CHEMICAL ANALYSIS OF TREES INFESTED BY THE ASIAN LONGHORNED BEETLE

Megan Choy, Michele Edelmann, Louis Kang, Yushen Qian, Craig Schindewolf, Robert Vitt, Bill Zhang, and Rachel Zhang

Team Project Leader: Jeremy Stanton
Team Project Assistant: Bhavik M. Shah

ABSTRACT

This study investigated the possible role of certain aspects of tree chemistry and anatomy in the attraction and sustenance of the Asian Longhorned Beetle (*Anoplophora glabripennis*) pest. Trees in the experimental group included Northern Red Oak (*Quercus rubra*), Shagbark Hickory (*Carya ovata*), Sugar Maple (*Acer saccharum*), and Willow (*Salix sp.*), all known targets of the Asian Longhorned Beetle (ALB). An evergreen control, Eastern Pine (*Pinus Strobus*), was also present. Some aspects of tree chemistry tested include reducing sugar content, protein content, and lignin content. A feature of tree anatomy that was examined was total cross-sectional vessel area relative to total cross-sectional area of the stem. The team hypothesized that reducing sugar content, protein content, and the ratio of total vessel area to total area of the stem cross-section would have a positive correlation with degree of known beetle infestation, while the lignin content would will have negative correlation. The study found that experimental data of sugar, protein, and lignin content yielded inconclusive results. It found a slight positive correlation with vessel area, but more studies are needed to verify the significance of this finding, retest for sugar, protein, and lignin concentration, and investigate other variables such as tree extractives, seasonal effects on tree chemistry, and interdependence of all variables.

INTRODUCTION

Asian Longhorned Beetle and its Effects

The ALB is around 1.9 –3.2 centimeters in length with a white-speckled black shell and black and white striped antennae (Figure 1). The ALB life cycle includes six stages: egg, early stage larva, mid stage larva, late stage larva, pupa, and adult (Figure 2) [1]. In the late summer and early fall, the female beetles chew their way into the host tree in order to lay eggs under the bark [2]. A female usually lays thirty to seventy eggs which hatch in ten to fifteen days [3]. The female beetle lays an average of thirty-five eggs per lifetime [3]. Once the egg hatches the larva bores deeper into its host, feeding off the tree's cellulose, protein, simple sugars, and other nutrients. At some point, the late stage larva creates a pupal chamber in the heartwood of the tree, when it is approximately five centimeters long. It stays in this chamber and transforms into a pupa. Feeding ceases in this phase. Later, the pupa becomes an adult beetle. The adults proceed to lay eggs and,

Figure 1:
Adult Female ALB

hence, create another cycle of destruction. The most damage is done to the tree during the larva phase. The pest spends ninety percent of its lifetime inside its host [4].

In China, many infected trees containing grubs and pupa were used to construct cargo boxes which were shipped to the United States [5]. Once in the country, the beetles fly a short distance to neighboring trees that will become their new hosts. The ALB first appeared in the United States in Brooklyn, New York in August of 1996 [4]. Since then, many trees in and around port cities such as New York and Chicago have been killed as a result of this infestation [4].

Figure 2:
Stages of the ALB

Today, the ALB continues to devastate hardwood trees in the Western Hemisphere. However, the beetle does not seem to target softwood trees. Popular ALB hosts include maple trees (*Acer* spp.), alders (*Alnus* spp.), birches (*Betula* spp.), elms (*Ulmus* spp.), horsechestnut (*Aesculus* spp.), poplars (*Populus* spp.), and willows (*Salix* spp.). Among these, maple trees are known to be the most targeted. The ALB is a potential threat to America's natural hardwood forests and agricultural industries. Many infected trees show signs of yellowing and wilting even in ideal climates as a result of ALB infestation. Sawdust is created as the beetles create eggholes or exit sites which usually measure one centimeter between the farthest points on the hole edge. Often sap may flow out of these holes. Both the sawdust and exit and egg holes are typical signs of infestation (Figure 3).

Figure 3: Egg (O) and Exit (E) Sites

The destruction of America's hardwood trees could have a crippling effect on tourism, maple syrup, nursery, lumber, and furniture industries. Many experts speculate that the damage could cost the U.S. up to forty billion dollars. The ALB problem, similar to other pest problems such as Dutch elm disease, chestnut blight, and the gypsy moth, is extremely detrimental. Unlike the others, ALB infestation often results in the complete destruction or removal of tree populations.

The United States Department of Agriculture (USDA) has been making strides in preventing further annihilation of trees by the ALB. Together with the Animal and Plant Health Inspection Service (APHIS), the USDA encourages state governments to take action against infestation. Currently, they are refining detection and control methods for ALB and gathering information on the dispersal potential of adult ALB to establish quarantine boundaries [6]. Host preference and susceptibility indices are also being developed [6]. Such information will be utilized in this study to see if there are correlations between preferred trees and certain chemical and/or anatomical factors.

In October of 2002, infestation was first identified in the Jersey City area of New Jersey [2]. A 9-acre area of the city then was quarantined (Figure 4), in which over one-hundred trees were bug-ridden [2]. The quarantined area is bounded by the Hudson River to the east, Hoboken to the north, Summit Avenue to the west, and Grand Street to the south [2].

Figure 4: Jersey City Quarantine Area

Wood Chemistry

To predict whether the ALB's selection of certain trees has a chemical basis, it was necessary to examine certain aspects of wood chemistry, and then see if the data coincided with hypothesized correlations between tree chemistry types and beetle selection. This analysis requires a general understanding of wood chemistry. Although different tree species exhibit slightly unique tree chemistry, they all share the same basic wood composition. Wood's major components are cellulose, hemicelluloses, lignin, and extractives. Glycoproteins, another component, are entwined around microfibrils of cellulose which compose the primary cell wall (Figure 5).

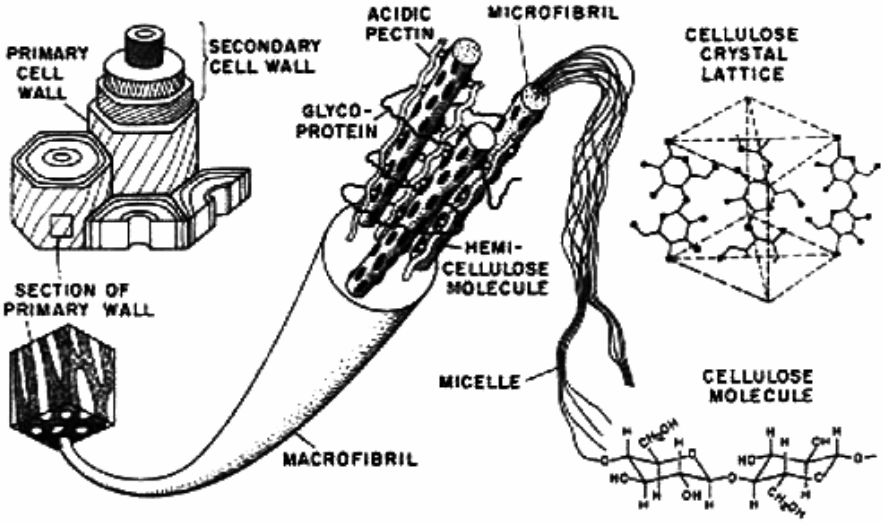

Figure 5: Cell wall ultrastructure

Cellulose, which makes up approximately fifty percent of the weight of the wood, consists of a few hundred to over six thousand β-D-glucose monomers, covalently bonded together by β-glycosidic linkages [7]. The polar OH groups of the glucose monomers cause the formation of many hydrogen bonds between polymers, holding them in a rigid crystal lattice ideal as a structural component of cell walls [8]. Cellulose's chemical composition is identical in all trees, both hardwoods and softwoods [7].

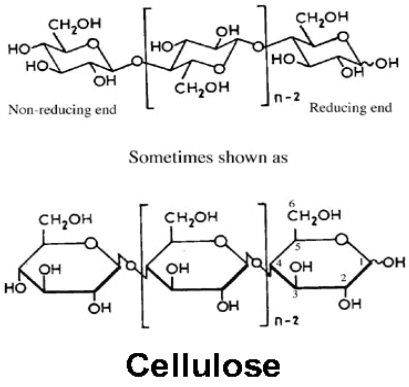

Figure 6: Chemical structure of cellulose

Hemicelluloses, make up approximately 20-30% of the weight of the wood [9]. They are mostly found in stalks or other supporting tissues of woody plants [10]. Like cellulose, they play an important role in maintaining the structure of plant cell walls [9]. In contrast to cellulose, however, which is homogenous throughout the plant, hemicelluloses differ in amount and composition among the stems, branches, roots, and bark [9]. Structurally, hemicelluloses consist of short, sometimes extensively branched chains of five-carbon and six-carbon sugars and uronic acid [7]. Common in hemicelluloses five-carbon sugars include D-xylose and L-arabinose [7]. Common six-carbon sugars include D-galactose, D-glucose, and D-mannose [7]. Some hemicelluloses are easily hydrolyzed and readily soluble in water [7]. Hydrolysis of hemicelluloses in hardwood trees usually yields five-carbon sugar constituents, such as xylose, while the process in softwoods yields mostly six-carbon sugars [7]. Hemicelluloses are often chemically bonded to lignin and adhere to cellulose via hydrogen bonds and van der Waals forces [9].

Lignin is the amorphous "glue" that holds much of the tree together. Comprising 25-35% of the weight of wood, it provides strength and stability to the wood by covalently bonding with hemicelluloses [7]. Lignin aromatic monomers, formed by an irreversible dehydration reaction, combine in an elaborate three-dimensional network. The monomer types that compose lignin differ among tree species [11].

Both the alcoholic and phenolic OH groups on the rings allow the monomers to bind to each other. They can also react with aldehyde or ketone groups. Figure 7 illustrates a possible bonding structure of several lignin monomers:

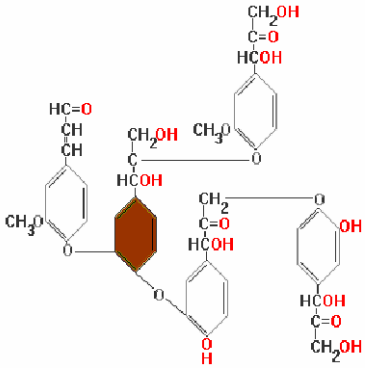

Figure 7: Lignin molecule

The available functional groups of one monomer may form links and cross links with other monomers, forming a lignin network that is highly stable. These networks may vary in size and may have vastly different molecular weights. Together with the hemicelluloses, they add both flexibility and additional strength to the tissues of wood [11].

Extractives, the final component, amount to 3-5% of wood weight, and they usually have low molecular weights [7]. Many characteristics of trees that we normally see, smell, or feel in trees are primarily due to the presence of different extractives in their wood. For example, certain extractives may give a tree a specific scent or color. Some scents attracts or repels insects [12]. Moreover, other wood characteristics such as density, hardness, compressive strength, and permeability to liquids are dependent on extractives.

Extractives can be separated into two main classes: organic and inorganic. Organic extractives are sticky, volatile, and include aromatics, tanning compounds, stilbenes, flavonoids, terpenes, aliphatic acids, and alcohols. The inorganic compounds are minerals absorbed from the soil; they include potassium, calcium, and magnesium. Although extractives make up only a small fraction of a tree's weight, their variety among trees allows for the exhibition of different characteristics and properties [7].

It is uncertain why the ALB prefers certain trees as hosts over others. According to research by Smith, Bancroft, and Tropp, woody tissue characteristics, such as nutritional content, secondary substances, and structural features, may play a role in ALB attraction and survival. From this, the team hypothesized that the ALB is attracted and sustained by trees with greater sugar and protein content. It was also hypothesized that trees with lower lignin content, offering less resistance to burrowing, would be favorable for ALB larvae infestation. In this investigation, the scanning electron microscope was also used to observe slight differences in the structure of the trees. The researchers predicted that trees with more vessel space are favorable hosts because of access to nutrients and less matter to burrow. The intent of this project is to further analyze the chemical and anatomical reasons factors underlying the ALB's preference for certain tree varieties—that is whether preferred tree varieties contain higher amounts of sugar and protein, lower amounts of lignin, and more vessel space.

MATERIALS AND METHODOLOGY

Each pair of experimenters was randomly assigned a bundle of branches from one of the following hardwood trees: Sugar Maple (*Acer saccharum*), Shagbark Hickory (*Carya ovata*), and Willow (*Salix sp.*), all known targets of the ALB, and Northern Red Oak (*Quercus rubra*), a control. An evergreen, Eastern White Pine (*Pinus Strobus*), control later was added to the group. The trees were labeled from one to five, respectively.

Phase I—Grinding

Tree samples were chopped up into course pulp with kitchen blenders. A few branch cross-sections, cut by a pruning saw, and bark samples were set aside for their later viewing under the Scanning Electron Microscope (SEM).

Phase II—Reducing Sugars Test

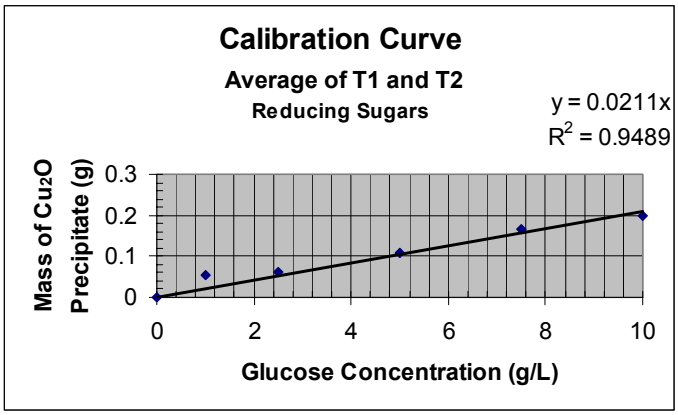

Figure 8: Reducing Sugars Calibration Curve

During the second part of this project, the team tested the amount of reducing sugar contained in each tree sample. The researchers speculated that trees with higher sugar concentration would be superior hosts for the beetles because of the increased food supply. In order to determine the sugar concentration in the trees, the team created a calibration curve using five standard solutions of the following concentrations: 1g/mL, 2.5g/mL, 5g/mL, 7.5g/mL, and 10g/mL (Figure 8). The solutions were prepared by adding varying amounts of dextrose into volumetric flasks.

Figure 9: Reducing Sugars Reaction

Ten milliliter samples of each of the standard solutions were reacted with Benedict's solution, a basic solution containing sodium citrate (dehydrate), sodium carbonate (anhydrous), cupric sulfate (pentahydrate), and water (Figure 9). The formation of a fine red precipitate, cuprous oxide, indicated the progress of the reaction. To stimulate the reaction, samples were heated in a microwave for a total of 30 seconds, with stirring at 10 second intervals. Additional Benedict's solution was added and the sample reheated until a constant blue color remained, signifying that the reaction was complete. After a 24-hour standing period, the samples were filtered gravimetrically and excess Benedict's solution was washed off of the filter paper with de-ionized water. The filter paper was dried in a 160 ºC oven and later massed. Finally, the precipitate masses were plotted to make a curve, using Microsoft Excel with regression analysis.

Water was added to 6.5 grams of tree pulp and then heated in 10 second intervals for 30 seconds in a microwave (high setting) to extract sugars. The resulting mixture was filtered to yield a 10 mL filtrate that was then treated with Benedict's solution and the precipitate collected in a similar fashion.

Phase III—Lignin Isolation

In order to isolate the lignin content from each tree sample, the linkages between the lignin and cellulose/hemicelluloses were exposed first to cellulase enzyme, and secondly, to hydrochloric acid, HCl, which was used to break linkages left intact by the enzyme.

For the first part of the lignin isolation—enzyme digestion—acetate buffer (pH = 4.5) was prepared by adding 1.75 mL glacial acetic acid, 1.6g sodium acetate, and 10g of NaCl to a beaker of 100mL de-ionized water and then filling the beaker to the 500-mL mark. Next, 250-mL beakers were filled with 6.5 g of the appropriate dried tree pulp. Fifty milliliters of acetate buffer and 1.219g cellulase enzyme were then added to each beaker. Next, the beakers were heated and stirred, at 40ºC for 48 hours on hot plates with magnetic stirrers. After heating, the samples were decanted and washed three times with de-ionized water.

For the second part of the lignin isolation—acid cleavage—the sample, now mostly lignin, was added to 50 mL 0.05M HCl and boiled for two hours, during which additional acid was added to replace the volume of solution which evaporated. After boiling, the beakers were decanted, then washed three times with de-ionized water until the lignin was almost completely cellulose/hemicellulose free (92% pure). Lastly, the lignin was dried on watch glasses overnight in a 160 ºC oven, and massed.

Phase IV—Protein Analysis

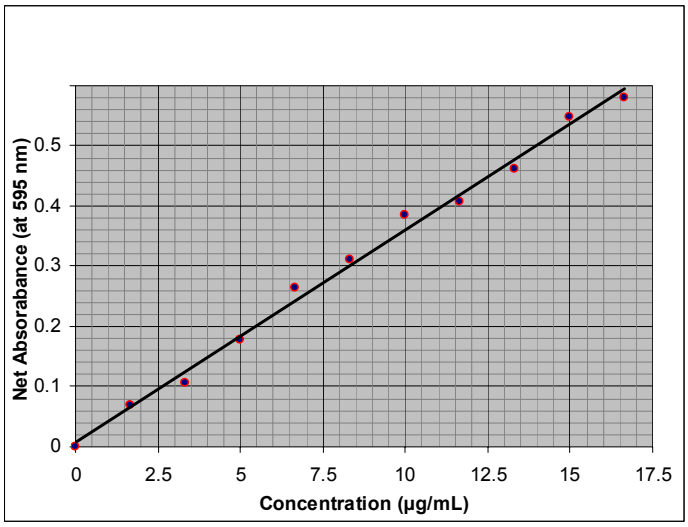

Figure 10: Protein Calibration Curve

This phase of the experiment involves analysis of the protein content of the tree samples. The researchers prepared and tested the protein content of standard solutions, which served as

calibration points. The protein analyses of the tree samples were then compared to the calibration curve (Figure 10).

A relevant equation is Beer's Law, which states $A = \epsilon bc$. In other words, the absorbance of a solution is directly proportional to the concentration of a solution. The researchers stained the protein solutions with a Bradford protein assay. The Bradford reagent is a dye, named Brilliant Blue G, mixed in phosphoric acid and methanol. Upon contact with protein, the Bradford reagent forms a blue complex of varying intensity that depends on the concentration of the protein. Then, the researchers used a Beckman DU® 530 UV-visible spectrophotometer to measure the absorbance of the solution. The spectrophotometer was set to a wavelength detection of 595 nm. From the test standard absorbance data, the researchers constructed a calibration curve to evaluate the protein sample of the trees. Microsoft Excel and its regression analysis was used to create the calibration curve.

The test samples required the use of Bovine Serum Albumim (BSA) as the protein source. Test standards included sample concentrations from 0 to 16.667 μg/mL of BSA, with increasing interval concentrations of 1.667 μg/mL. The team created these concentrations by adding 5 mL of Bradford reagent to each milliliter of BSA sample. The 1-mL BSA samples were of varying concentrations of 0 to 100 μg/mL with increasing interval concentrations of 10 μg/mL. When added to the Bradford reagent, the total volume became 6 mL instead of the original 1 mL. Therefore, the original concentrations were divided by 6 to obtain the final correct concentrations.

A 5 mL assay of the Bradford reagent was then added to the standard solutions. A greater concentration of BSA would lead to a bluer color. The resulting solutions were then transferred to cuvette cells in preparation for spectrophotometer analysis. The 11 results are then used as data points to generate a calibration curve.

To extract the protein from the tree, the researchers added 10 mL of water to 1 g of tree pulp. After 30 seconds in the microwave on high heat, the researchers filtered the congregate to collect the protein-containing filtrate.

To measure the protein in the trees, the researchers ran three samples for each tree. The first sample, a blank, consisted of 5 mL of pure Bradford reagent with 1 mL of de-ionized water. The second sample, another blank, the researchers ran consisted of 5 mL of de-ionized water added to 1 mL of tree extract. The final sample consisted of 1 mL of tree extract added to 5 mL of Bradford solution. The researchers then used the two blanks to determine the correct amount of absorption in the third sample. The net absorbance of the protein is the absorbance of the third sample minus the sum of absorbance of the two blanks.

Phase V—Scanning Electron Microscope

In this phase of the team project, the researchers examined tree samples with the Scanning Electron Microscope (SEM). The reason why the team used the SEM is that not only does it give us more detailed images than the conventional light microscopes, but that also these images are three-dimensional. This feature becomes very useful when team members have to measure the distances between specific points in the sample, i.e. between tree vessels. The

hypothesis for this phase is that greater spacing between the vessels would lead to greater mobility of the ALB larvae; since more movement allows the ALB to more easily burrow through the host tree, it would mark that tree species as a viable target.

To start each group broke a branch of the sample tree that was saved from a previous phase in half. Next, a small cylindrical cross section was cut out of one of the sections. It is important to note that the surface of the cut should be kept as smooth as possible and the other side of the cut should not be so jagged such that the stump cannot stand on its own. The height of the stump was made to be about 1.5 centimeter. The team then peeled a piece of bark from the other section of the branch. The bark was prepared to be very thin, and was a few centimeters long. The researchers then handed cross section and bark to Dr. Miyamoto who afterwards coated both pieces with gold and palladium with a sputter coater. The coating was not too thick so that the electrons from the SEM cannot penetrate, was well furnished nevertheless. Each sample was later placed inside the scanning electron microscope. The team member working with the machine first created a vacuum within the object chamber and then tried to find appropriate images by using the magnifying and focusing features of the SEM. He or she saved the images onto a computer and wrote down the potential difference between the two plates inside the Scanning Electron Microscope and the magnification and the size of the images. When analyzing the images, the group took note of various characteristics of the samples, such as size of the vessels, spacing between the vessels, and the existence of phenols.

RESULTS

Phase II—Reducing Sugars Test

Table 1: Sugar Concentrations

Tree Sample		**Sugar Concentration (g/L)**
Maple	A	1.898
	B	1.983
Hickory	A	2.239
	B	1.855
Willow	A	2.697
	B	2.000
Red Oak	A	0.633
	B	4.140
Pine	A	43.960
	B	33.415

The data shows that the pine tree has the highest concentration of sugar (Table 1). The sugar concentrations determined for maple, hickory, and willow are very similar to each other. There is a large discrepancy between the two values for the red oak.

Phase III—Lignin Isolation

Table 2: Lignin Masses

Tree	Maple	Hickory	Willow	R. Oak	Pine
Mass of Dried Lignin (g)	4.2923	5.0093	4.2606	4.4918	3.4575

The data shows that the maple, the willow, and the red oak had about similar amounts of lignin extracted (Table 2). The hickory showed a higher level of lignin compared to the maple, the willow, and the oak, and the pine showed a lower level of lignin compared to the three.

Phase IV—Protein Analysis

Table 3: Protein Analysis of Tree Samples

Tree Number	**Absorbance of Diluted Tree Solution**	**Absorbance of Diluted Bradford Solution**	**Absorbance of Tree + Bradford Solution**	**Net Absorbance of Tree**	**Protein Concentration (µg/mL)**
Maple	0.024	0.423	0.694	0.247	6.798
Hickory	0.018	0.423	0.590	0.149	4.014
Willow	0.081	0.423	1.042	0.538	15.605
Red Oak	0.029	0.423	0.716	0.264	7.281
Pine	0.055	0.440	0.872	0.377	10.491

According to the results, the willow has the highest concentration of protein (Table 3). The hickory has the lowest concentration.

Phase V—SEM

Table 4: Measurements of Vessels and Distances Between Vessels under SEM

	Maple	Hickory	Willow	Red Oak	Pine
Avg. Diam.	5.341	9.396	11.393	9.326	9.396
Avg. Dist.	11.008	23.126	15.618	29.340	8.805
Diam./Dist.	0.485169	0.406309	0.729453	0.317857	1.067155

The data showed that the willow contained vessels with the largest average diameter whereas those of the maple tree had the smallest average diameter (Table 4). The red oak contained vessels with the largest average distance between them, whereas those of the pine had the smallest average distance between them. The pine had the largest diameter to distance ratio, whereas the red oak has the smallest.

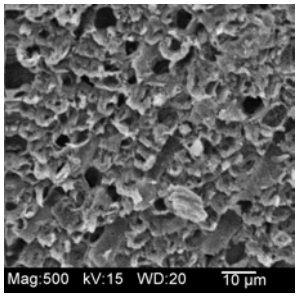

Figure 11.1
Tree 1

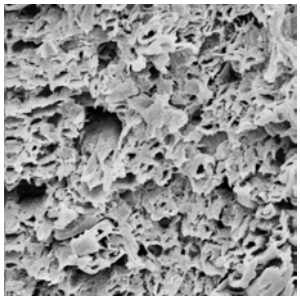

Figure 11.2
Tree 2

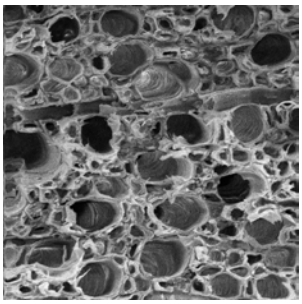

Figure 11.3
Tree 3

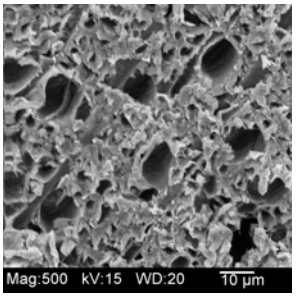

Figure 11.4
Tree 4

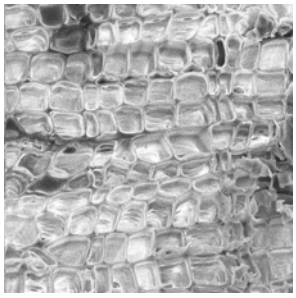

Figure 11.5
Tree 5

Figures 11.1-11.5: SEM Images of Tree Cross-Sections under the same magnification. Scale bar = 10 µm in Figure 11.1.

DISCUSSION

Phase II Discussion

Results from the reducing sugars test were inconclusive. Because the team did not test enough samples from each tree, it could not produce any statistically significant data from which to draw firm conclusions. There were also a few procedural errors that the team did not anticipate.

One of the main issues the team did not account for was the mass of the *dry* filter paper. Because the filter paper lost moisture when it was heated with the precipitates in the oven, the final precipitate masses were lower than they should been. To fix the discrepancy, the team added a correction factor, obtained from subtracting the average mass of ten pieces of filter paper before heating in the oven from the average mass after heating. However, the correction factor was only an average and was not accurate for each individual filter paper. In order to more accurately execute this phase of the project in the future, researchers will have to dry the filter paper before using it.

A second item the team overlooked was that the grinding procedure may not have exposed enough surface area for the complete extraction of the reducing sugars. If a finer pulp mixture were used, more surface area would have made it possible for the water to dissolve more sugar.

Phase III—Lignin Isolation

Results from the lignin isolation were inconclusive. Measurements of the lignin content in the experimental group were substantially higher to that of the evergreen control (the Shagbark Hickory sample containing higher lignin content than the other trees in the experimental group). The implications of this finding are uncertain, however, because it was reasonably assumed that lignin content would influence which trees were selected by the ALB—higher lignin content representing a greater obstacle to the ALB's boring. Yet this appears to not be the case; perhaps lignin content is not as important a factor as the team hypothesized. It is also quite possible that the methods used to isolate lignin were not sufficiently precise. Regrettably, most parts of isolation were vulnerable to error: the grinding of the tree pulp, the acid hydrolysis, and the decanting at the end of either sub-stage of phase three.

First, the grinding of the tree samples unwittingly included the tree bark as well as the woody stems. The outer layer of bark in woody-stemmed trees, called the periderm, consists a layer of cork external to the cambium from which it arises. Cells of the cambium deposit a waxy material called suberin in their walls before becoming mature cork cells, which are dead [13]. Thus, because the bark was grounded with the rest of the pulp, and the lignin isolation was not specific for suberin removal, it is probable that this substance may have influenced the masses of the final lignin products. Other substances could have influenced the final masses of the lignin products as well, which were altogether too heavy (lignin content in woody stems is 35%; data collected suggested twice that number). Also, the grounding process may have produced more accurate results if the tree samples were ground to a finer pulp. More surface area would have facilitated the enzymatic digestion and acid hydrolysis of phase three.

Second, the acid hydrolysis may not have been as effective as anticipated in removing impurities from the lignin. It is probable that the solvent used to dissolve the HCl may have been inadequate for the isolation. In an experiment by D.S. Argyropoulos et al., impure lignin was added to HCl dissolved in a dioxane-water solvent, whereas the present experiment used only a water solvent [14].

Third, the accuracy of the decanting of the impure lignin mixture at the end of either sub-stage of phase three fell short of expectations. Because lignin fibers could be very small and almost indistinguishable in the brown solution of cellulose/hemicelluloses that resulted at the end of either sub-stage, it is possible that some of the lignin was decanted as well. Similarly, it is likely that some of the digested cellulose/hemicelluloses was retained in the lignin product even after decanting, trapped within the mass of partially purified lignin.

Phase IV—Protein Analysis

Results from the protein test were inconclusive. The concentration difference between the five tree samples, however great, does not show any definitive pattern. The experimental data implies that the protein content of the trees follows the order: Willow, Pine, Red Oak, Sugar Maple, and Shagbark Hickory, descending. According to the data, the two tree species in the team's collection that are known to be most targeted by the ALB - the sugar maple and the shagbark hickory – have relatively low concentrations of proteins. Furthermore, the red oak and

the pine controls showed high concentrations of proteins [3]. The tree that showed the greatest amount of protein was the willow, but earlier studies have showed that the willow is less frequently targeted than either the sugar maple or the shagbark hickory [3]; hence, it is the team's opinion that this seemingly contradictory result receives further considerations.

Several factors may account for the ambiguous data. First of all, there is no definitive evidence that the ALB requires protein from trees as part of its diet. Even if it does, there was no mechanism in this experiment to determine which protein the beetle specifically feeds on, or the amount of that protein the beetle requires.

Furthermore, the Bradford protein assay is limited in its ability to detect protein. The Bradford reagent interacts with only. Therefore absorbance data for a solution that contains proteins that do not contain cationic amino acids and/or hydrophobic amino acids will be inaccurate.

There are also other factors involved that potentially affected the team's data. Heating the grounded tree pulp in water may extract proteins, but the process can also denature the proteins, damaging their shape and function, or worse, breaking the proteins down into individual amino acids. There was much room for human and experimental error to occur, whether it was extraction, filtration, or massing. Furthermore, the team did not consider the extractives in the tree, which are responsible for 5% of the total tree mass. Furthermore, it may not be possible to draw a valid comparison between the evergreen control and the rest of the samples; Tree 5 was a softwood, whereas trees 1-4 were all hardwoods.

Phase V—SEM

From the assembled data tables of average distance between the vessels and the average diameters of the vessels, a few important points can be made. First of all, the data suggests that there is no immediate correlation between the distance between the vessels alone and how much a tree species is targeted. The team had expected the distance between the vessels to increase from tree one to tree five because the shorter the distance would mean that the vessels are on average closer together and thus in turn show that there is more space in that specific species; this is a consequence of the fact that the vessels are xylems and have very little matter in them. The researchers' data does not completely support this fact. Although the distance between any two vessels is about 11 microns in tree one, the sugar maple, the distance between vessels in the pine is even smaller, around 8.8 microns on average. These two numbers contradict each other: while the sugar has low inter-vesicular distance and is attacked very often by the ALB, the pine has a small distance as well but is rarely, if ever targeted.

This leads to the next step in the analysis of the SEM images. The team realizes that the diameter of individual vessels can contribute to the fact of whether species are targeted are not. The reasoning that is behind this is that while the distance between vessels shows how much space there is in a tree sample, this value of this distance can vary with respect to the vessels themselves. In other words, if the distance between vessels in a sample is small, but at the same time the vessels themselves are very small, the combination of these two factors does not necessarily equate to large empty space as the distance itself would suggest. The review of data

again does not support the original hypothesis. For example, in tree two, the individual vessels are relatively small (8.193 microns) and the distance between vessels are fairly large (23.126 microns). These two together indicate that tree two has very little vessel space and is therefore very densely packed. Tree two however, is the Shagbark Hickory, a species that is among the top targets for the ALB. Since the phase hypothesis states that more vessel space facilitates the entry of the ALB into the heartwood, the analysis of diameter and length alone does not defend this proposition.

The researchers next analyzed the distance between the vessels and the diameter of the vessels together. It is not enough to discuss the team's SEM results jointly, but consider them to be independent of each other; rather, both the distance and the diameter must be taken into account as one set of data. It is then the team decides to create a new factor, the diameter divided by length, or the d/l factor. Using the average diameter of the tree sample as a uniform number for all the vessels in that sample, each vessel can be considered as a perfect circle with diameter d. Then the comparison between distance and diameter is made simple: as the ratio of d/l approaches one, the vessels will seen as being closer and closer together until finally d/l is one, diameter is equal numerically to the distance between the vessels, and the vessels are physically touching one another. The d/l ratio provides a standard from which the team can compare the trees; the higher the d/l, the closer the vessels are to each other, and the more space exists in the sample. Reviewing the data shows that the team's hypothesis is somewhat accurate, that is, the first three trees, the Sugar Maple, the Shagbark Hickory, and the Willow all have higher d/l ratios than the last tree, the Red Oak (.318) This fits well with observations, as the Red Oak is rarely ever attacked by the ALB and in fact serves as a hardwood control for this project. There are still, nevertheless, factors and data that are unexplained. First of all, the Willow has the highest d/l (.704), but it is widely acknowledged that the Sugar Maple (.473) is the prime target for the ALB. On this point the researchers believes that since the d/l ratio is not the only factor that determines whether a tree species is a fit host for the ALB, the SEM data only becomes useful when it is used in combination with the reducing sugar analysis, the protein analysis, and the lignin analysis. All of these factors can drastically affect the attractiveness of a tree species to the ALB. Another serious problem involves the fact that the d/l ratio for tree five is greater than one. Since this is a mathematical impossibility, this can only be attributed to human error and perhaps procedural mistakes.

Finally the team would like to emphasize that where the images are taken were completely arbitrary. It was agreed that the image must be that of the heartwood; however, its placement in the heartwood was determined that the individual working with that tree sample. Minute differences and small discrepancies should not be overemphasized since this particular research entails an extremely small group of sample trees.

CONCLUSION

The data did not support nor disprove the hypotheses. The beetles were not specifically attracted to trees with a higher sugar or protein content. Also, there did not seem to be a correlation between lignin content and the tree species that are known to be popular hosts for the ALB. There were numerous errors made in the experimental process. Additionally, due to time constraints, the team was unable to test additional samples to ensure accuracy and precision.

Future research should improve the shortcomings of this project. In this experiment, the samples were only taken from trees during the summer. The researchers did not consider the time variable, that is, how wood chemistry varies throughout the year and its effect on the degree of infestation, particularly with regards to sugar content. Also, numerous samples of each host species should be tested in order to prevent inaccurate data. Furthermore, gas chromatography and mass spectrophotometry could be used to analyze volatile organic extractives – a variable that was not considered in this project. This project shows that more progress must be made in order to address the ALB threat to the nation's forestry and dependent industries.

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