Lycorma delicatula mitochondrial DNA in relation to feeding patterns based on monosaccharide concentrations of host plants

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Personal Section

Since 2014 I have watched forests and farms struggle against the spotted lanternfly. As I saw my community struggle I decided to take action. I organized "scraping parties," where I taught others the dangers of the spotted lanternfly and how to scrape their egg masses. Additionally, I created a YouTube video and many flyers that teach about the importance of eradicating the spotted lanternfly. I even planted over 600 grapevines at a local vineyard, to make up for the ones lost due to the spotted lanternfly.

I started my community outreach in 2017 after one of my 9th grade Introduction to Agriculture classes where we learned about the spotted lanternfly. Mr. Siefert, my teacher, explained the damages spotted lanternflies were causing to the environment and economy by feeding on the phloem sap of trees. I listened to his lecture intently and had many questions, but he explained that there was little known about the spotted lanternfly even though it arrived in the United States in 2014. This baffled me, the spotted lanternfly had been damaging our forests and farms for over 3 years and yet there was little known about the pest.

When I stated this to Mr. Siefert, he told me that I could make the difference. He explained that even though I was a high school student I was able to make discoveries that scientists around the world were unable to make, and this changed my life. I began to realize that research I conducted would greatly impact not only the local farms and forests but also people all over the world that relied on the products our forests and farms provided.

I began conducting research on the spotted lanternfly to find the gaps in what was understood of spotted lanternflies. After reading an article about how methyl benzoate eradicates stinkbugs, which have many similarities with the spotted lanternflies, I decided to conduct a project that would determine if methyl benzoate could be used as a control method for the spotted lanternflies. I planned to grow the spotted lanternflies from eggs in incubators and test different concentrations of methyl benzoate on various experimental groups.

I quickly learned that spotted lanternflies do not survive for a length of time in a lab setting, especially without a food source that supplies phloem sap. I needed them to survive to run my tests, so I tested hatching spotted lanternflies in an area that has tomatoes plants for the spotted lanternflies to feed on. As the spotted lanternflies nymphs hatched, I noticed that they avoided the tomatoes, ultimately choosing to die over feeding on them. I wondered how could a pest that is so particular with feeding, rapidly take over our forests and farms? There had to be a feeding pattern that had yet to be discovered.

I changed the focus of my project to determine the feeding pattern of the spotted lanternfly, using phloem sap from the spotted lanternflies' host plants. I extracted the phloem sap from host plants and tested their monosaccharide concentration using High Performance Liquid Chromatography (HPLC). By doing so I discovered that spotted lanternflies prefer to feed on plants with a higher concentration of sucrose and a low concentration of fructose and glucose.While testing the monosaccharide concentration in host plants to discover the spotted

lanternflies feeding pattern, I began to wonder were there other patterns that spotted lanternflies possess?

In order to look for more patterns I tested the spotted lanternflies on a molecular level and compared my results to results in China, India, and Vietnam. I discovered that the spotted lanternflies in the United States had the same mitochondrial DNA (mtDNA) as spotted lanternflies in Asia.

Over the last few years I have become passionate about science research. I have learned that in science you are going to fail. And you can choose to let failure set you back or push you forward. I allowed my failure to push we forward as I looked to understand the reason for my failure. By doing so I discovered many traits involving the spotted lanternfly and have used these traits to create a potential eradication method. I can now see the change I am making for homeowners, forestry industries, and farmers. This difference is changing their lives for the better, which is why I continue my studies.

Science research is more than finding new information and testing hypotheses in the lab. Science is more than a textbook, and a class. Science is about making discoveries and presenting these discoveries to the world. My studies have made an impact on a community of people. These people have supported my research and by doing so have changed my life.

Abstract

Lycorma delicatula (Spotted Lanternfly) has become more propionate in the United States causing tree and financial damage to farmers and therefore the United States government. In order to eliminate *Lycorma delicatula* it is important to understand what attracts them to certain trees and plants. Through High Performance Liquid Chromatography (HPLC) on *Ailanthus altissima* (Tree of Heaven), *Betula nigra* (River Birch), *Pyrus calleryana* (Callery Pear), *Acer rubrum* (Red Maple) and *Acer saccharum* (Sugar Maple) it was determined that *Lycorma delicatula* are attracted plants and trees with a higher concentration of sucrose and a lower concentration of fructose and glucose.

When comparing *Lycorma delicatula* feeding patterns in the United States to *Lycorma delicatula* feeding patterns in their native countries, China, Vietnam and Japan, it was shown that there was a difference in behavior. In order to see if that difference is also shown in the DNA; mitochondrial DNA (mtDNA) ND2 and ND6 was extracted from *Lycorma delicatula* 1st instars (nymphs). Only the mtDNA ND6 was successfully amplified and sequenced. Once sequenced the mtDNA ND6 was compared to mtDNA ND6 of *Lycorma delicatula* in China, Japan, and Korea. It was then determined that *Lycorma delicatula* in the United States and *Lycorma delicatula* in China, Korea, and Japan do not vary in DNA, specifically mtDNA ND6.

Without any DNA variance in mtDNA ND6 it can be determined that mtDNA ND6 has not been mutated in *L*ycorma *delicatula* since its arrival in the United States. If the DNA of *L*ycorma *delicatula* is not mutating it can be concluded that *L*ycorma *delicatula* is adapting to the environment through its feeding patterns quickly enough that it is not requiring a DNA mutation. For the United States this shows that even though the feeding pattern is distinctive now it may change quickly. Suggestions for United States citizens remain the same with the increase in protecting vegetation with 'prime' monosaccharide concentrations.

Introduction

Lycorma delicatula, otherwise known as the Spotted Lanternfly, is a part of the fulgoridae family. *L. delicatula* was first discovered in the United States in Berks County, PA, on September 22, 2014. This invasive insect has migrated to the United States from their native countries: China, India, Vietnam and Japan. The pest can also be found in Korea as an invasive species (Jung, 2017).

L. delicatula is a planthopper, which means that the pest spreads its egg masses throughout many species of trees. The egg masses are laid once the weather turns cold, usually in the beginning of November. When the temperatures reach freezing, the adult *L. delicatula* die off, but the eggs survive throughout the winter temperatures. Once the eggs hatch in April or May *L. delicatula* use their proboscis to feed on different species of trees (Cooperband, 2019). *L. delicatula* has no known pattern to what species of trees and vines that they feed on, which makes *L. delicatula* polyphagy (Hao, 2017). *L. delicatula* does have preferences such as fruit trees (including grape vines), red and sugar maple, oak, walnut, sycamore, willow and tulip poplar trees. The *L. delicatula* host plant is also an invasive species native to China, *Ailanthus*

altissima, otherwise known as the tree of heaven (Dara, 2017). It is believed but not proven that *L. delicatula* prefer species that have smooth bark (Hao, 2017). Many of the *L. delicatula* preferred plants have a variety of texture. Red maple, apple, tree of heaven, and sycamore trees have a smooth texture. Sugar maple, oak, walnut, tulip poplar and willow have a slightly rougher texture. Preference on texture of bark could save some trees while making another tree more vulnerable.

The vulnerability pertains to the risk of fungal infections which is created when *L. delicatula* uses their proboscis to feed on the phloem sap of trees. The proboscis of the *L. delicatula* creates a cavity starting at the outer most layer of bark to the phloem sap channel. The phloem sap contains a high concentration of sugars and nutrients that are necessities for many populations, which cause an infections. These infections are most commonly witting and sooty mold. As the fungal infection takes over and takes away from the trees nutrients the tree quickly suffers (Jung, 2017).

As *L. delicatula* rapidly grow in population size the risk for infections in vegetation also increases at an unhealthy rate. The Pennsylvania Department of Agriculture has predicted an 18-20 billion dollar loss caused by *L. delicatula* (Phillips, 2018). This loss in the economy and in the environment has caused the need for eradication methods. It is important to understand the complexity of *L. delicatula* to create an efficient eradication method. The understanding of L. *delicatula* feeding patterns will aid in effective control methods. Comparing the mtDNA of *L. delicatula* in the United States to *L. delicatula* in native countries will aid in finding similarities and differences that can be utilized in the construction of efficient eradication methods.

Literature Review

As stated in the introduction *L. delicatula* feed on the phloem sap of vegetation. Phloem sap carries carbohydrates, and high levels of proteins (Shah, 2016). When feeding on the phloem sap insects of the Hemiptera order, such as *L. delicatula*, gather energy, carbon, and nitrogen in the form of free amino acids (Douglas, 2006). The carbohydrates that *L. delicatula* gather from the phloem sap contain different sugars such as glucose, fructose, and sucrose.

It has been shown that *L. delicatula* prefer to feed on trees that contain sucrose and fructose, but *L. delicatula* can survive on fructose and glucose. *L. delicatula* can survive for only a short time on mannose, galactose, and raffinose pentahydrate, as sucrose and fructose are necessary for *L. delicatula* survival (Lee, 2009).

In past research High Performance Liquid Chromatography (HPLC) was used in order to find sugar concentrations in different hosts of *L. delicatula*. HPLC starts with a solvent, in this testing a 75% acetonitrile and 25% deionized (DI) water was used as a solvent. As the solvent is pumped through the HPLC machine the injector (in this case the sap) is added. This test required the use of a 250x4.6mm Luna 5u NH2 column. This column uses Azanide (NH2) in order to slow the sugar particles, glucose, sucrose, and fructose at different rates of time. A detector

measures the rate of the sugar particles as they pass through the column. The detector produces a graph of the retention time over the concentration of sugar. Using the graph a liner chart and equation can be written in order to find the exact percentage of sugar in the material, in this case the sucrose, fructose, and glucose in phloem sap samples.

Conducting HPLC testing on other hosts of *L. delicatula* will lead to finding a feeding pattern. In order to compare the feeding pattern, and generally the way that *L. delicatula* from the United States survives to *L. delicatula* in China, Korea, and Japan, results from DNA sequencing will be placed and evaluated next to results found in an experiment which sequenced *L. delicatula* DNA in China, Korea, and Japan.

Using the methods from this similar test conducted in Korea will result in larger accuracy. In Korea the test was completed by DNA sequencing. Much of the DNA sequencing such as primers, temperatures, additives, and measurements will be replicated in this experiment. This will insure the amplification and sequencing of the correct section of DNA nucleotides allowing comparison results to be accurate.

In the experiment conducted in Korea results indicated little genetic difference between populations. Between *L. delicatula* populations north and south of the Yangtze River there was a 1.6 percent difference. Researchers, Zhang, Zhao, Wang, and Qin, determined that this means that *L. delicatula* north of the Yangtze River have migrated south. While it is already known that *L. delicatula* in the United States has migrated from Asia it is necessary to find a relationship between DNA. Knowing if *L. delicatula* has mutations will lead to better understanding of feeding patterns (Zhang, 2019).

Materials and Methods

Experiment #1: Concentration of monosaccharides in host plants

Hypothesis:

By using HPLC in my previous research it was found that hosts of *L. delicatula, Acer rubrum* and *Acer saccharum*, otherwise known as red maple and sugar maple, contain close to zero percent of glucose and fructose but both contain close to two percent of sucrose. This has led to the hypothesis that *L. delicatula* prefer to feed on vegetation that contains two percent sucrose and close to zero percent fructose and glucose.

Sap Collection:

In order to find a pattern between the monosaccharides that *L. delicatula* feeds on multiple sap samples were collected near the beginning of March (once the days were above freezing and the nights were below freezing). These sap samples were about 2mL and were extracted using a 5/16" drill bit, 5/16" ecolo taps, and 36" drop lines. The phloem sap samples

were collected from *A. altissima, B. nigra, P. callertana, A. saccharum* and *A. rubrum* samples. The concentration of the monosaccharides, sucrose, fructose, and glucose, in each of the dependent variables (the sap samples) were found using HPLC. The previous experiment had concluded *L. delicatula* preferred to feed when the tree has a concentration of two percent sucrose and close to zero percent of glucose and fructose. The reason that more phloem sap was extracted from *A. saccharum* and *A. rubrum* is due to the fact that different weather affects the concentration of monosaccharides in a tree and concentrations vary from tree to tree.

HPLC:

To create the data that can be compared to my previous research this experiment took place in a university laboratory, and utilized a High Performance Liquid Chromatography (HPLC) machine. The model of the HPLC was Hewlett Packard, series 1100. The column that was utilized was a Luna 5u NH2, with the solvent being 75% Acetonitrile and 25% deionized water. To start five standards were created and ran through the machine. These standards were the base for the experiment and were used as independent variables due to that fact that the percentage of sucrose, glucose and fructose in each of the standards was already known. In standard one the fructose level was .985%, glucose was .992%, and sucrose was 1.0173%. In standard two fructose was 1.97%, glucose was 1.984% and sucrose was 2.034%. In standard three fructose was 2.956%, glucose was 2.973%, and the sucrose was 3.052%. In standard four fructose was 3.94%, glucose was 3.968% and the sucrose was 4.069%. In standard five fructose was 4.93%, glucose was 4.96%, and the sucrose was 5.09%. After the standards that contained fructose, glucose, and sucrose went through HPLC it was necessary to run three lone standards. The lone standards allowed the retention time of each monosaccharide to be determined. In the first lone standard it contained about 5% sucrose and the second lone standard contained about 5% fructose, while the third lone standard contain about 5% glucose. The information gathered from the HPLC was utilized to create liner charts that created an equation. This equation allows the actual monosaccharide concentration from the host plant to be identified.

Once the standards were established and ran through the HPLC machine the samples of sap were ran through, with no dilution needed. HPLC generated the retention time and peak areas which were recorded.

Interpreting Monosaccharide Concentrations:

The HPLC machine created a graph of all the data sets and provided the peak areas for each of the monosaccharides in the standards. By using the known information (standard percentages) and the peak areas, a liner chart of each of the sugar concentrations was created. These charts were able to create equations in standard form (y=mx+b) for each of the data sets. In these equations y represents the peak areas while x represents the percentage of a specific monosaccharide in the concentration. For sucrose the equation is y=174354x+202.18. The fructose equation is y=163807x+124.59, and the glucose equation is y=162365x+171.25.

By using these equations and the peak area, that was found for the sucrose, glucose, and fructose levels in each of the sap samples, as the y, the percentage of each specific monosaccharide was able to be found. Once the peak area is put in, as y, the equation is then solved for x using algebra. Once x is found it is then multiplied by 100 to make it into percentage form.

Experiment #2: DNA Sequencing

Hypothesis:

Since there is a difference in the feeding of *L. delicatula* in the United States when compared to *L. delicatula* in China, Korea and Japan it is hypothesized that the DNA of *L. delicatula* in the United States has mutated meaning that the coding of the sequenced DNA from *L. delicatula* in the United States will be different then the DNA of *L. delicatula* in China, Korea, and Japan.

Mitochondrial DNA Extraction:

In order to extract mitochondrial DNA (mtDNA) from *L. delicatula* it was necessary to obtain *L. delicatula* instars (nymphs). These instars were hatched in an incubator that was kept at 21.6 degrees Celsius and between 70%-80% humidity. After they were deceased an instar was collected and placed into a 1.5mL tube. 300uL of lysis solution was added to the tube before the instar was turned to powder using a pestle for 2-5 minutes. The tube was incubated in a heat block at 65 degrees Celsius for 10 minutes, and then was centrifuged for one minute at maximum speed. 150uL of the supernatant and 3uL of silica resin was transferred and mixed into a new 1.5mL tube. This tube was then placed into the heat block for five minutes at 57 degrees Celsius which was followed by centrifuging for 30 seconds at maximum speed. All of the supernatant was removed and 500uL of ice-cold wash buffer was added and mixed into the pellet. This tube was then centrifuged again for 30 seconds at maximum speed, followed by adding 500uL of ice-cold wash buffer, and centrifuging again for 30 seconds at maximum speed. Using a micropipette all of the supernatant was removed and 100uL was added and mixed into the silica resin, which was centrifuged for 30 seconds at maximum speed. 90uL of the supernatant was added to a new 1.5mL tube which was then stored at -20 degrees Celsius. This process was done twice on two different instars which were kept in separate tubes.

Mitochondrial DNA Amplification:

Once the extraction and purification process was complete for both instars the amplification process was started. To start 25nmol of the primers ND2-238F (5'-AATTGCCCCATTAATGAAAGA-3'), ND2-866R (5'-TTTGATTTGGTTATTGTA-3'), and primers ND6-87F (5'TCAAACAGCCTTAATG TGCAG-3'), ND6-480R (5'-

TGGTCCTTCAAATGTTCTTACG-3') were obtained from Genewiz. The primers were made into a 100uL stock, which was what was utilized for the continuation of the tests. In order to get the correct DNA amplified it was necessary to create multiple samples. The first instar was titled sample A while the second instar was titled sample B. The first tube was sample A ND2 and held 12.5uL of master mix, 1uL of each ND2-238F and ND2-866R primer, 5uL of the extracted DNA from sample A, and 5.5uL of sterile dH2O. The second tube was sample A ND6 and held 12.5uL of master mix, 1uL of each ND6-87F and ND6-480R primer, 5uL of the extracted DNA from sample A, and 5.5uL of sterile dH2O. The third tube was sample B ND2 and held 12.5uL of master mix, 1uL of each ND2-238F and ND2-866R primer, 5uL of extracted DNA from sample B and 5.5uL of sterile dH2O. The forth tube was sample B ND6 and held 12.5uL of master mix, 1uL of each ND6-87F and ND6-480R primer, 5uL of extracted DNA from sample B, and 5.5uL of sterile dH2O.

Polymerase Chain Reaction (PCR):

Once the DNA samples contained the primer, polymerase chain reaction (PCR) was utilized to finish the amplification process. Using a thermal cycler the amplified mitochondrial DNA (mtDNA) was initially denatured at 94 degrees Celsius for three minutes. After initial denaturing, 35 continuous cycles of three different stages were set. The first stage was denature at 94 degrees Celsius for 30 seconds. The second stage was anneal at 54 degrees Celsius for 45 seconds. The final stage in this cycle was 72 degrees Celsius for 45 seconds. The holding temperature was kept at four degrees Celsius until they was able to be removed from the PCR machine. Once removed the samples were held at -20 degrees Celsius.

Electrophoresis:

After the PCR cycles were completed the samples went through electrophoresis in order to be sure that the DNA amplified. A Hi-Low DNA marker was loaded into the first channel (on the far left). This DNA marker provided a rough estimate of how many base pairs were in the DNA that was amplified. Each band that was created from the DNA marker after electrophoresis had a specific number of base pairs which is already known. It was previously known that *L. delicatula* ND2 mtDNA has 552 base pairs while *L. delicatula* ND6 mtDNA has 337 base pairs.

Before the samples were loaded into the electrophoresis 5uL of 6x loading dye was added to each sample in order to be able see the DNA samples move through the gel. Sample A ND2 was loaded into the channel next to the DNA marker. Next to Sample A ND2 was Sample A ND6 followed by Sample B ND2 and then Sample B ND6. After being loaded into the electrophoresis gel the top of the electrophoresis machine, which holds the negative and positive charge was placed on. The machine was then run at 130 volts for about half an hour, which allowed the amplified dyed DNA to travel half way through the gel (see figure 2.2).

The gel was taken out of the electrophoresis machine and was placed in a UV-transilluminator. The image from the UV-transilluminator can be seen in figure 2.3. The UV-transilluminator showed that only Sample B ND6 had successfully amplified (for more information refer to results section Experiment #2: DNA Sequencing figure 2.3)

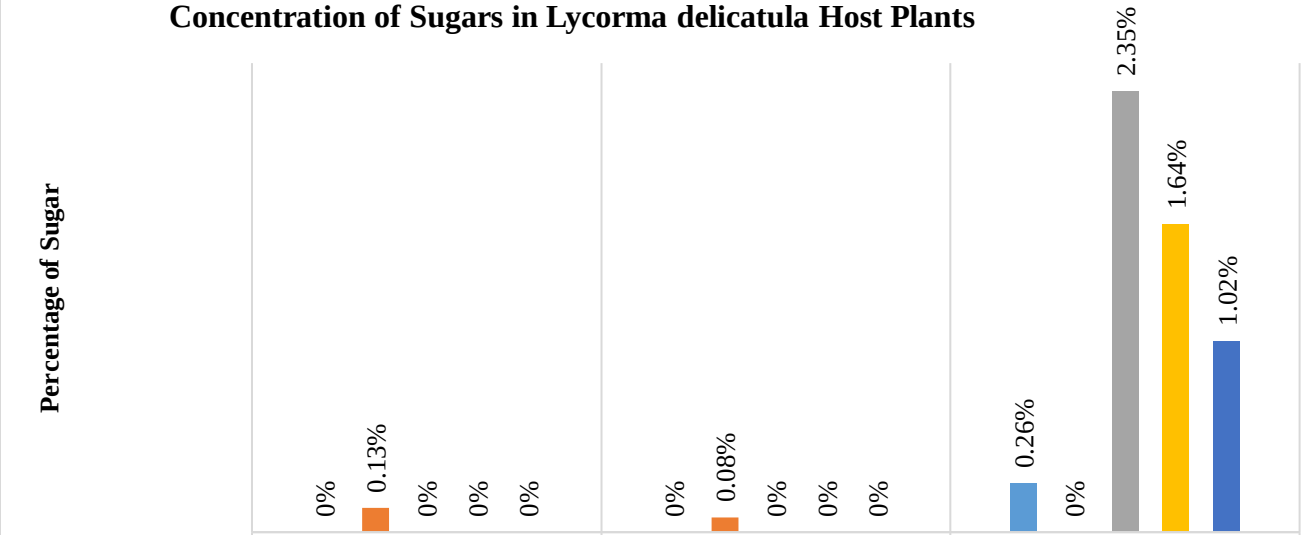
Collecting Sequencing Data

After running electrophoresis and determining that only Sample B ND6 mtDNA had successfully amplified 20uL of the sample was collected. This sample was then mailed to a Genewiz lab in New Jersey. It is at this lab that the ND6 mtDNA was sequenced. These sequenced results were used to generate charts that were then analyzed and compared to results from *L. delicatula* ND6 mtDNA in China, Japan, and Korea.

Results

Experiment #1: Concentration of monosaccharides in host plants

In figure 1.1 the HPLC results for *A. altissma*, *B. nigra*, *P. calleryana*, *A. saccharum* and *A. rubrum* are shown. The HPLC results for *A. altissma* showed 0% fructose, 0% glucose and 0.26% sucrose, while the HPLC results for *B. nigra*, showed 0.13% fructose, 0.08% glucose and 0% sucrose. *P. calleryana* contained 0% fructose, 0% glucose and 2.35% sucrose. The results from this round of HPLC showed that *A. saccharum* contained 0% fructose, 0% glucose, and 1.64% sucrose while *A. rubrum* contained 0% fructose, 0% glucose, and 1.02% sucrose.



	Fructose	Glucose	Sucrose
Tree Of Heaven	0%	0%	0.26%
River Birch	0.13%	0.08%	0%
Calary Pear	0%	0%	2.35%
Sugar Maple	0%	0%	1.64%
Red Maple	0%	0%	1.02%

Figure 1.1 (Above):

The figure shows the concentration of fructose, glucose and sucrose in Tree of Heaven, River Birch, Callery Pear, Sugar Maple and Red Maple. In this figure each tree is represented by a different color. The Tree of Heaven is represented by light blue, River Birch red, Callery Pear green, Sugar Maple purple and Red Maple turquoise.

Experiment #2: DNA Sequencing

The following figures depict the DNA sequencing process. Results from electropheresis showed that ND6 mtDNA extraction from the second sample of *L. delicatula* was successful. The results show the amplified DNA has about 330 base pairs, which is the correct amount for *L. delicatula* ND6 mtDNA. After sequencing the results stated that *L. delicatula* ND6 mtDNA in the United States is 99.7 percent similar to *L. delicatula* ND6 mtDNA in China, Japan, and Korea.

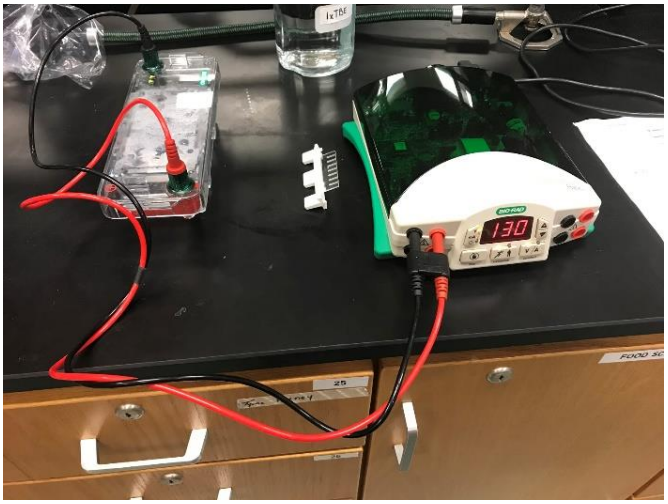


Figure 2.1 (Above):

This image shows the electrophoresis machine while it was running. Since DNA is negatively charged it is attracted to the positive charge which is coming from the red wire.

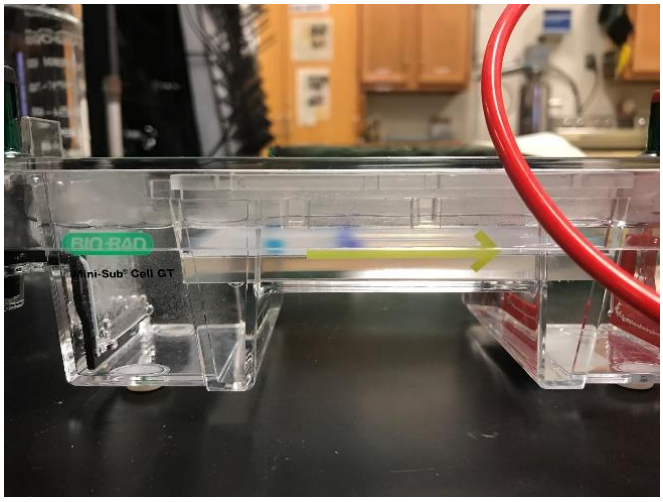


Figure 2.2 (Above):

This image shows the electrophoresis machine after a half an hour of running. The dark blue line is the amplified dyed DNA which is half way through the gel.

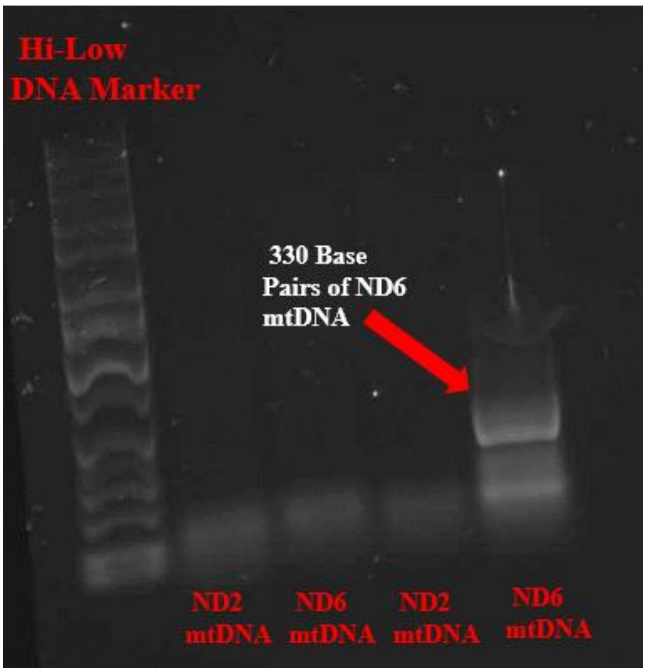


Figure 2.3 (Above):

In this figure is results from electrophoresis. This figure shows faint lines which is the amplified DNA. These lines are the result of a standard Hi-Low DNA marker, which helps determine the number of base pairs in a sample. On the far right side of the figure there is a bright stripe which is in the 330 base pair region. This stripe is from a sample of DNA which contained the ND6 primer. This stripe is followed by a fainter stripe which is the primer from that sample. The faint three lines between the ladder and the sample results are the primers from the other samples. This means that only sample B contained mtDNA, specifically ND6 mtDNA.

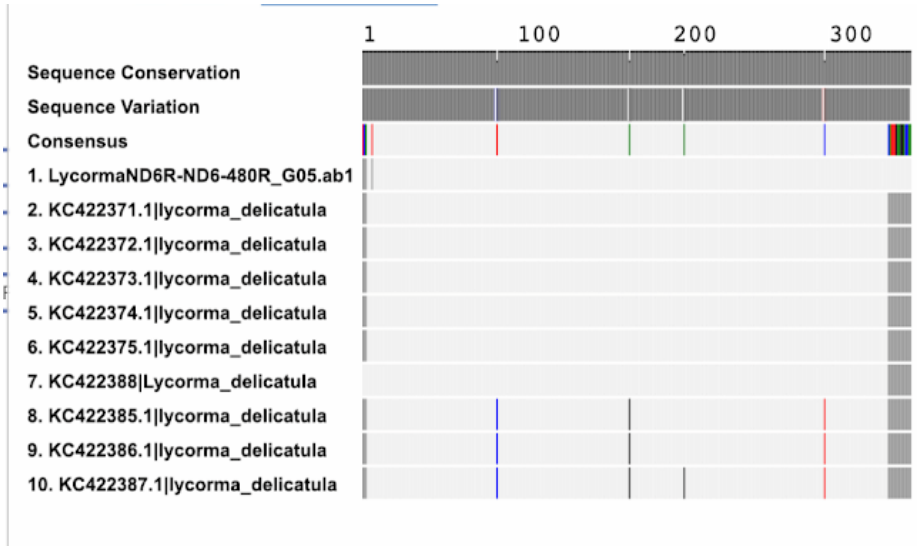


Figure 2.4 (Above):

In this figure the mtDNA of *L. delicatula* in the United States is shown as number 1. In 2-10 the mtDNA of *L. delicatula* from different countries is shown. The closer the numbers are to 1 the more similar the mtDNA is to *L. delicatula* from the United States. The consensus line is mtDNA that satisfies the mtDNA for each of the samples. The colorful vertical lines show that there is various between the different mtDNA samples.

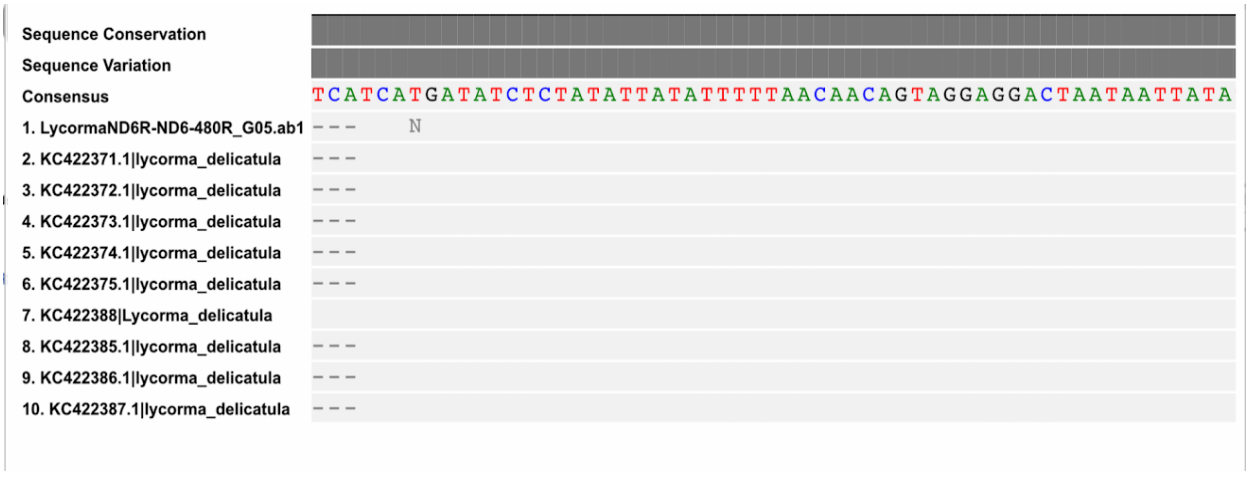


Figure 2.5 (Above):

This figure is a piece of figure as 2.4 but shows the nucleotide letters. The N in row 1 (mtDNA sequence of *L. delicatula* in the United States) is the only difference between the consensus and rows 2-7. The N simply means that the nucleotide was unable to be identified during sequencing.

	C	1	2	3	4	5	6	7	8	9	10
C	-	99.71	100.00	100.00	100.00	100.00	100.00	100.00	99.10	99.10	98.80
1	99.71	-	99.70	99.70	99.70	99.70	99.70	99.70	98.80	98.80	98.50
2	100.00	99.70	-	100.00	100.00	100.00	100.00	100.00	99.10	99.10	98.80
3	100.00	99.70	100.00	-	100.00	100.00	100.00	100.00	99.10	99.10	98.80
4	100.00	99.70	100.00	100.00	-	100.00	100.00	100.00	99.10	99.10	98.80
5	100.00	99.70	100.00	100.00	100.00	-	100.00	100.00	99.10	99.10	98.80
6	100.00	99.70	100.00	100.00	100.00	100.00	-	100.00	99.10	99.10	98.80
7	100.00	99.70	100.00	100.00	100.00	100.00	100.00	-	99.10	99.10	98.80
8	99.10	98.80	99.10	99.10	99.10	99.10	99.10	99.10	-	100.00	99.70
9	99.10	98.80	99.10	99.10	99.10	99.10	99.10	99.10	100.00	-	99.70
10	98.80	98.50	98.80	98.80	98.80	98.80	98.80	98.80	99.70	99.70	-

Figure 2.6 (Above):

In this figures the numbers (1-10) mean the same as the numbers (1-10) in figures 2.4 and 2.5. This figure shows the percentage of similarity between each of the mtDNA samples as well as the consensus mtDNA. Row 1 holds the mtDNA from *L. delicatula* in the United States which is about 99.7% the same as *L. delicatula* in Japan and Korea. Compared to *L. delicatula* in China it is only 98.8% the same.

Discussion and Conclusions

Experiment #1: Concentration of monosaccharides in host plants

The data collected from *A. altissima, B. nigra, P. calleryana, A. rubrum* and *A. saccharum* reject the hypothesis that *L. delicatula* is attracted to plants with 2% of sucrose and 0% of fructose and glucose. By analyzing the results a stronger conclusion about *L. delicatula* attraction to monosaccharides in host plants can be made. The results show that each plant that *L. delicatula* primarily feeds on contains a higher concentration of sucrose and lower concentrations of fructose, and glucose.

In a broader scope these results show that *L. delicatula* is attracted to trees because of their monosaccharide concentration. This means that *L. delicatula* decide on which trees to feed on because of the monosaccharides in a trees phloem sap. This is the very reason that some trees have a significantly larger population of *L. delicatula* than other trees such as coniferous trees. Although the sap concentration for coniferous trees is unknown it can be concluded that the sap

concentration has higher fructose and glucose levels and lower sucrose levels based on the conclusions made about *L. delicatula* feeding patterns through HPLC testing.

Due to the fact that *L. delicatula* feed on trees because of their monosaccharide concentration it can be concluded farther that *L. delicatula* do not choice to feed on plants at random. This means that certain plants that have higher concentrations of sucrose and lower concentrations of fructose and glucose are at a higher risk of being prayed on. Meaning those plants need to be better protected from *L. delicatula*. Not only can certain plants be better protected using previously created methods but these results also can lead to new methods of trapping *L. delicatula.* Trapping mechanisms that would attract *L. delicatula* would need to contain a higher concentration of sucrose and lower concentrations of fructose and glucose.

By analyzing the data that was gathered and comparing the results to prior knowledge a correlation directly to the allegory can be made. The allegory states that *L. delicatula* prefer to feed on vegetation that have smooth bark. Each vegetation that has *L. delicatula* "prime" monosaccharide concentrations (higher concentration of sucrose and lower concentration of fructose and glucose) also has smooth bark. While vegetation, such as the river birch (*B. nigra*), that have rough bark also do not have *L. delicatula "*prime" monosaccharide concentrations. Based on the correlation between the bark texture and the monosaccharide concentration a conclusion can be drawn that *L. delicatula* prefer to feed on smooth bark not only because of the bark texture but due to the monosaccharide concentration in the vegetation.

Note: *Lycorma delicatula* does not feed during the winter, when the sap was collected. Due to this fact more sap will be collected in the spring and will be tested by HPLC. This will hopefully provide the same results therefore a stronger conclusion can be made.

Experiment #2 DNA Sequencing

The collected data rejects the prior hypothesis that *L. delicatula* in the United States had an adaptation in ND6 mtDNA which lead to different feeding patterns then of *L. delicatula* in China, Japan, and Korea.

The sequenced results when compared ND6 mtDNA sequencing in Genebank and DNA Subway showed that *L. delicatula* ND6 mtDNA from the United States was on average 99.7% the same as *L. delicatula* ND6 mtDNA in South Korea, 99.7% similar to those in Japan, and 98.7% similar to the *L. delicatula* in China. The data was gathered in five different locations in South Korea, those locations and the percentage that was similar to the data gather in the United States were Seoul (99.7%), Incheon (99.7%), Suwon (99.7%), Cheonan (99.7%), and Cheongyang (99.7%). In Japan only one location gather data which was Hakusan (99.7%). In China there was three locations that data was gathered. These locations were Ningbo (98.8%), Tiantai (98.8%) and Linan (98.5%) (This data can be seen in Figure 2.6).

It cannot be fully confirmed but these slight differences in the ND6 mtDNA could be due to the one unknown in the sequence gathered in the United States. This unknown nucleotide would cause the ND6 mtDNA to not be 100% accurate to the ND6 mtDNA in China, Japan, and Korea. These results show that the difference in feeding patterns between *L. delicatula* in the

United States and *L. delicatula* in China, Japan, and Korea are not due to a difference in *L. delicatula ND6* mtDNA. Since there is 2.2 billion base pairs in *L. delicatula* DNA it is possible that a different strand of DNA has changed and that may be the reason for the different feeding patterns between countries that *L. delicatula* is native to and countries that *L. delicatula* is invasive to. This study has only found that the difference in feeding pattern did not cause a change in *L. delicatula* ND6 mtDNA.

With the ND6 mtDNA of *L. delicatula* in the United States being very similar to the ND6 mtDNA of *L. delicatula* in China, Japan, and Korea it can be supported that the difference in feeding patterns is just due to the way *L. delicatula* evolved to the environment of the United States. This does not mean that *L. delicatula* ND6 mtDNA will never adapt with *L. delicatula* feeding behavior only that ND6 mtDNA has not adapted at this time.

Experiment #1 and Experiment #2:

Based on the HPLC results the hypothesis about *L. delicatula* attraction to monosaccharide concentration is rejected. The hypothesis about the mtDNA sequencing was rejected since the mtDNA are on average 99.4% similar meaning that the ND6 mtDNA has not adapted.

This shows that *L. delicatula* have a certain and distinct feeding pattern and although this feeding pattern varies from *L. delicatula* feeding pattern in China, Korea, and Japan it is not caused by an adaption in the ND6 mtDNA. This means that either there is an adaptation in a different part in the DNA or that *L. delicatula* naturally adapted to the United States environment in order to get the nutrients *L. delicatula* requires to survive.

In order to make a stronger conclusion more sap will be extracted from other host plants during the spring and summer months when *L. delicatula* is feeding on this vegetation. This will be able to decipher if *L. delicatula* is truly attracted to vegetation with a certain monosaccharide concentration or if the vegetation that was tested was just similar because vegetation is just starting to make phloem sap after their dormancy of the winter. Another point that will be tested is *L. delicatula* attraction to physical appearance of a plant. In order to do this bark samples will be collected for host plants and the preference of *L. delicatula* will be monitored. By comparing the monosaccharide attraction and the bark attraction a stronger conclusion about *L. delicatula* feeding pattern can be made.

Another way to make a stronger conclusion is by extracting a testing more DNA. This will allow more than a small fraction of *L. delicatula* DNA to be compared to the DNA of *L. delicatula* in China, Korea, and Japan. By having more DNA to analyze there is a greater possibility to find variance in the DNA and therefore make a stronger conclusion.

Further research is being conducted to find a biological control method. The research conducted in this experiment is beneficial to aid in finding patterns that can be used to find an effective control method. This research helped to determine what control methods would work on *L. delicatula.* Results showed that *L. delicatula* is rapidly changing and there is a wide but specific feeding pattern. By using this knowledge gained a proper and effective control method

has been hypothesized. This hypothesized control method is effective in *Aedes aegypti* populations and is known as *Wolbachia*. Studies have shown that this method causes a 95.55 percent reduction in treated populations and a 97.1 percent reduction in egg hatching rates. In order for *Wolbachia* to work it is necessary for males to be infected and the females to be uninfected.

Which is why future research that is being conducted starts with testing wild *L. delicatula* for *Wolbachia* through DNA tests. If *Wolbachia* has not infected wild *L. delicatula* populations, further tests will be run in order to find the effectiveness of *Wolbachia* on *L. delicatula*. When the effectiveness of *Wolbachia* is determined male *L. delicatula* will be infected and released throughout wild populations. Once infected males mate with uninfected females it will result in embryonic lethality, meaning that the eggs will be unable to hatch. This is known as the incompatible insect technique and has proven to have a healthier impact on treated species than the sterile insect technique. This future direction for this project is promising and may allow the economy and environment to recover from this pest.

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