CHEMICAL ANALYSIS OF TREES INFESTED BY THE ASIAN LONGHORNED BEETLE

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ABSTRACT

In the past few years, the Asian Longhorned Beetle (*Anoplophora glabripennis*) has infested various species of trees throughout the United States, and it threatens to do billions of dollars worth of damage. This study sought to identify various factors that may affect the selectivity of the Asian Longhorned Beetle (ALB) for specific host tree species. Trees studied included the Sugar Maple (*Acer sacrum*), White Willow (*Salix alba*), and Hackberry (*Celtis spp.*), all of which are known hosts of the ALB. The Eastern White Pine (*Pinus strobus*) functioned as a softwood control, and the Northern Red Oak (*Quercus rubra*) functioned as a hardwood control. The study focused on reducing sugar, protein, lignin, and extractive levels as well as the ratio of vascular vessel space to wood material. The team hypothesized that high sugar and protein content, low lignin content, and relatively little vessel space would lead to high levels of ALB infestation. Although experimental results for sugar and protein content were inconclusive, it appeared that the ALB marginally prefers trees with more lignin and less vessel spaces. For the extractives evaluation, the team investigated possible correlations between ALB infestation rates and the presence of certain compounds; dibutyl phthalate and benzenemethanol were identified as possible repellants of the ALB, but more studies are needed to determine the validity of these preliminary results. Possible sources of error include, but are not limited to the interdependency of variables, the use of different tree parts, and procedural and experimental inaccuracies.

INTRODUCTION

The Asian Longhorned Beetle

The ALB originated in China and entered the US in wooden packaging materials [1]. It was first discovered in North America in Brooklyn, NY in 1996, but it was soon found in Chicago, IL in 1998 and Jersey City, NJ in 2002. Eradicating the ALB is of great importance because the tree species that it attacks are worth billions of dollars to the lumber, Maple syrup, and tourism industries. Moreover, the ALB has the potential to disrupt the forest ecosystem [2].

Current methods to counter the ALB problem include increased inspections, quarantines of infected areas, preventive insecticide treatments, and the destruction of infected trees [2]. The most popular hosts for the ALB are hardwood trees such as Maple (*Acer spp.*), poplar (*Populus spp.*), and Willow (*Salix spp.*) [3]. Although few trees have been formally identified as infested, over eight thousand trees in New Jersey alone have been destroyed in the effort to stop the ALB [2].

Fig. 1 - The ALB
Picture courtesy of
Tree Canada Foundation

Adult beetles are 1.9 to 3.2 centimeters long and have black bodies with mottled white spots (see Fig. 1). The antennae have black and white segments that are 1 ½ to 2 ½ times its body length. The feet and the antennae have a bluish tinge [3]. Adult beetles are generally observable from June to October. The female beetle, after mating, chews out holes in the tree bark and deposits one egg in each hole. Typically, each female lays thirty to seventy eggs. Once the eggs hatch ten to fifteen days later, the young larvae bore through the bark and feed off the tree's vascular system before eventually burrowing into the tree's heartwood [4]. After the larvae mature, they chew their way out of the tree, leaving circular exit holes approximately one centimeter in diameter in the trunks and branches [3].

ALB infestations are very hard to detect because the larvae attack the trees from the inside. Since a single ALB may target many different trees, merely destroying trees that are definitively known to be infested is not enough; in order to ensure that a local outbreak has been completely contained, all possible host trees in the area of the infestation must be cut down.

Cellulose and Hemicelluloses

Cellulose is a polysaccharide composed of long chains of glucose with β-glycosidic linkages. A β-glycosidic linkage is a bond in which each glucose molecule is oriented with its hydroxyl group on the same side as the hydroxyl group of the glucose molecule next to it (see Fig. 2). Because of the orientation of the hydroxyl groups, hydrogen bonding between polysaccharide chains is possible. Cellulose is particularly resistant to most forms of chemical degradation because its β-pleated sheet structure prevents bending of a cellulose molecule necessary for hydrolysis to occur. The bonds between cellulose and lignin, a compound that fortifies the wood, are also highly important for maintaining a stable structure for vascular plants.

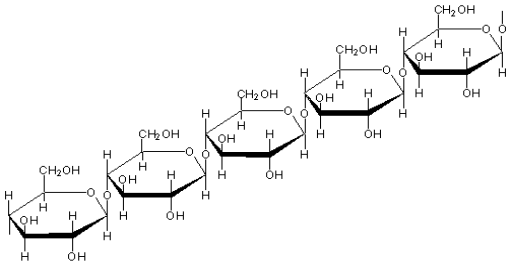

Fig. 2 - The structure of cellulose
Picture courtesy of Royal Society of Chemistry

Hemicelluloses, other polysaccharides, are shorter polymers than cellulose, and comprise between twenty and twenty-five percent of a tree's wood mass [4]. They are composed of branched chains of fifty to three hundred five- or six-carbon sugars [5]. The five-carbon sugars, such as D-xylose and L-arabinose, are usually found in hardwood trees, whereas the six-carbon sugars, including D-galactose, D-glucose, and D-mannose, are more common to softwood trees [4, 6].

Although both cellulose and hemicelluloses maintain the structure of the plant cell wall, no chemical bonds exist between the two substances. Instead, cellulose and hemicelluloses adhere through hydrogen bonds and van der Waals forces.

The ALB has digestive enzymes known as cellulases that can break down both cellulose and hemicelluloses [7]. Both cellulose and hemicelluloses are potential sources of nutrition for the ALB, suggesting that trees with higher concentrations of cellulose and hemicelluloses will attract the ALB more than trees with lower concentrations of these compounds.

Lignin

Lignin is a key component of trees that is derived from sugar through the removal of water. Lignin provides between twenty-five and thirty-five percent of the mass of a tree and strengthens a tree's cell walls. It consists of a complex polymer, which is made up of molecules that bond through their hydroxyl groups to aldehyde, ketone, or hydroxyl groups on different molecules. Inside the cell walls of trees, lignin and hemicelluloses are covalently bonded to create a matrix that is responsible for the strength of the cell wall [4]. Although its structure is known to contain many carbon atoms in the form of benzene rings (see Fig. 3), the exact chemical formula of lignin can differ based upon the species of tree.

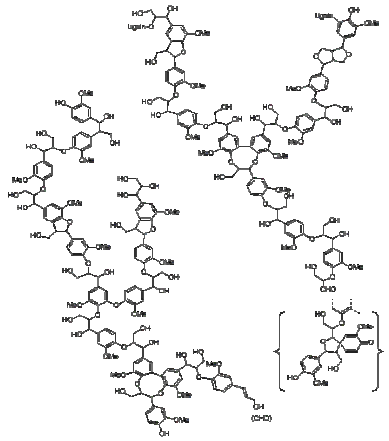

Fig. 3 - Lignin Molecule
Picture Courtesy of Helsinki, Finland: *"Structure of Softwood Lignin"*

Lignin may be a factor in the ALB's selection of a host species. The team hypothesized that the ALB is less likely to infest trees with more lignin because lignin inhibits the ALB's ability to burrow into the tree.

Proteins

Constructed from amino acids, proteins in trees include hydroxyproline-rich glycoproteins, glycine-rich proteins, and proline-rich proteins [8]. While all trees have proteins that guard against certain pathogens and pests, none of the tree species tested have previously been exposed to the alien ALB, so they are assumed to be equally vulnerable in this study. Most organisms need to obtain proteins from food sources to perform many of the functions necessary for life, so even though proteins compose less than one percent of the mass of wood, they may still play an important role in an ALB's diet. The team hypothesized that the ALB is more likely to infest a tree that has a high concentration of protein.

Extractives

Specific organic and inorganic compounds are essential to the survival of trees; they help to protect wood from decay and pests, boost the structural integrity of the tree, provide distinct colors and odors unique to each species, and seem to play important roles in the symbiotic relationships between trees and their environments. Present in roughly the same proportions in all species of trees, inorganic compounds are unlikely to affect the attraction of the ALB to a particular species of tree, and their presence is therefore not the main focus of this study. Organic compounds, however, are present in varying amounts in different species of trees, and their concentrations depend on the particular climate and environmental stresses placed on a tree.

Many of these compounds, commonly referred to as extractives when isolated in the laboratory, are produced by natural metabolic processes of trees, but others come from non-metabolic processes or external sources such as microorganisms.

Volatile organic compounds generally makeup three to five percent by weight of wood material, and their presence may be important in evaluating why the ALB targets certain species of trees. Few studies have yet to be completed on the specific relationship between volatile organic compounds and the ALB, but preliminary studies have shown some promising results. In a study conducted in 1999, "cis-3-hexen-1-ol baited traps attracted a significantly higher number of beetles then unbaited traps." In a similar study from 2000, "cis-3-hexen-1-ol also attracted a significantly higher number of beetles. Traps baited individually with 1-butanol, 1-pentanol, or 2-pentanol caught few beetles, but the combination of the three compounds attracted significantly more beetles than all other treatments, including cis-3-hexen-1-ol" [9].

In this study, the team analyzed which extractives were present in each particular tree sample. The team suspects that the ALB is attracted or repelled, at least in part, by specific extractives present in a tree, and hopes to find evidence of this correlation through its study.

MATERIALS AND METHODS

Tree Descriptions

Five species of trees were used in this study: the Eastern White Pine (*Pinus strobus*), Sugar Maple (*Acer sacrum*), Northern Red Oak (*Quercus rubra*), White Willow (*Salix alba*), and Hackberry (*Celtis spp.*). These trees were selected from the University of Vermont's website on the ALB; the Maple, Willow, and Hackberry were classified as known hosts whereas the Pine and the Oak were classified as non-hosts. All the samples were taken from Jersey City with the exception of the Pine, which was taken from the Drew University campus. These trees were randomly assigned to eliminate any experimental bias. The samples were ground to a pulp using standard household blenders.

Reducing Sugars Test

Reducing sugars are compounds that have aldehydes or hemi-acetals that can be oxidized to carboxylic acids by the copper (II) ion. The amount of the reducing sugar present in a solution may be determined from the color and density of the precipitate formed after adding Benedict's solution, an aqueous solution of $CuSO_4$, $NaCO_3$, and sodium citrate. The team determined the amount of reducing sugar in each tree sample by first creating a standard calibration curve using five standard solutions (10 g/L, 7.5 g/L, 5 g/L, 2.5 g/L, and 1 g/L) made from a stock dextrose (glucose) solution. First, 10 mL of each standard solution was reacted with 10 mL of Benedict's solution. Each solution was heated in a microwave for thirty second intervals to initiate the reaction, the formation of the red copper precipitate copper (I) oxide. Benedict's solution was added in small increments to the standard solutions and heated until a blue color persisted, indicating that the reaction was complete. The standards were then vacuum filtrated to collect the precipitate. Multiple filtrations were necessary since the copper (I) oxide is a fine precipitate. The filter papers were washed with deionized water to remove any trace Benedict's solution and then placed in a drying oven overnight. The precipitate was massed and a calibration curve was

created using a spreadsheet program. To isolate the sugar in each tree sample, 1.00 g of tree pulp was measured, heated with a total of 10 mL of water in 4 mL, 3 mL, and 3 mL increments, and filtered after each heating. The filtrate was then reacted with Benedict's solution and collected using the same procedure as used for the standard solutions. Based on the amount of precipitate collected for each sample, the sugar concentration was determined using the standard calibration curve.

Lignin Isolation

To break down all the cellulose into its soluble sugar components, 1.219 g of cellulase enzyme from *Aspergillus niger* were added to 6.50 g of crushed dried tree sample. In order to maintain optimal conditions for the cellulase, 50 mL of an acetate buffer of pH 4.5 were added while the mixture was stirred at 40^{o}C for forty-eight hours using a hot plate and magnetic stirrer. The mixture was then decanted and rinsed three times with deionized water to remove any remaining sugar, while the solid lignin remained at the bottom of the beaker. The lignin was boiled in 100 mL of 0.05 M hydrochloric acid for two hours to break down any remaining associations between lignin and hemicelluloses. The mixture was again decanted to remove any remaining sugars. The lignin was then washed three times with deionized water, dried overnight in a 100^{o}C oven, and massed.

Protein Content Analysis

The protein content of the five tree samples was determined using a Bradford protein assay and a spectrophotometer. Beer's law, modeled by the equation A = abc, states that the light absorbance (A) of a solution is directly proportional to the distance the light beam must traverse (b) and the concentration (c) of the solution by the constant of proportionality (a). Using this law, the team created a calibration curve to which experimental data obtained from the tree samples was compared.

The experiment involved using standard solutions of Bovine serum albumin (BSA), a commonly used protein for similar experiments. 1 mL BSA solutions of known concentrations ranging from 10 to 100 μg/mL increasing in increments of 10 μg/mL were added to 5 mL of Bradford solution in test tubes. A blank solution of 5 mL of Bradford solution and 1 mL of deionized water and a blank solution of 5 mL of deionized water and 1 mL of tree extract were also created. The resulting solutions were transferred to cuvettes and analyzed in a spectrophotometer. The data obtained was used to create a calibration curve for the extractions from the tree samples. The two blank solutions were used to correct for the absorption values of the Bradford solution and the tree extract.

To isolate the protein in each tree sample, 1.00 g of tree pulp was measured, heated with a total of 10 mL of water in 4 mL, 3 mL, and 3 mL increments, and filtered after each heating. The filtrate collected was reacted with 5 mL of Bradford solution for a minimum of five minutes and then placed in the spectrophotometer. To determine the protein concentration of each sample, the collected data was compared to the standard calibration curve.

Scanning Electron Microscopy

Samples of each tree were prepared by carefully cutting a lateral cross section from a piece of tree branch. The surfaces of the cut samples were kept as smooth as possible. Each sample was then mounted on an individual aluminum stub. Since the scanning electron microscope (SEM) uses electrons to generate images, all samples were coated with a thin layer of a 40% palladium and 60% gold mixture with a sputter coater.

Each sample was placed within the SEM, and images were gathered at 15 kV and a distance of 10 cm. An image of the central core was recorded at 700X, and images of the inner, middle, and outer layers were recorded at 500X. All the images were analyzed using the Java program ImageJ to estimate the area of vessel space and the total area of the image. For each tree, the ratio of the total vessel space to total area of each image was taken, representing the percentage of vessel space.

Gas Chromatograph Mass Spectrometer Analysis of Extractives

To determine any other compounds present in the tree sample, a gas chromatograph mass spectrometer (GCMS) was used. In order to extract the volatile organic compounds (VOCs) from the tree, two 1.00 g samples of freshly ground tree pulp were each placed in a different type of solvent. Cyclohexane, a non-polar solvent, dissolved the non-polar compounds present in one of the tree samples, while ethyl acetate, a polar solvent, dissolved the polar compounds in the other.

The tree samples were dissolved in 5 mL of the respective solvent, and each was placed in a Hirsch funnel and vacuum filtrated. This process was repeated two more times to yield a 15 mL solution. These solutions were poured into test tubes and the solvents were evaporated off using Pasteur pipettes and rubber tubing connected to the high-pressure air source of the fume hoods. After all the solvent was evaporated off, 2 mL of methylene chloride was added to each test tube. The contents were then poured into capped vials.

Using a 10 μL syringe, approximately 1 μL of the contents was injected into the GCMS according to standard procedure. This process was completed for each the vials prepared. The second run for each tree sample in each solvent involved the addition of anthracene to standardize results and account for any differences in injection times.

RESULTS

Reducing Sugars Test

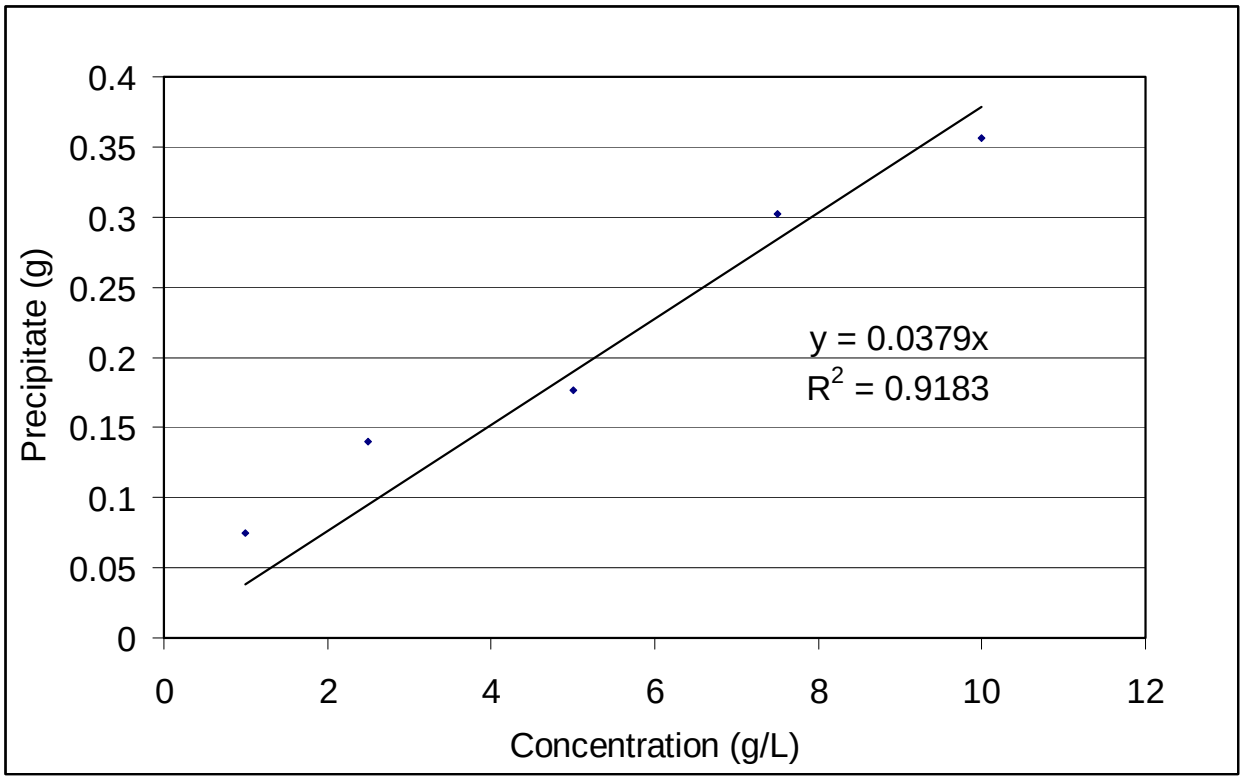

Fig. 4 – Reducing Sugars Calibration Curve

Table 1: Reducing Sugars

	Oak	Maple	Pine	Hackberry	Willow
Sample 1 (g)	0.17214	-	0.05064	-	-
Sample 2 (g)	0.19242	0.04874	0.03382	0.04566	0.1769
Average (g)	0.18228	0.04874	0.04223	0.04566	0.1769
Concentration (g/L)	4.8094987	1.286016	1.114248	1.204749	4.667546

Table 1 shows that the Oak had the highest concentration of sugar, followed by the Willow, Maple, Hackberry, and Pine. The values were calculated using the equation in Fig. 4.

Lignin Isolation

Table 2: Lignin Content

	Oak	Maple	Pine	Hackberry	Willow
Sample 1 (g)	4.1542	5.1503	4.1425	4.5701	4.8141
Sample 2 (g)	3.7753	5.001	3.8685	3.8942	3.9165
Average (g)	3.96475	5.07565	4.0055	4.23215	4.3653

Table 2 shows that Maple had the most lignin, followed by Willow, Hackberry, Pine, and Oak.

Protein Content Analysis

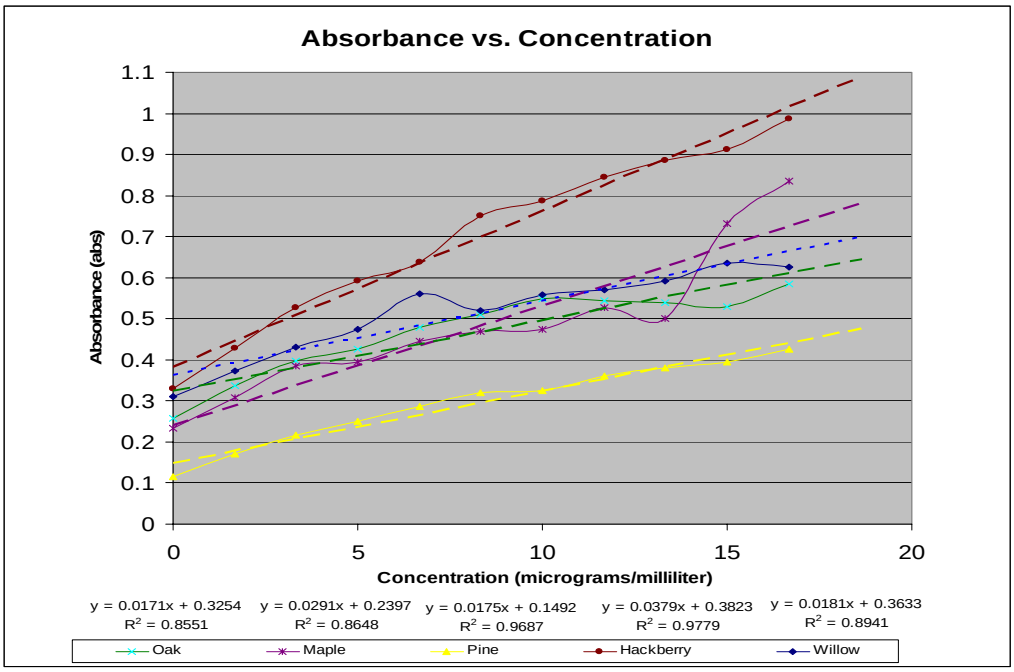

Fig. 5 – Protein Calibration Curve

Table 3: Protein Content

	Abs of Diluted Tree	Abs of Tree A	Abs of Tree B	Net Abs A	Net Abs B	Protein Conc. A (µg/mL)	Protein Conc. B (µg/mL)	Avg. Protein Conc. (µg/mL)
Oak	0.125	0.613	0.591	0.488	0.466	9.509	8.222	8.865
Maple	0.049	0.862	0.916	0.813	0.867	19.701	21.557	20.629
Pine	0.033	0.541	0.533	0.508	0.5	20.503	20.046	20.274
Hackberry	0.038	0.8865	0.946	0.8485	0.908	12.301	13.871	13.086
Willow	0.104	1.227	1.205	1.123	1.101	41.972	40.757	41.365

Table 3 shows that the protein concentrations, listed here in descending order, are Willow, Maple, Pine, Hackberry, and Oak. The values were calculated using the equations in Fig. 5.

SEM

Table 4: Percentage of Vessel Space

	Oak	Maple	Pine	Hackberry	Willow
Area Vessel Space (μm^2)	35169.5	24871.4	19143.9	14760.4	13661.5
Total Area (μm^2)	90982.5	88196.5	66151.9	91285.9	79620.2
Percent Vessel Space	38.7	28.2	28.9	16.2	17.2

Table 4 shows that Oak had the highest percentage of vessel space while both Maple and Pine were roughly ten percent less. Hackberry and Willow had even lower proportions of vessel spaces to total area; they were approximately sixteen and seventeen percent, respectively. In ascending order of percent vessel space, the trees are Hackberry, Willow, Maple, Pine, and Oak.

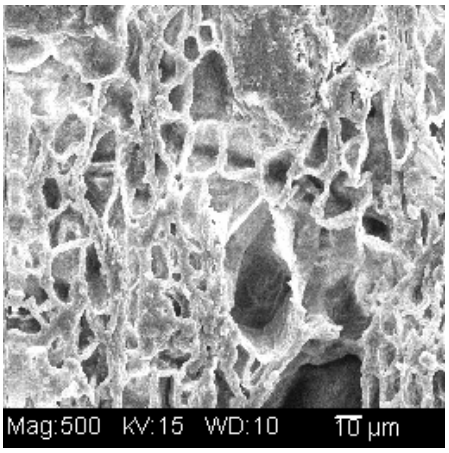

Fig. 6a - Oak

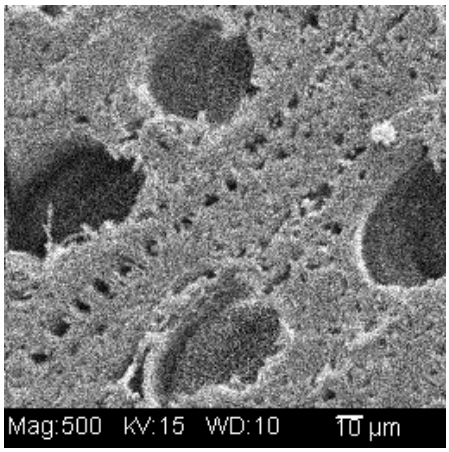

Fig. 6b - Willow

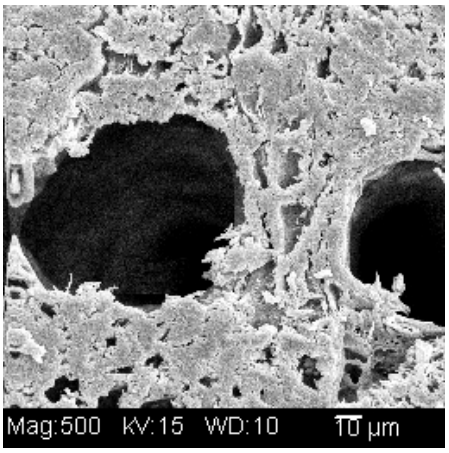

Fig. 6c - Hackberry

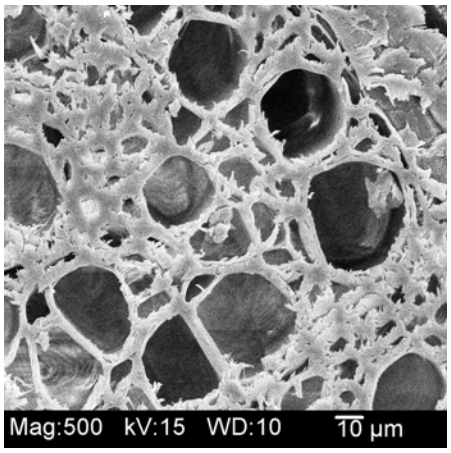

Fig. 6d - Maple

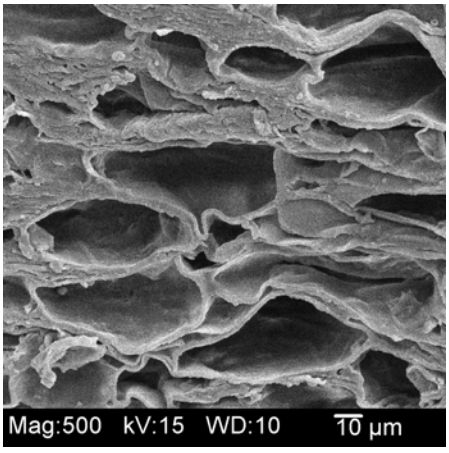

Fig. 6e - Pine

Fig. 6a-6e – SEM Images

GCMS Analysis of Extractives

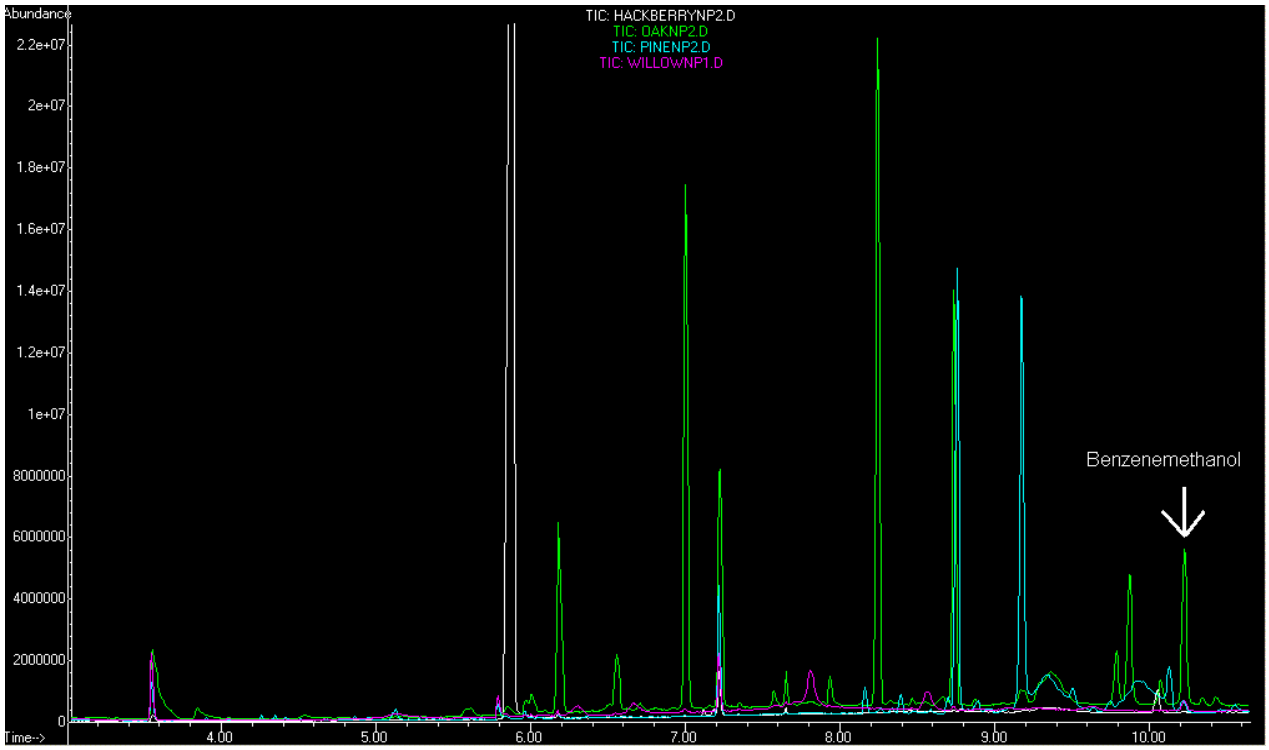

Fig. 7a - Non-polar with Benzenemethanol Shown

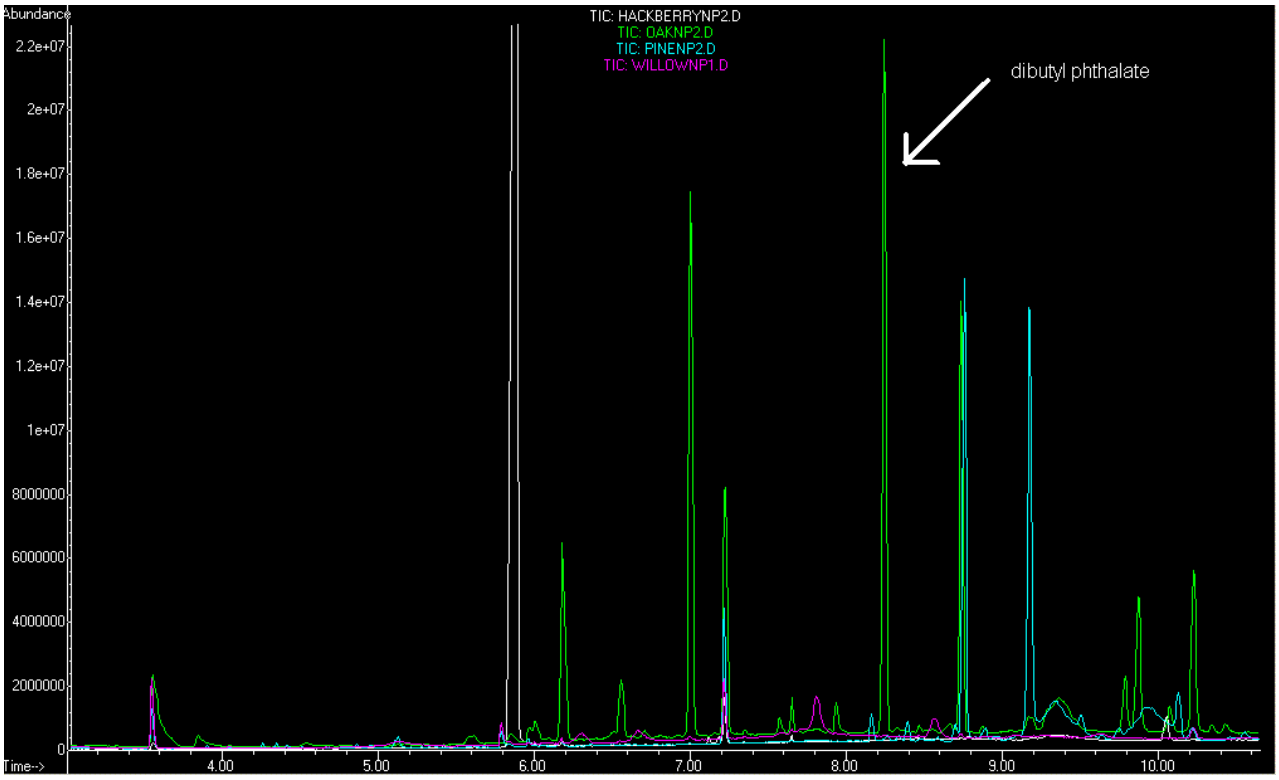

Fig. 7b - Non-polar with Dibutyl Phthalate Shown

Fig. 7a-7b – Labeled Gas Chromatographs

Figures 7a and 7b show the two compounds of interest that were present in Oak.

DISCUSSION

General Discussion

The results obtained through the team's experiments did not confirm all of the suggested hypotheses. One of the reasons why the team might not have produced the expected results is that the hypotheses themselves were flawed. The variables examined may not have had a direct correlation with the ALB's selection of trees. Additionally, the experiments only studied one variable at a time; multiple factors may contribute together to the ALB's selection of host species. Without accounting for this possible interdependency, the results may have no real significance. Another possibility is that the trees that the team tested may have all met a minimum threshold level for the different compounds required for the ALB to survive, so the gathered data may ultimately be irrelevant.

Some experimental errors also may have accounted for discrepancies in the team's data. The team used a limited number of trials for each experiment due to a lack of time and materials, so proper statistical analysis was not possible. To produce conclusive data, the team would have had to perform the experiments with several samples from different sections of each tree; the team only worked with samples from the topmost branches of each tree, which may provide an inaccurate representation of the trees. Fresher tree samples would also contribute more accurate data concerning the chemical makeup of the trees.

Other experimental error may be attributed to the grinding process. Appropriate grinding of the trees was vital to every experiment with the exception SEM. Some trees were grinded to a finer pulp, which resulted in a higher surface area than those trees that were grinded to a coarser pulp. Several procedures also required the use of vacuum filtration, and incomplete filtration may have led to inconsistencies in the reducing sugars, proteins, and extractives tests.

Reducing Sugars Test

The team hypothesized that a higher concentration of sugar would attract the ALB more, suggesting that the Pine, the tree least preferred by the ALB, would have the lowest concentration of sugar while the Maple, the tree most preferred by the ALB, would have the highest concentration of sugar. The results indicated, however, that the Oak had the highest amount of sugar, followed by the Willow, Maple, Hackberry, and Pine. The results suggest that sugar concentration is not a factor in the selection of trees by the ALB.

The data was skewed due to a variety of experimental errors. Some of the tree sample and standard solutions boiled over the sides of the flask when heated in the microwave. Decanting of the solutions was only done for certain samples; for those solutions that were siphoned, suspended precipitate could have been inadvertently removed. Additionally, the filter papers placed in the drying oven often were burnt, potentially altering the calculated masses of the precipitate. While a correction factor was taken into account for the moisture originally in the filter paper, the team did not consider the daily differences in humidity, which could have been

remedied by drying the filter paper before filtration. All of these errors could have been compounded by the use of diluted Benedict's solution, which increased the amount of solution that needed to be filtered.

Many opportunities for conceptual errors also existed. The Benedict's solution tested only for aldoses, yet ketoses may have also been present in the tree samples. The ALB may prefer ketoses to aldoses or one specific monomer over another, and neither of these factors was taken into account. Other reactions may also have added extraneous mass to the precipitate content that was collected through filtration. With so many possible sources of error, the data from the reducing sugars test should be considered inconclusive.

Lignin Isolation

The team hypothesized that a lower concentration of lignin would attract the ALB. According to this hypothesis, the Pine tree should have contained the most lignin while the Maple should have contained the least. However, the data provided opposite results, showing that the Maple contained the most lignin, followed by the Willow, the Hackberry, the Pine, and the Oak.

The team initially hypothesized that the ALB prefers less lignin because more lignin would hinder the beetle from burrowing into the heartwood of the tree. The results show that this may not be a valid idea. The ALB may prefer trees with more lignin because of the stability the lignin gives to the wood. Once the ALB reaches the heartwood of a tree, it uses the lignin present there to create a protective sawdust casing for itself. Data also indicates that the ALB may favor lignin over nutrients found in the vessel spaces of the trees. Such preliminary findings may explain why a tree like the Pine tree is not attacked by the ALB, especially when one considers that the Pine tree does not contain any heartwood. This would also explain why the Maple, which has the most lignin of all the trees analyzed, is the most frequently attacked by the ALB.

Several experimental and conceptual errors could account for these unexpected results. The decanting process may have removed some lignin or failed to remove all the sugar remnants, thereby distorting the actual lignin content. Certain extractives may also have been unknowingly massed with the lignin. Therefore, each tree's lignin sample would have accumulated extra mass dependent on the amount of extractives present in each tree.

Protein Content Analysis

The team hypothesized that the ALB would be more likely to infest trees with higher protein content. The basis for this hypothesis was the assumption that the ALB breaks down proteins to obtain the amino acids necessary for its metabolism. Though the results did show that the two trees with the highest infestation rates also had higher protein content than the other three, the results also showed that the Pine, the tree predicted to have the least amount of protein, had the third highest concentration. The data suggests that protein content may not play a fundamental role in the ALB's selection of host trees.

Several experimental and conceptual errors could have affected the accuracy of the data. The experimental errors include the discrepancies between the timing of the addition of Bradford solution to the cuvettes and the running of the samples through the spectrophotometer, as well as deviation in the calibration curves due the addition of too much water in the filtration of tree samples. As the BSA and tree samples react with the Bradford solution over time, the absorbance of the solution changes. Therefore, the variations between the timing would result in inconsistent data that does not accurately represent the protein concentration of the tree samples. Too much water would decrease the concentration of protein in the samples to be tested. Additionally, the Bradford Reagent reacts only with protein residues containing arginine, histidine, lysine, tyrosine, tryptophan, and phenylalanine; as a result, it is likely that some protein content remained undetected during the experiment.

SEM

The team hypothesized that trees with a small percentage of vessel space are more likely to attract the ALB since the ALB may prefer cellulose and lignin found in the heartwood. According to the data gathered in this experiment, in ascending order of percent vessel space, the trees are Hackberry, Willow, Maple, Pine, and Oak. While the data does not directly parallel the actual order of ALB infestation of trees, listed here in ascending order: Pine, Oak, Hackberry, Willow, and Maple, there is still an overall pattern. The data may support the hypothesis as it may show that trees with more vessel space are less prone to ALB infestation.

The procedure used to quantify percent vessel space may have contributed to a variety of errors. In choosing images to capture and record, the process was somewhat subjective; estimation of what constituted an "average" percentage of vessel space within each part of the sample was somewhat inaccurate. Instead, an entire image of the whole cross section should have been obtained. Moreover, a better image analysis program would have yielded more precise area results than the freehand tool method utilized in this experiment.

Gas Chromatograph Mass Spectrometer Analysis of Extractives

The team hypothesized that certain organic compounds present in the trees may serve as potential deterrents or attractants for the ALB. In previous studies, researchers identified cis-3-hexen-1-ol as a compound that drew a large number of beetles in a controlled environment. The team attempted to identify compounds that were similar to this in structure and/or properties along with any other unique compounds. After analyzing the gas chromatographs and mass spectrums of the extractives, certain compounds appeared to be potential targets for further investigation, although the team failed to find any cis-3-hexen-1-ol in the samples.

The team observed a significant peak in the gas chromatograph of Oak, which was identified as dibutyl phthalate. Dibutyl phthalate (DBP) is a known pesticide and an ingredient included in a many insect repellents [10]. The presence of DBP could potentially be a factor in the ALB's avoidance of the Oak as a host. Further investigation of the effect of DBP on the ALB should include observing the interactions of the ALB with the isolated compound DBP, a branch of Oak, and a branch of Oak, in which the DBP has been completely removed.

Another significant compound that was identified in both the Oak and the Willow was benzenemethanol. Studies have shown that benzenemethanol serves as a feeding deterrent for certain types of beetles, specifically the bark beetle [11]. This behavior has not yet been observed in the ALB, but further research may or may not show that the ALB reacts similarly. The presence of this compound may explain why the ALB does not prefer the Oak or Willow as much as it prefers other trees that do not contain this compound.

Although the gas chromatograph mass spectrometer is extremely accurate, experimental error may have still existed. At the time of the experiment, the branches may have been removed from the tree for over a week. As a result, the extremely volatile compounds may have evaporated even before the VOCs were extracted. Similarly, some VOCs may have evaporated along with the solvent during air evaporation. In future research, branches should be removed from live trees immediately before experimentation.

The leaves of trees are scented, suggesting that they have volatile organic compounds. The leaves should also be analyzed for extractives that may attract or repel the ALB. Perhaps better software with a more extensive database of the mass spectrums of compounds could be utilized in the future as well. With the older software that the team used, it is possible that some of the compounds detected in the GCMS were improperly identified or went unidentified.

CONCLUSION

While the experimental results did not definitively support or refute the team's hypotheses, they did provide some preliminary information that future studies can utilize. The reducing sugar and protein content results did not appear to relate to the ALB's selection of host trees; it is possible that their role is minimal or their presence in all species is above a certain, required threshold level. High lignin content and relatively low vessel space, however, appeared to be conducive for the ALB. These results may suggest that the ALB prefers trees with more wood material, specifically trees with heartwood high in cellulose and lignin content. From the GCMS, dibutyl phthalate and benzenemethanol were identified as possible repellants of the ALB.

Future research in which our identified sources of error are corrected should provide more meaningful and conclusive results. Due to time constraints, the team was unable to completely verify its results to ensure accuracy and precision. Human experimental error was magnified by the lack of consistency in tree samples acquired and tested. The interdependency of variables was also not considered as much as it perhaps should have been. Further studies should account for all these shortcomings to acquire results that are more conclusive.

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APPENDICES

Appendix A - Gas Chromatograph Mass Spectrometer Specifications and Settings

Equipment: Agilent Technologies 6890N Network GC System
5973 Network Mass Selective Detector
Temperature: 130°C to 230°C at 15°C/min
Time: 10.67 minutes
Split Ratio: 20:1
Helium Gas Rate: 1.2 mL/min