beetle from Asia that discovered in the USA in 2002 and have decimated *Fraxinus* (ash) forests throughout the country. The ash trees of Stevenson University in Owings Mills, MD have not been spared from EAB infestation. This research study sought to understand how important ash trees are to the overall local forest, how EAB have affected those ash trees and the forest habitat, and considered ways to restore the forest because of EAB infestation. Trees and canopy cover were surveyed in 5 randomly placed plots throughout a 6-hectare section of riparian forest. Individual ash trees were examined for EAB exit holes and status rating. This study found that ash trees are the most important tree in the forest but with spatial variation. Majority of the ash trees are dead with signs of EAB infestation, but canopy openness does not correlate with EAB ash infestation. There are opportunities for future studies that could be conducted on the local forest with our data as a starting point. One potential study could analyze the genome of the four living ash trees that were found. Restoration options include planting already present non-ash tree seedlings in certain plots to restore the canopy, injecting the 4 living ash trees in plot 4 with insecticide, and releasing non-native parasitoid wasps to reduce EAB populations. We could also collect and mail seeds from the living ash trees to a US Forest Service project in Ohio to preserve and understand EAB-resistant ash trees. From this study, we learned how important Ash trees are to Stevenson's ecosystem and that the ash population has been severely affected by EAB infestation.

II. Introduction

Invasive species have become a widespread phenomenon ever since the world became more connected through trading and transportation (Bright, 1999). Invasive species introduction takes place when a species not endemic to the local environment escapes – or is purposely released – and can cause major disruptions in the habitat they invade. In most cases, since invasive species have little to no predators in the areas in which they take over, invasive species' ranges will become larger to occupy a whole ecosystem, state, and even across the country. Invasive species have many detrimental direct and indirect effects on the ecosystems they invade (Lovett et al., 2006). Some of these effects include tree defoliation, displacing native species, creating canopy gaps that make it easier for invasive plants to take over, and tree death (Lovett et al., 2006).

The emerald ash borer (EAB), an insect native to Asia, is an invasive insect that was first identified in the USA in Michigan in 2002 (Gandhi et al., 2008). Hence the name, EAB's main source of food is the ash tree (*Fraxinus* spp.) (Wei et al., 2007). EAB kill ash trees in their larval stage by boring into and feeding on the tree's cambium and leaving winding tracks throughout the tree, which cuts off food transportation (Poland & McCullough, 2006). When the EAB has reached maturity, they will chew their way out of the ash tree and leave D-shaped holes in the bark, commonly referred to as "exit holes" (Wei et al., 2007. Once an ash tree has been invaded by EAB larvae, it can die within the next 1-3 years and results in a mortality rate of 95-98% (Poland & McCullough, 2006; VanHassent, D., 2018). Since the time that EAB have inhabited Michigan, they have caused nearly 100% ash tree (*Fraxinus americana)* death in the area they were first discovered (Gandhi et al., 2008). The following ash tree species are now on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species as critically endangered as of 2017: *Fraxinus americana L., Fraxinus nigra L., Fraxinus pennsylvanica* Marshall, F*raxinus profunda,* and *Fraxinus quadrangulata* (Whittemore et al., 2018). Each of these species listed were of Least Concern 15 years prior to EAB invasion (Whittemore et al., 2018).

EAB can cause other negative effects on the forest besides killing ash trees. Defoliation following the death of ash trees caused by EAB leaves gaps in the forest canopy that open the understory to more light (Baron & Rubin, 2020). This added light availability to the forest understory creates an ideal habitat for invasive plants, which favor high light conditions (Gandhi & Herms, 2009; Baron & Rubin, 2020). While both native and invasive plants favor high light availability, invasive plants grow significantly more than native plants under high light conditions because of the new advantage the invasive plants have over the native plants (Chen et al., 2019). EAB invasion can also lead to a change in the forest microenvironment, forest temperature, and moisture (Twery, 1990; Stadler et al., 2006). This decimation caused by EAB could lead to an "invasional meltdown" in many forest communities throughout the United States. Coined by Simberloff and Von Holle (1999), the term "invasional meltdown" refers to the way that nonindigenous species facilitates the invasion of other nonindigenous species that leads to even more detrimental ecosystem effects than if only one invasive species existed in that area (Simberloff & Von Holle, 1999). It is crucial to understand EABs effects on afflicted ash forests in the United States to start conserving and protecting these critically endangered trees – and the forest as a whole - from further destruction and possible extinction.

Stevenson University in Owings Mills, Maryland, USA, has clusters of ash trees scattered throughout the property. Most of the ash trees present in this area are dead, and majority of dead ash trees have signs of EAB infestation. Gaps in the canopy are easily recognizable above these dead ash trees, along with numerous species of invasive plants in the understory, including Japanese Stilt-grass (*Microstegium vimineum*) and Mile-A-Minute (*Persicaria perfoliata*). Our research objective is to identify common tree species, examine ash tree population abundance and health; determine most abundant mid and understory vegetation; characterize soil moisture, organic matter, and pH; and propose ways to conserve Stevenson University's local forest.

III. Methods

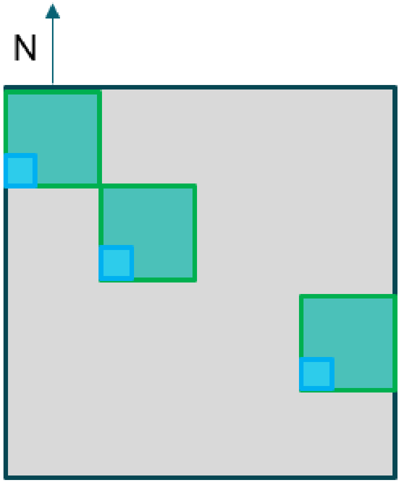
The forest area that was surveyed is a deciduous riparian forest in Owings Mills, MD, USA. Owings Mills is a developed suburban/urban area with patches of riparian and upland forest. Owings Mills falls within the Piedmont Region of MD, characterized by deciduous forests, hilly areas, floodplains, and igneous and metamorphic parent material (Brush et al. 1980). The mean temperature of this area is 13 C, and seasonal temperatures vary from 2 C to 24 C (Lemmons, 2021; DiLisio & Bready, 1999). Annual precipitation is approximately 109 cm (Lemmons, 2021). Common tree species found in this region include the white oak (*Quercus alba*), white ash (*Fraxinus americana*), red maple (*Acer rubrum*), and tulip poplar (*Liriodendron tulipifera*) (Brush et al., 1980). The Gwynns Falls stream runs adjacent the stand for about 500m and is an important tributary to the Patapsco River and Baltimore Harbor (Doheny, 1999). The total area of our research plot is approximately 6 hectares. Picture 1 is an aerial view of the forest surveyed along with markers for each plot that was established.

Picture 1: Aerial view of forest under study and yellow pins at each plot (Google Earth). Total area of forest was 6 hectares.

This research study will generally follow the EREN protocol that outlines methods to study a permanent forest plot (Keurs et al., 2014). Five 20x20m plots facing northeast from 5 points were randomly established for this research study, placed randomly throughout the forest. Within each plot, three 5x5m subplots and one 1x1m miniplot were established to survey midstory and understory (Picture 2).

Every tree in each plot was identified and measured for DBH larger than 2.5 cm in diameter. Trees were categorized as alive (A) or dead (D). Each ash tree's health will be scaled on a range from 1-5, with 1 being the most healthy and 5 being the most unhealthy (Kuers et al., 2014; Picture 3). Ash trees will also be examined for EAB exit holes between 1.25-1.75 m high, according to the EREN EAB protocol (Dolan & Kilgore, 2014).

Importance values for trees was calculated using excel by summing the relative density and the relative dominance of each species. Relative density is the tally of trees of a species and relative dominance is the sum of the DBH's of a species. A hemispherical photo was taken of the forest canopy at each plot corner with a fisheye lens on a modern cell phone and examined in the Gap Light Analyzer online software to determine percent tree canopy coverage as detailed by Promis et al. (2011; Fraser et al., 1999; https://www.caryinstitute.org/science/our-scientists/dr-charles-d-canham/gap-light-analyzer-gla).



Picture 2: Model of 20x20m plot (black square) with 3 random 5x5m subplots (green squares) and 3 1x1m miniplots (blue squares).

Picture 3: Ash canopy rating visual scale. 1 being the healthiest with full foliage and 5 being the least healthy with no leaves.

IV. Results

Overstory Importance Values

The most important tree across all the plots was the white ash tree, with the highest relative density and relative dominance (Figure 1). Almost all ash trees across all plots were dead. Red maple was a close second for importance value. In plots 1 and 2, white ash was the most important tree (Figures 2 & 3). Red maple, black walnut, and willow trees were the most important trees for plots 3, 4, and 5, respectively (Figures 4, 5, & 6). The average white ash importance value was 60, and its average relative dominance was higher than its relative density (Figure 7). The most variation occurred in white ash's importance value, which ranged from ~22-98, which shows the amount of spatial variation within the forest (Figure 7).

Canopy Openness

There was a large amount of variation in canopy openness within each plot (Figure 8). Plot 3 had the most open canopy (~53%) and plot 1 had the least open canopy (~39). Plot 5 had the most variation with canopy openness ranging from ~21-70%.

Ash Tree Tallies, Status Rating, & EAB Exit holes

There were only 4 living ash trees found, and all of them were in plot 4 (Figure 9) out of a total of 22 ash trees across all plots. In plot 4, 2 ash trees had a status rating of 2 and another 2 had a status rating of 3 (Figure 10). All the other ash trees had a status rating of 5 (Figure 10). The tally of EAB exit holes varied within and throughout each plot and the overall average of EAB exit holes per tree was ~9 (Figure 11). The plot that had the most exit holes was plot 1 and plot 2 had the least exit holes (Figure 11).

Overstory Importance Value - Graphs

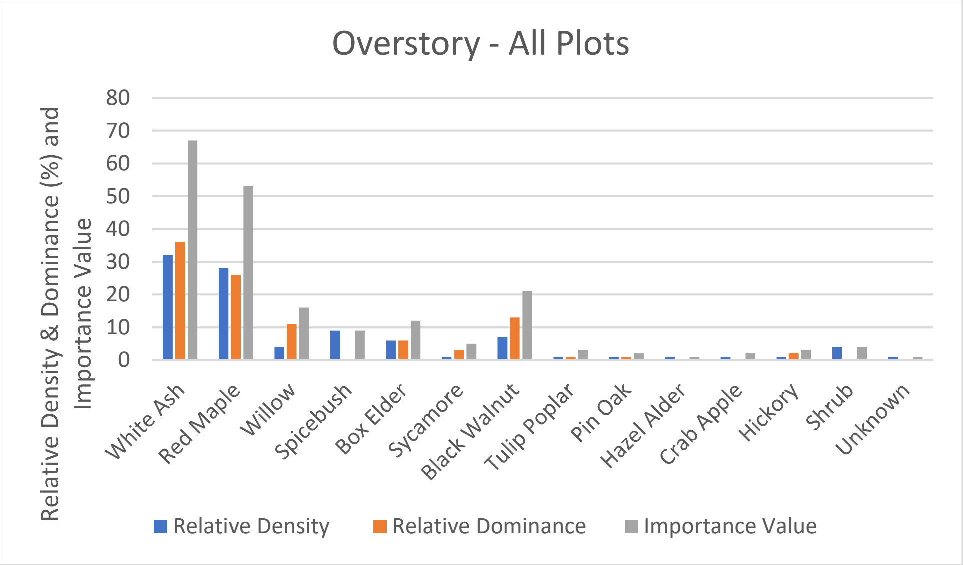


Figure 1: Importance value (IV) data for all tree species across all plots, including relative density and relative dominance. IV is the sum of relative density and relative dominance. White ash is the most important tree across all of the plots, with the highest relative density and relative dominance out of all the other species.

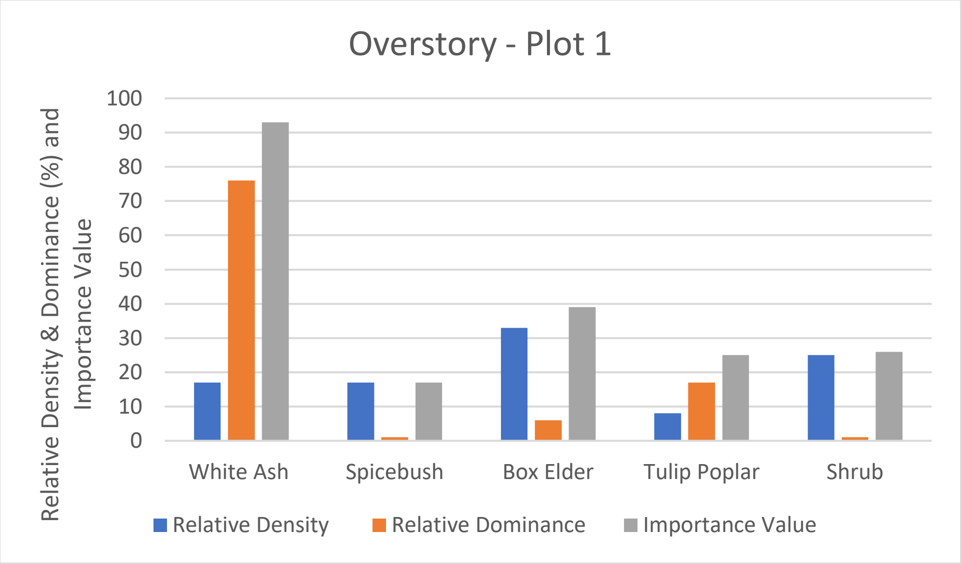


Figure 2: IV for trees in Plot 1. White ash is the most important tree due to its high relative dominance.

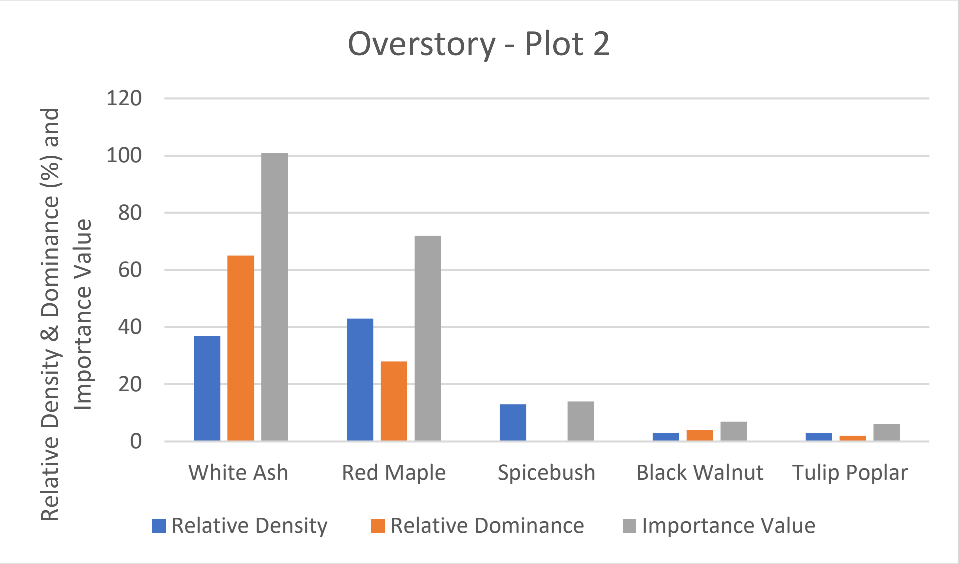


Figure 3: IV for trees in Plot 2. White ash is the most important tree due to its high relative dominance.

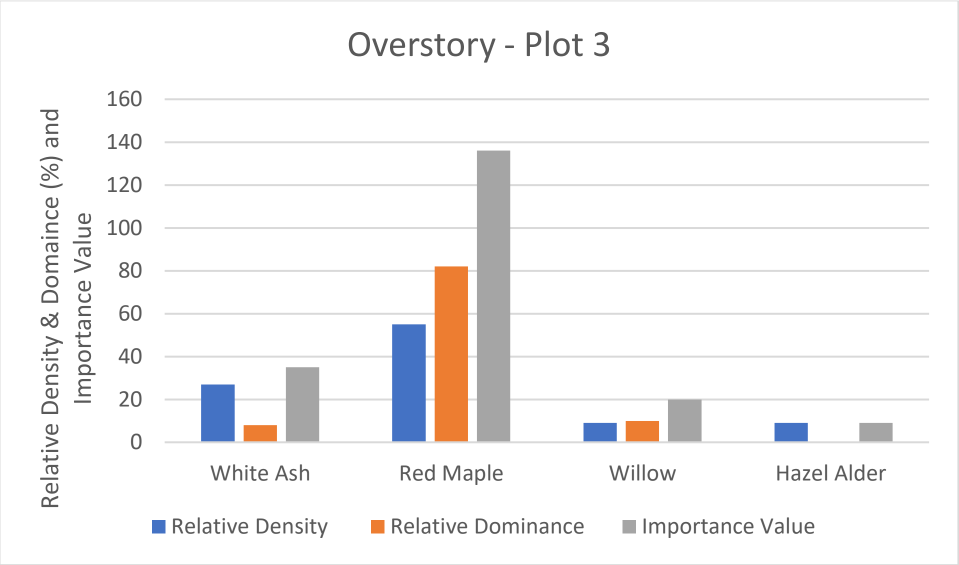


Figure 4: IV for trees in plot 3. Red maple is the most important tree due to its high relative dominance and relative density.

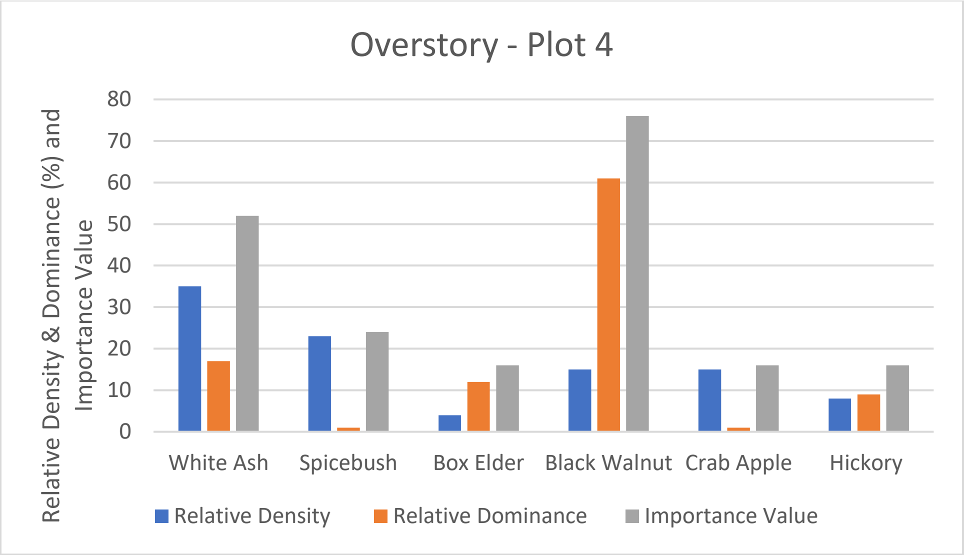


Figure 5: IV for trees in plot 4. Black walnut is the most important tree due to its high relative dominance.

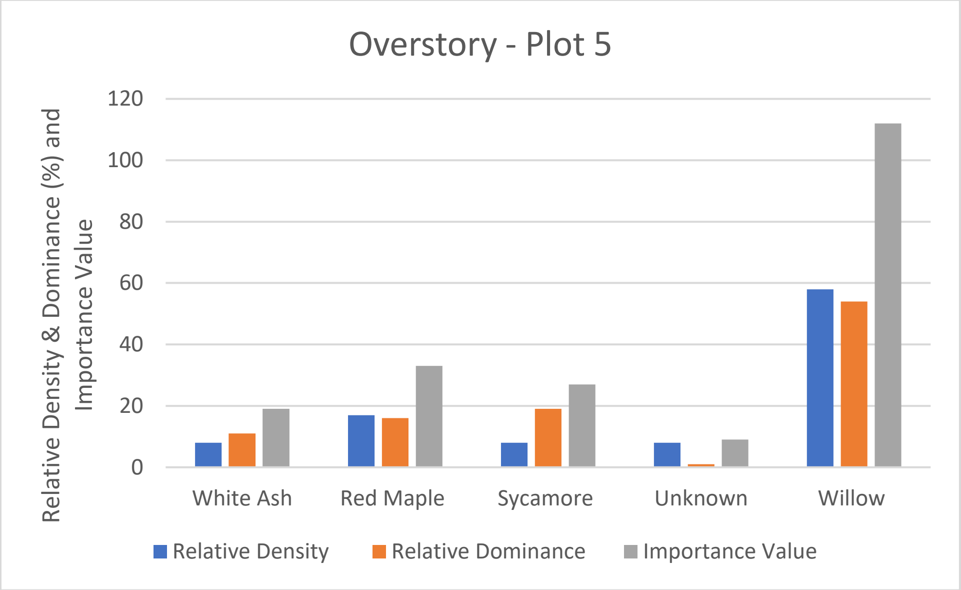


Figure 6: IV for trees in plot 5. Willow is the most important tree due to its high relative density and relative dominance.

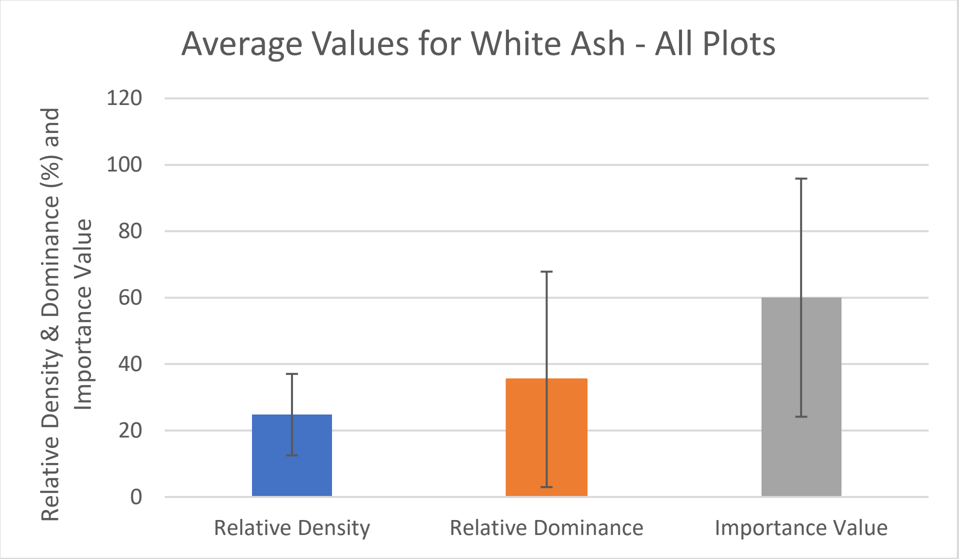


Figure 7: Relative density, relative dominance, and importance value averages for white ash trees in all plots.

Canopy Openness - Graph

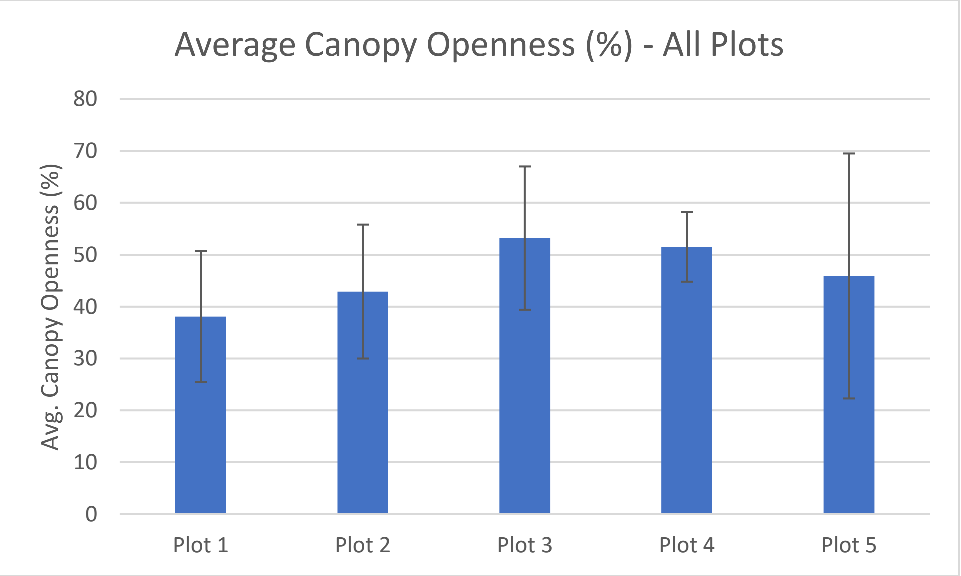


Figure 8: Percentage of average canopy openness in each plot. Averages were taken from the analysis of 4 canopy photos in the software Gap Light Analyzer.

Ash Status Rating & EAB Exit Holes - Graphs

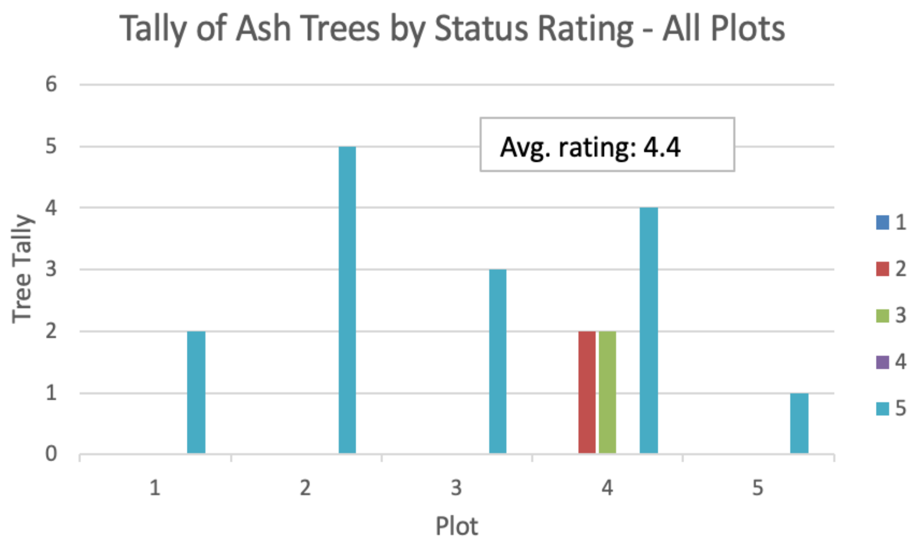


Figure 9: Tally of ash trees by canopy rating by plot, with 1 meaning a healthy, full canopy and 5 meaning a completely dead, open canopy.

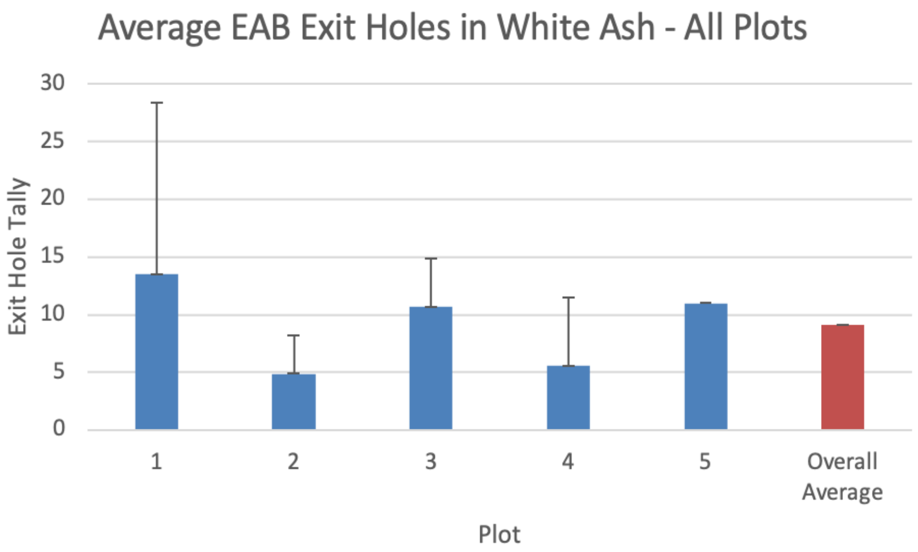


Figure 10: Average EAB exit holes per plot. Average exit holes per plot was ~9. There was variation throughout each plot with plot 1 having the most variation.

V. Discussion

Overstory Importance Values

It was expected that white ash trees were the most important in the forest since most of Maryland's ash trees grow in riparian forests, like the forest we studied (VanHassent, D., 2018). It was also expected that there would be variation between what species was the most important between plots. We did, however, expect white ash to be important in more plots than just plots 1 and 2. However, white ash's average importance value was ~60 (Figure 7), so this data supports the findings in Figure 1 that white ash is the most important tree in the forest.

Canopy Openness

The canopy openness results were not what we expected to find, with no clear correlation between canopy openness and ash importance. White ash is the most important tree in plot 1 (Figure 2) yet plot 1 had the most closed canopy (Figure 8). Since most of the white ash trees we surveyed were dead with no canopy, we expected that plot 1 would have the most open canopy compared to the other plots. These unexpected results could be since, in plot 1, white ash trees were the most important due to their relative dominance (size), not relative density (Figure 2). If white ash trees were the densest tree in plot 1, it would have most likely had a very open canopy. Box elder had the highest relative density in this plot, so this could explain why even though white ash was the most important in plot 1, plot 1 still had the most closed canopy.

The most important species for plot 2 was white ash (Figure 3), and yet plot 2 came in as a close 4th for the most open canopy. However, plot 2 is like plot 1 in that the white ash trees there were the most important because of their high relative dominance, not relative density (Figure 3). Red maple trees had a higher relative density than the ash trees in plot 2, which supports this comparison to plot 1(Figure 3).

In plot 3, red maple trees had the highest relative density and relative dominance while white ash came in second for relative density and a close third for relative dominance (Figure 4). With this in mind, we can hypothesize that plot 3 had the most open canopy because the red maple trees had lost some of their leaves since this study was conducted in mid-autumn. We think that if this study had been conducted in the summer, plot 3 would have had a more closed canopy since the red maple trees would have all their leaves. Future studies could look at canopy openness throughout each plot again during the summer and determine if there are any differences.

With these previous analyses in mind, we would expect plot 4 to have more of an open canopy than what it does (~51%) since there are more ash trees than there are black walnuts, which are the most important tree in that plot (Figure 5). However, black walnut trees had a greater relative dominance than the white ash trees, so the former trees could have a wider canopy because of their larger DBH's.

Plot 5 had the third most closed canopy (~45%) (Figure 8) and its most important tree was the willow (Figure 6). Willow trees dominated this plot and were the largest (Figure 6). No further analysis of this plot can be made.

A similar study was conducted in Ohio, where the researchers were trying to find a link between EAB ash infestation and increased canopy openness. Not unlike our results, these researchers found a small increase in canopy cover despite greater ash mortality (Kreutzweiser et al., 2020). These researchers used the same method of identifying canopy openness but with a different software, so our findings can be compared to theirs. Kreutzweiser et al. believe that the interreference of taller trees with fuller canopies impacted their results (2020). Two other studies also found no link between ash tree death caused by EAB and increased canopy openness (Dolan & Kilgore, 2018; Hoven et al., 2020).

Our study could have been impacted by that, and because of some minor inconveniences with the software. Slight breakages in cloud cover the day the canopy photos were taken were interpreted as leaves in the software, and it was not possible to get rid of these breaks without getting rid of a significant portion of the actual canopy. Further studies could take pictures of the canopy on a day with clear skies and investigate canopy openness in relation to EAB infestation in-depth.

Ash Status Rating & EAB Exit holes

There were 22 ash trees in total for all the plots (Figure 10). Only 4 ash trees were alive, and all 4 of those were in plot 4 (Figure 10). From this data, we estimate that only about 18.18% of ash trees in the forest are currently alive. However, this estimate is quite high given that the 4 live ash trees that were found were outliers compared to the other plots. It is likely that the true value of live ash trees in the riparian forest is much lower. Spatial variation of ash trees also connects with this since ash trees were the most important species in only plots 1 and 2 and that all of the live ash trees were concentration in one plot. It is possible that the 4 living ash trees have a genetic resistance to EAB and that is why they are all concentrated in the same area. Research is being conducted on this very issue. One study found a slight link between ash tree pedigrees and genetic resistance to EAB (Steiner et al., 2019). Future studies could sequence the DNA of the 4 living ash trees and compare it to the DNA of other dead ash trees throughout the forest to identify any significant variations.

18 ash trees had a status cover of 5, 2 ashes had a rating of 2, and 2 ashes had a rating of 3 (Figure 10). Refer to Picture 3 for what each status rating looks like. It is likely that majority of ash trees had a status rating of 5 because of EAB infestation. The overall average exit holes found throughout each plot was ~9, and there was a lot of variation between plots (Figure 11). There seems to be no correlation between plot canopy cover openness and the amount of EAB exit holes found. For example, plot 1 had the most exit holes and the most closed canopy (Figures 11 & 8). Plot 2 had the least exit holes and the second-most closed canopy (Figures 11 & 8). There could be no correlation because it seems that no matter how many exit holes there are, EAB infestation still results in dead ash trees.

We could not find any studies that investigated the relationship between number of EAB exit holes and ash tree canopy. Our study was slightly limited in that the EREN protocol only called for counting EAB exit holes between 1.25 and 1.75m of the tree, so exit holes that were not located within this section were not counted. It is highly likely that each ash tree had many more EAB exit holes than what was counted since EAB do not seem to have a preference for where they emerge from the tree, and no studies to our knowledge have been done on the issue. Future studies could investigate if there is a link between the amount of EAB exit holes on an ash tree and its canopy status rating.

VI. Restoration Options

Tree Seedlings

To restore the canopy to at least 50% in plots 1, 2, and 5, we propose growing tree seedlings in the SU greenhouse and then planting them throughout the established plots. We recommend planting box elder (*Acer negundo*), red maple (*Acer rubrum*), and willow (*Salix*) seedlings in plots 1, 2, and 5 respectively. These species were chosen based off the importance values in Figures 2, 3, and 6. Even though white ash trees are the most important tree in plot 1, we do not recommend planting these as it could lead to further EAB infestation and ash tree death. This suggestion is supported by multiple studies which found that not only is restoring ash forests not cost effective, but there is also no way to guarantee these seedlings will regenerate due to the major changes EAB had on the forest (Kashian & Witter, 2011; Sadof et al., 2011).

We believe that planting the tree species mentioned will have positive effects on the forest canopy. We aim to develop a more closed canopy throughout the SU forest because, as mentioned in the introduction, increased light availability in the understory can lead to increased growth of non-native invasive plants (Chen et al., 2019).

Ash Tree Insecticide Injection

We propose that the four living ash trees in plot 4 be injected with insecticide to prevent potential EAB infestation. Researchers suggest that the best way to prevent EAB infestation is to inject ash trees with insecticide in the spring before they show symptoms of infestation (Herms et al., 2019). Flower et al. suggests injecting emamectin benzoate to best protect ash trees from infestation (2015). A research study found that the number of ash trees injected with the same insecticide matters more than the size of the trees (Mercader et al., 2015). These findings could pose a challenge when applied to SU's forest but investigating whether insecticide could prevent EAB infestation in the currently living ash trees is something that should be investigated to apply to other regions of the forest studied and beyond.

Injecting insecticide would be a long-term commitment, with ash trees usually being treated once every three years to continually protect them from EAB infestation (Herms et al., 2019). In terms of monetary investment, since the cost of injecting ash trees with insecticide varies, the exact amount of money this option would require cannot be determined at this time. Researchers are developing "cost calculators" which can assist landowners with determining the cost of restoring an ash stand or forest, and we could potentially use one of these in the future (Sadof et al., 2011).

Parasitoid Wasps

Researchers are looking into various biological controls for EAB, and a popular method being studied is releasing non-native parasitoid wasps where there are signs of EAB. One recently conducted novel study found that releasing two species of parasitoid wasps – *S. galinae* and *T. planipennisi* – can live in the same location and successfully reduce the EAB population (Duan et al., 2019). However, it is important to note that the researchers suggest that these two wasp species can protect ash saplings and ash trees that have not yet reached maturity. It is unclear from this study if these wasp species can protect mature ash trees from EAB infestation. Another researcher suggests taking an adaptive management approach to local ash tree stands by combining various restoration options - including insecticide injection and parasitoid wasps - and observing the changes that happen as a result (McCullough, 2019).

Future studies could release the two mentioned parasitoid wasps in plot 4 and monitor the populations of those wasps compared to the population of EAB.

Forest Service Ash Project

The final option for restoring the local ash forests goes beyond our plot and to Delaware, Ohio where Forest Service researchers are asking people to send in ash tree seeds from ashes that have been untouched or scarcely affected by local EAB infestation. Seed samples of the 4 living ash trees in plot 4 could be mailed to this project to help in their efforts of researching and conserving these mysteriously EAB-resistant ash trees. Some of these seeds could also be kept at Stevenson for research by students and faculty.

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