

Drivers of herbivore damage on tree seedlings at Powdermill Nature Reserve, PA,  
USA

Undergraduate Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation “with Research  
Distinction in Biology” in the undergraduate colleges of The Ohio State University

by

Kaiyang Xu

The Ohio State University

April 2014

Project Advisor: Dr. Liza Comita, Department of Evolution, Ecology, and  
Organismal Biology

## Abstract

Herbivores can cause major damage and affect survival of tree seedlings. The factors that cause variation in herbivore damage rates among individual plants are not well known. Most prior studies have focused on single factors that affect herbivore damage rates on plants. The purpose of this study is to test which variables affect herbivore damage rates on tree seedlings. Specifically, I was interested in testing whether abiotic variables, such as elevation and moisture levels (as measured by distance from stream), and biotic variables (such as the density of conspecifics and plant species diversity), influence herbivore damage rates. I took data from thirty-seven 20×20 meter plots at Powdermill Nature Reserve (PNR) in southwestern Pennsylvania (40°09'S, 79°16'W). I took photos of tree seedling leaves and quantified percent herbivore damage in the photos using Image J software for 296 tree seedlings of 22 species. I ran Spearman rank correlations and a generalized linear regression model to test for effects of distance from stream, elevation, seedling diversity and conspecific neighbor density on percent herbivore damage. I found large variation in herbivore damage rates among species (0.373-10.8%) and among individuals (0-40.6%). The herbivore damage rate was particularly high for American basswood (*Tilia americana*) seedlings. From the multiple linear regression model, I found the biotic factors had significant, but minimal effects on herbivore damage rate, while the abiotic variables had no significant effects on herbivore damage rate. In related biotic factors, height had a positive relationship with herbivore damage and species richness had a negative relationship with herbivore damage. My results suggest that herbivore damage is not influenced by abiotic factors such as elevation and moisture level. It shows that biotic factors can affect the relationship between herbivores and tree seedlings, although the effects were small. This study helps eliminate some of the potential abiotic factors that could affect herbivore damage rates, and also indicates

that further studies are needed to determine what other abiotic elements can drive variation in herbivore damage rates.

## **Introduction**

Plants are important components of communities, ecosystems and food webs. (Maron and Crone 2006; Long et al. 2003). In the natural world, plants face competition and attack from natural enemies, such as herbivorous insects. Herbivores have often been viewed in the plant community system as a type of disturbance (Grime 1979, Tilman 1982), and they can influence vegetation by directly consuming plants tissues and also by indirectly affecting nutrient cycling and soil disturbance (Crawley 1983). Most herbivores primarily consume the leaves of plants, rather than other tissues of plants such as stems and roots (Cain et al 2011). They especially prefer the young leaves, since they have weak antiherbivore defenses (Coley 1980, 1983, Crawley 1983, Raupp and Denno 1983, Lowman 1985). Herbivores can change the nutrient requirements of plants by consuming leaf tissues, which will reduce photosynthesis, then change the resource availability of other plants, which could affect the results of competitive interactions (Louda et al 1990; Huntly 1991). Herbivores can cause significant plant mortality by attacking seed and seedlings (Harper 1977; Meiners and Martinkovic 2002; Crawley 1989, 1992, 1997; Louda 1989; Gange 1990; Marquis 1992; Strauss and Zangrel 2002). By influencing the survival of plants, herbivores can impact plant regeneration and affect plant species coexistence (Hulme 1996). Another ecological consequence caused by herbivores is that they reduce the competitive ability of plant species, which then can lead to an increase in plant diversity since the lower competitive ability will have more resource ability for heterospecific species (Louda et al. 1990; Pacala and Crawley 1992). As a plant species becomes rare, herbivores will attack plants of the species less and this mechanism will lead the increase of plant diversity (Pacala and Crawley 1992; Janzen 1970; Connell 1971).

Abiotic and biotic factors are not totally independent variables; for example, solar energy varies with latitude, which affects species richness (Rohde 1992). In the complex ecological environment, herbivore damage on plants can be impacted by both abiotic and biotic factors, but it is still unclear what factors (biotic and abiotic) influence herbivore damage rates. Studies of interactions between herbivores and plants have a long history in ecology, and ecologists have obtained many valuable consequences of herbivore-plant interactions such as how it influences species composition, local ecosystem and food webs. Additionally, previous studies have generally focused on either abiotic or biotic factors in isolation, but not both in tandem. Abiotic factors include all nonliving chemical or physical factors in the environment such as temperature and moisture level. At low latitudes, where it is warmer, plants suffer higher rates of herbivore attack (Salazar and Marquis 2012, Donzhansky 1950, MacArthur 1972, Pennings and Silliman 2005). Temperature affects insect activity, since insects are cold-blooded organisms whose body temperature will change with the temperature of the environment (Mellanby 1939). Elevation-related temperature can influence herbivore-feeding activities and higher elevation plant species may suffer higher herbivore damage than lower elevation plant species (Koptur 1985). Increasing soil moisture levels will lead to an increase in herbivore damage rate (Hagstrum and Milliken 1988, Louda et al 1986) by reducing plants' antiherbivore defense (Katijua and Ward 2006). Air moisture level can influence herbivores metabolism rate, so they prefer to stay at an optimal air moisture level place to maintain optimal metabolism rate (Tanaka 2000).

Biotic factors are factors created by a living thing or any living component within an environment in which the action of the organism affects the life of another organism. Many previous studies reported biotic factors affected the herbivore damage rate of plants, such as

natural enemies of plants (Elton 1973). According to the Janzen-Connell hypothesis, plant individuals are expected to suffer a higher rate of herbivore damage at higher conspecific density since it has higher food resource availability (Janzen 1970 and Connell 1971). Furthermore, many studies focused on the herbivores activities on particular plants species, but herbivores attack almost every plant in the natural world. However, herbivores are not randomly picking their “host” plants (target). Some insects have specific host preferences. Therefore, different species suffered significant differences in herbivore damage due to variability of antiherbivore defense intensity (Katijua and Ward 2006). The antiherbivore defense mechanism can be caused by different life history and growth forms of plant species (Cruz and Dirzo 1987). Many herbivores specialized on only one or a few plants (Cain et al. 2011). This narrow diet range caused the variation of intensity of herbivore damage in plants species at certain area, since herbivores have preference on food which could cause some plants species suffer higher herbivores attack.

While many biotic and abiotic factors were singly studied, few studies consider the abiotic and biotic factors together that have impact on the herbivore-plant interactions. Thus, there is not a comprehensive study to explain abiotic and biotic drivers of herbivores attack; in particular, the influence of elevation on herbivory remains largely unknown. Furthermore, most of the previous studies were conducted in tropical forests due to its high species richness. In contrast, few studies have looked at the factors influencing herbivore damage in temperate forest. This is important because it is possible that temperate forests will have different relationships of herbivore damage rates of plants than in tropical forests. Herbivore damage will have significant effects on survival of seedlings, since the young individuals have lower resistance to herbivore damage (Aide 1993). I also wanted to discover the relationship between plants seedling size and

herbivore damage percentage, since generally larger plant individuals would be easily found by herbivores and has more leaves on it which means it has more food resource available for herbivores.

In this study, I explored both abiotic and biotic drivers of herbivore damage on tree seedlings at Powdermill Nature Reserve, a temperate forest in southwestern Pennsylvania. Since most of the herbivores feed on leaves (Cain et al. 2011), I measured the leaf damage on tree seedlings. I tested whether the percentage of leaf area lost to herbivores was related to biotic factors—specifically, species richness, conspecific neighbor density and height of tree seedlings. I also explored this relationship with abiotic factors— specifically, distance from stream and elevation. I generated multiple hypotheses: (1) How does herbivore damage rates vary across species within the community? (2) Herbivore damage rate increases with conspecific seedling density; (3) As elevation increases, herbivore damage rate decreases; (4) At a certain range where the herbivores have optimal metabolism rate of distance from the stream will occur the highest herbivore damage; (5) Larger tree seedlings will have higher herbivore damage and (6) Higher species richness will result in lower herbivore damage.

## **Methods**

### *Study site*

The study was carried out between July and August 2013 at Powdermill Nature Reserve (PNR), which was established in 1956 by the Carnegie Museum of Natural History. It is an approximately 900 ha temperate deciduous forest, located in Westmoreland County, Pennsylvania (40°09'S, 79°16'W), between the westernmost ridges of the Allegheny Mountains in southwestern Pennsylvania (Mulvihill and Chandler, 1990). *Acer* spp., *Liriodendron*

*tulipifera*, *Quercus* spp. and *Carya* spp. dominate the PNR forest. My plots at PNR has elevation ranging from 394-474.5 meters and 1100 mm of annual precipitation (NCDC 2012).

Temperatures in the area range from an average low of -20°C in January to an average high of 33°C in July (NCDC 2013, based on years 2001 - 2009). Researchers at PNR conducted a vegetation survey from 2006 to 2008 to generate vegetation maps.. They divided the PNR region into 647 plots to record the vegetation composition and marked the centers of those plots with steel rebar (Fig.1).

### *Study Species*

For my study of herbivory, my database contained 22 different species—*Acer pensylvanicum*, *Acer rubrum*, *Acer saccharum*, *Amelanchier laevis*, *Betula lenta*, *Carpinus caroliniana*, *Carya cordiformes*, *Carya ovata*, *Cornus florida*, *Crataegus* spp., *Fagus grandifolia*, *Fraxinus americana*, *Liriodendron tulipifera*, *Magnolia acuminata*, *Nyssa sylvatica*, *Ostrya virginiana*, *Prunus serotina*, *Quercus alba*, *Quercus rubra*, *Sassafras albidum*, *Tilia americana*, and *Viburnum dentatum*.

### *Data collection*

To monitor tree seedlings, I randomly picked sampling plots from the PNR vegetation survey plots. I only selected plots that had been forested since 1939, based on historical aerial imagery. The plots were centered at the rebar posts, which were set up for the original vegetation survey. Around the centers, I established two 20-meter transects oriented north to south and west to east, and then used the four points (N, S, W, E) to set four corner posts. I marked plots with nine polyvinyl chloride (PVC) pipes hammered into the ground at each cross point (Fig. 2). I



established a total of thirty-seven 20×20-meter plots at PNR. As Fig. 2 demonstrates, each 20×20-meter plot contained four 1×10-meter subplots along the north-south axis. Subplots near the centers were placed two meters away from the center, and plots further from center were placed 4.5 meters away from the center. I separated each small plot into ten adjacent 1m<sup>2</sup> quadrants. This resulted in a total of 40 1×1 m quadrats per 20×20-m plot. I considered all individuals with < 1cm diameter at breast height (DBH) and ≥ 7 cm tall as tree seedlings. Tree seedlings were identify to species and measured using a straightedge.

Of the above-defined seedlings, I selected tree seedlings with > 20 cm height as focal individuals for the herbivory study. I photographed the leaves of individual tree seedlings > 20 cm in height. I sampled a maximum of five individuals for every 10×1m subplot and took enough photos to capture all the leaves on an individual until a maximum of 5 photos for each individual. However, many plots contained fewer than five individuals > 20 cm tall. I prepared a white board as a background for the photos. After I flattened the leaves on the white board, I used a laminated transparency as a cover board, then used an opaque parasol to minimize the reflection from the sun and adjusted camera angles to avoid flashlight spots on leaves. A 7×7cm paper note was labeled on the white board to record the position, species, photo ID and served as a scale bar in the following analysis of each picture.

In total, I recorded 298 individuals, 4459 leaves (~121 per plot), and 683 photos. Each photograph was analyzed with the software program Image J (National Institute of Health, 2013) to calculate total leaf area and damaged leaf area (cm<sup>2</sup>). Leaves were manually adjusted to fill in missing leaf portions and paint herbivore damaged area white by Image J software. Brush, threshold, and binary functions were then used to convert the photos into black and white for separating damaged (white) and undamaged (black) areas. All photographs were standardized

using the paper note for a scalebar. Image J calculated leaf damage area (cm<sup>2</sup>) and total leaf area (cm<sup>2</sup>) of each processed photo. Leaf damage area was divided by total leaf area for all leaves of an individual combined to calculate percentage (%) of leaf area lost for each seedling.

### *Environmental Data*

Distance from stream and elevation were components of potential abiotic factors measured. I obtained those data from the PNR GIS office. Species richness, conspecific seedling density, and heterospecific seedling density within each 20×20 m plots were calculated from the seedling census data as potential biotic factors.

### *Data analysis*

To explore the relationship between herbivore damage (%) and abiotic and biotic factors individually, I did a Spearman's rank correlation test. I then used a multiple linear regression model to find the relationship of variables (biotic and abiotic) and herbivore damage. Because the percent herbivore damage data were not normally distributed, for the linear regression model I transformed the herbivore damage percentage to a log-scale and added 0.0001 to all herbivore damage values to avoid the error caused by individuals with zero herbivore damage. I also ran the ANOVA test to find the relationship between the herbivore damage (%) among different tree seedling species in log scale. To determine herbivore interactions with different species of tree seedlings, I calculated the herbivore damage rates of each species (22 species). Differences in herbivore damage rates among species were compared using ANOVA. I generated a bar plot to compare mean herbivore damage rates among the 22 tree seedling species in my sample. Herbivores are often specialized on certain plants (Begon et al. 2005) and different herbivore

species may respond differently to the abiotic and biotic factors tests. Therefore, I did separate linear regression models for the six most abundant species in my sample—*Acer pensylvanicum*, *Nyssa sylvatica*, *Fraxinus americana*, *Prunus serotina*, *Quercus rubra* and *Acer rubrum*. The data were analyzed using the R statistical programming environment (R Core Development Team, 2013).

## Results

In total, data on herbivore damage were collected for 298 seedlings of 22 species. Herbivore damage percentage varied with species from 0% to 10.8%, and average percentage herbivore damage across all species was 2.5% (Table 2 and Fig. 3). Most species had herbivore damage percentage less than 10% except for *Tilia americana* (average percentage damage 10.8%, Table 2 and Fig. 3). Figure 5 showed the trend of herbivore damage percentage which has a higher frequency in the middle range of herbivore damage and extremely high at 0% in all species. A clear normal distribution trend is shown in the histogram of log scale of herbivore damage percentage (Fig.4).

When testing for a relationship between individual variables and herbivore damage using Spearman rank correlations, I found that only seedling height and species richness were significantly related to percent leaf area lost to herbivory (Linear Regression Model,  $p$ -value $<0.05$ , Fig 5, Table 4). Similar results were obtained using a multiple regression model that included all independent variables (Table 1). As hypothesized, herbivore damage percentage increased with tree seedling height ( $p<0.05$ , Table 1 and Table 4, Fig. 5). Herbivore damage percentage increased as species richness decreased ( $p<0.05$ , Table.1 and Table.4, Fig. 5). Conspecific seedling density, elevation, distance from the stream and heterospecific seedling

density did not affect the herbivore damage percentage (all  $p > 0.05$ , Table.1, Table.4 and Table.5, Fig. 5). From Table 5, it illustrated that different tree seedling species have no significant difference in herbivore damage percentage ( $p > 0.05$ ).

In the six most abundant species (i.e., *Acer pensylvanicum*, *Nyssa sylvatica*, *Fraxinus smreicana*, *Prunus serotina*, *Quercus rubra* and *Acer rubrum*), there were no significant relationships between herbivore damage and the abiotic and biotic variables (all  $P > 0.05$ , Table.3).

## **Discussion**

### *Summary of Results*

My results show that biotic factors influence herbivore damage on tree seedlings at PNR, and that abiotic factors have no relationship with herbivore-plant interactions at PNR. Specifically, higher species diversity leads to a lower percentage of damaged tree seedlings, and a higher percentage of tree seedlings have herbivore damage at the PNR temperate forest. However, both of these effects were quite small. Other factors in my hypotheses – conspecific species density, distance from the stream and elevation – have no relationship to herbivore damage. Additionally, I found no relationship among the herbivore damage among different species of tree seedlings.

### *Variation among different Species*

Different tree seedling species have different antiherbivore defense types, so herbivore damage on individuals partially depends on species (Coley 1988). Explanations for these varying defenses were proposed by many studies. The evolutionary responses to resource limits in habitats might cause this variation among species (Janzen 1974; Grime 1977, 1979; McKey

1979). The main anti-herbivore defense chemicals are tannins and lignins (Coley 1988). If tree seedlings invest too many resources producing tannins and lignins, it will slow the growth rate of individuals (Coley, 1986). Another potential reason that might cause this not significant result is that this is an evolutionary strategic difference among species, which means that there will be larger differences in the amounts of antiherbivore chemicals and growth rates between older individual tree seedlings. It means that differences of herbivore damage of older tree seedlings will larger than differences of herbivore damage of young tree seedlings. Therefore, my sampled tree seedling species might not have many differences in antiherbivore defenses and herbivore damage, since they are young seedlings.

#### *Biotic Factors – Conspecific Species Density, Species Richness, and Height of Individuals*

Several previous studies addressed how herbivores influence tree seedlings' conspecific species density and species richness. Janzen (1970) and Connell (1971) hypothesized that enemies that eat tree seeds and seedlings will concentrate their foraging efforts on areas of high density or close distance to parent tree; individuals surrounded by other species are more likely to survive than those in groups of high conspecific density, and after a long time this creates an even distribution of individuals surrounded by dissimilar individuals. Another model called the herd immunity model predicts that the greatest number of seedlings will survive in areas with the most other species. Some herbivores attack several similar species of tree seedlings, but they may not find their desired species among inedible species (Mayers and Pimm, 1997; Peters et al, 2003). The resource concentration hypothesis explains that herbivore damage will be greatest where resources are most concentrated. This would be in monospecific (low diversity) stands. Stands of high diversity in contrast would have less concentrated

resources, and therefore buffer against herbivore attack (Root 1970?). All these three models support my results, which show the negative relationship between species richness and herbivore damage. However, surprisingly, the conspecific species density had no relationship to herbivore damage. Based on the Janzen-Connell hypothesis, the herd immunity model and the resource concentration hypothesis, if herbivore damage related to species richness, conspecific species density should have a positive relationship with herbivore damage. My conspecific species density data only contained data for tree seedling individuals; data for adult trees and seed individuals was omitted. Therefore, the effects of adult densities was not addressed.

As I hypothesized, the heights of the tree seedlings has a positive relationship with herbivore damage. Larger tree seedling individuals are more easily found by herbivores, and herbivores choose larger individuals to obtain more food, so larger tree seedlings will attract more herbivores. Similar to the resource concentration hypothesis, I expect that the larger tree seedlings will have more and larger leaves, so herbivores will prefer to attack large tree seedling individuals.

#### *Abiotic Factors – Distance from the Stream and Elevation*

Temperature is an important element in the natural world and it can influence both herbivores and plants. Low temperatures reduce insect herbivore activity, so colder areas will likely have less herbivore damage to plants (Mellanby, 1939). Temperature decreases as elevation increases, so plants at high elevation will suffer fewer herbivore attacks. Another important pattern is that species richness decreases with increasing elevation (Brown, 1988; Rahbek, 1995; Brown & Lomolino, 1998). As I mentioned before, herbivore damage will be lower in high species richness areas (Mayer and Pimm, 1997). In contrast, my small range of elevation data found

nothing on this relationship between elevation and herbivore damage. Distance from the stream will affect both the air moisture level and the soil moisture level; I assumed that moisture levels increase as the distance to the stream decreases. In nature, both herbivores' metabolic rates (Tanaka, 2000) and plant antiherbivore chemical secretions (Katijua and Ward, 2006) are affected by moisture levels. Therefore, both elevation and distance from the stream could influence herbivore-plant interactions. However, none of my results showed that either the elevation or the distance from the stream have any influence on herbivore damage. My tree seedlings were sampled at elevation ranges between 394 and 474.5 meters. This small range has insignificant changes in temperature and temperature-related species richness. It might be due to the small range, so my results showed no significant relationship between herbivore damage and elevation. The distance from the stream in my data is measured from edge of plots to the stream, not from the tree seedling individuals to the stream, so it is not easy to estimate how this variable affected herbivore damage. Additionally, in my data I used the distance from the stream to predict the moisture level, but distance from the stream might not have the strong relationship to air and soil moisture levels that I predicted.

#### *Omitted Variables in Multiple Linear Regression Model*

In my multiple linear regression model, I tested five different variables (two abiotic factors and three biotic factors), but they are not all the variables that could influence the herbivore damage percentage. In the natural world, some environmental conditions are correlated: elevation affects temperature and moisture levels; precipitation affects moisture levels and sunlight; sunlight affects temperature; wind speed affects temperature; atmospheric pressure affects wind speed; and elevation influences atmospheric pressure. In my study, I tested several factors that influence

the herbivore-plant relationship, but many other variables are omitted that were addressed in previous studies. These include intensity of sunlight (Salgado-Laurte and Gianoli, 2010); soil nitrogen level (Ritchie et al, 1998); and other related factors. All of the omitted variables correlate to my tested variables. Hence, omitted variables may affect the accuracy of my statistical results.

### *Future Research*

In my study, most of the tested variables have no relationship to herbivore damage, but other previous studies showed a relationship among them. My methodology needs to be improved in terms of sample collection. Metcalfe et al (2013) provided a better methodology for the long-term; they marked the leaves and took photos of individual leaves several times every 2-3 months for two years, so that they could record the damage caused by large herbivores and track the changes in the plants over the long term. The best part of this method is they can study both small herbivore and large herbivore species' consumption of tree seedling leaves.

To develop my study, I would like to set up more plots, collect more tree seedling individuals and obtain more potentially related variables, such as aspect, sunlight and distances from adult trees. As I mentioned before, a small sample size and a small range in some variables might influence my results. I didn't choose species of tree seedlings as one of my independent variables in my multiple linear regression model, since I only have one or a few individuals for some species such as *Carya cordiformes* and *Viburnum dentatum*. Hence, expanding the dataset to include more individuals from each species would be the best way to improve my study. Next year, I can get the mortality data on sampled tree seedlings, which will provide direction for my future study. I can use mortality data to explore how herbivore damage affects the survivability



of tree seedlings at PNR. Furthermore, I can discover how herbivore damage influences the survivability of different tree seedling species, and which species can tolerate the highest herbivore damage percentage at PNR. Lastly, I look forward to seeing how herbivore damage affects species richness and if the areas of high herbivore damage will have an increasing species richness rate, as the Janzen-Connell hypothesis predicted.

## **Conclusion**

I found evidence that biotic variables significantly affect herbivore damage on tree seedlings. Larger seedlings had higher herbivore damage, and tree seedlings living in areas with higher species richness had lower herbivore damage. No evidence showed that the abiotic factors tested have significant influences on herbivore damage of tree seedlings. These results suggest a significant role of biotic factors effect on herbivores and tree seedlings.

## **Acknowledgement**

This study was conducted at Powdermill Nature Reserve and was supported by researchers there. I would like to thank to John Wenzel, James Whitacre at Powdermill Reserve and Liang Song from Ohio State Univerisity. I thank Dr. David Stetson and Dr. Alison Snow from Department of Evolution, Ecology and Organismal Biology at Ohio State University for providing valuable advice. I particularly thank Stephen Murphy for helping with collected data and provide valuable comments on writing and statistic analysis.

## Literature Cited

- Begon, M. C. R Townsend, and J. L. Harper. 2006. Ecology: From Individuals to Ecosystems 4th edition. Blackwell Publishing Ltd. Oxford. pp 267-274.
- Brown, J. H. 1988. Species diversity (ed. Myers, A. A and Giller, P.). Analytical biogeography - an integrated approach to the study of animal and plant distribution Chapman and Hall, New York, pp 57-89.
- Brown, J. H. and Lomolino, M. V. 1998. Biogeography (2nd edition). Sinauer Press, Sunderland, MA.
- Cain, M. L., W. D. Bowman, and S.D. Hacker. 2011. Ecology 2nd edition. Sinauer Associates, Inc. Sunderland. pp 262-281
- Chandler, C. R. and R. S. Mulvihill. 1990. Wing-shape variation and differential timing of migration in dark-eyed juncos. *The Condor* 91: pp. 54-61.
- Coley, P. D. 1986. Costs and benefits of defense by tannins in a neotropical tree. *Oecologia* 70: pp.238-241.
- Coley, P. D., 1988. Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia* 74:pp. 531-536.
- Coley, P.D. 1980. Effects of leaf age and plant life-history patterns on herbivory. *Nature* 284: 545-546.
- Coley, P.D. 1983. Herbivory and defensive characteristics of trees species in a lowland tropical forest. *Ecology Monographs* 53:209-233
- Coley, P. D. 1983. Intraspecific variation in herbivory on two tropical tree species. *Ecology* 64:426-433

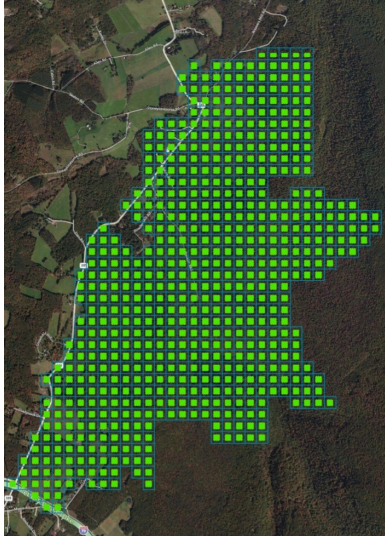
- Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. *Dynamics of Populations* (ed. Den Boer, P.J and G. Gradwell), pp. 298-312. New York, USA. Wageningen Center for Agricultural Publishing and Documentation.
- Crawley, M. J. 1983. *Herbivory: The Dynamics of Animal-Plant Interactions*. Blackwell Scientific Publications, Oxford.
- Crawley, M. J. 1989. Insect herbivores and plant population dynamics. *Annual Review of Entomology*. 34, 531-564.
- Crawley, M. J. 1992. Seed Predators and Plant Population Dynamics. *Seeds. The Ecology of Regeneration in Plant Communities* (ed. M.Fenner), pp. 157-191. CAB International, Wallingford.
- Crawley, M. J. 1997. Plant-herbivore dynamics. In *Plant ecology* (ed. M.J.Crawley). pp. 157-192. Wallingford, UK: CAB International.
- Cruz, M. D. L. and R. Dirzo. 1987. A survey of the standing levels of herbivory in seedling from a Mexican rain forest. *Biotropica* 19(2): 98-106.
- Dobzhansky, T. 1950. Evolution in the tropics. *American Scientist*, 38, 209-221.
- Elton C.S. 1973. The structure of invertebrate populations inside neotropical rain forest. *Journal of Animal Ecology*: 42:pp. 55-104.
- Gange, A.C. 1990. Effects of insect herbivory on herbaceous plants. In *Pests, pathogens and plant communities* (ed. J.J. Burdon and S.R. Leather), pp. 49-62. Oxford, UK: Blackwell Science.
- Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111: pp. 1169-1194.

- Grime, J. P. 1979. Plant Strategies and Vegetation Processes. Wiley & Sons Ltd, Chichester.
- Hagstrum, D. W. and G. A. Milliken. 1988. Quantitative analysis of temperature, moisture, and diet factors affecting insect development. *Annals of the Entomological Society of America* :81(4): pp. 539-546.
- Harper, J. L. (1977) Population Biology of Plants. Academic Press, London.
- Hulme, P. E. 1996. Herbivory, plant regeneration, and species coexistence. *Journal of Ecology* 84, 609-615.
- Huntly, N. 1991. Herbivores and the dynamics of communities and ecosystems. *Annual Review of Ecology. Syst.* 22, 47-503.
- Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forest. *American Naturalist* 104: pp. 501-529.
- Janzen, D. H. 1974. Tropical blackwater rivers, animals and mast fruiting by the Dipterocarpaceae. *Biotropica* 6: pp. 69-103.
- Katjiua, M. L. J. and D. Ward. 2006. Resistance and Tolerance of *Teriminalia sericea* Trees to Simulated Herbivore Damage Under Different Soil Nutrient and Moisture Conditions. *J Chem Ecol* 32:1431-1443.
- Long, Z. T., C.L. Mohler, and W.P. Carson. 2003. Extending the resource concentration hypothesis to plant communities: effects of litter and herbivores. *Ecology* 84(3): pp. 652-665.
- Louda, S.M. 1986. Insect herbivory in response to root-cutting and flooding stress on native crucifer under field conditions. *Acta Oecologica.* 7(1): pp. 37-53.

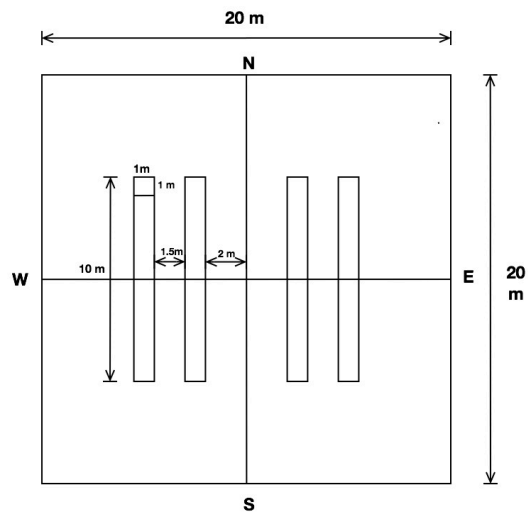
- Louda, S.M. 1989. Predation in the dynamics of seed regeneration. In Ecology of soil seed banks (ed. M.A. Leck, V.T. Parker and R.L. Simpson), pp.25-51. San Diego, CA: Academic Press.
- Louda, S.M., K.H. Keeler, and R.D. Holt. 1990 Herbivore influences on plant performance and competitive interaction. Perspectives on Plant Competition (eds J. B. Grace and D. Tilman), pp. 454-474. San Diego, Academic Press.
- Lowman, M.D. 1985. Temporal and spatial variability in insect grazing of the canopies of five Australian rainforest tree species. Australian Journal of Ecology 10:7-24.
- MacArthur, R.H. 1972. Geographical Ecology: Patterns in the Distribution of Species, Princeton University Press, Princeton, NJ, USA.
- Maron, J.L and E. Crone. 2006. Herbivory: effects on plant abundance, distribution and population growth. Proceedings of The Royal Society B 273: 2575-2584.
- Mayer, A. L. and S. L. Pimm. 1997. Tropical rainforests: diversity begets diversity. Current Biology 7: pp. R430-R432.
- McKey, D. B. 1979. The distribution of secondary compounds within plants. In: Rosenthal GA, (ed: Janzen, D. H.) Herbivores: their interaction with secondary plant metabolites. Academic Press, New York, pp. 55-133.
- Meiners, S.J. and M.J Martinkovic. 2002. Survival of and herbivore damage to a cohort of *Quercus rubra* planted across a forest-old-field edge. The American Midland Naturalist 147:247–255.
- Mellanby, K. 1939. The physiology and activity of the bed-bug (*Cimex lectularius* L.) in a natural infestation. Parasitology, Vol 31, issue 2: pp 200-211.

- Metcalf, D. B., G. P. Asner, R. E. Martin, J. E. Silva Espejo, W. H. Huasco, F. F. F. Amezcua, L. Carranza-Jimenez, D. F. G. Cabrera, L. D. Baca, F. Sinca, L. P. H. Quispe, I. A. Taype, L. E. Mora, A. R. Davila, M. M. Solorzano, B. L. P. Vilca, J. M. L. Roman, P. C. G. Bustios, N. S. Revilla, R. Tupayachi, C. A. J. Girardin, C. E. Doughty, and Y. Malhi. 2013. Herbivory makes major contributions to ecosystem carbon and nutrient cycling in tropical forests. *Ecology Letters*: doi: 10.1111/ele.12233.
- Pacala, S.W. and Crawley, M.J. 1992. Herbivores and plant diversity. *American Naturalist*, 140, 243-260.
- Pennings, S.C. and Silliman, B.R. 2005 Linking biogeography and community ecology: Latitude variation in plant–herbivore interaction strength. *Ecology*, 86: pp. 2310-2319.
- Peters, H. A. 2003. Neighbor-regulated mortality: the influence of positive and negative density dependence on tree populations in species-rich tropical forests. *Ecology Letter* 6: 757-765.
- Raupp, M.J. and R.F. Denno. 1983. Variable plants and herbivores in natural and managed systems. Academic Press. New York. USA. pp: 91-124
- Rehbeck, C. 1995. The elevational gradient of species richness: a uniform pattern? *Ecography*: 18(2): pp.200-205.
- Richie, M. E., D. Tilman, and J. M. H. Knops. 1998. Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology* 79(1):pp. 165-177.
- Robert J. Marquis. 1992. A Bite is a Bite is a Bite? Constraints on Response to Folivory in *Piper Arieianum* (Piperaceae). *Ecology*: 73: pp. 143–152.
- Rohde, K. 1992. Latitudinal gradients in species diversity: the search for the primary cause. *Oikos* 65: 514-527.

- Salazar, D. and R.J. Marquis. 2012. Herbivore pressure increases toward the equator. *Proceedings of the National Academy of Sciences* Vol. 109. No. 31:pp. 12616-12620.
- Salgado-Luate, C. and E. Gianoli. 2010. Herbivory on temperate rainforest seedlings in sun and shade: resistance, tolerance and habitat distribution. *PlosOne* (5)7: e11460.
- Strauss, S.Y. and A.R. Zangrel. 2002. Plant-insect interactions in terrestrial ecosystems. In *Plant-animal interactions. An evolutionary approach* (ed. C.M. Herrera and O. Pellmyr), pp. 77-106. Oxford, UK. Blackwell Science.
- Tilman, D. 1982. *Resource Competition and Community Structure*. Princeton University Press, Princeton, NJ.
- Willis, C., R. Condit, R.B. Foster and S.P. Hubbell. 1997. Strong density- and diversity- related effects help to maintain tree species diversity in a neotropical forest. *Proceedings of the National Academy of Sciences* 94: pp. 1252-1257.
- Wills, C and D.R. Green. 1994. A genetic herd-immunity model for the maintenances of MHC polymorphism. *Immunol Recolution* 143: 263-292.



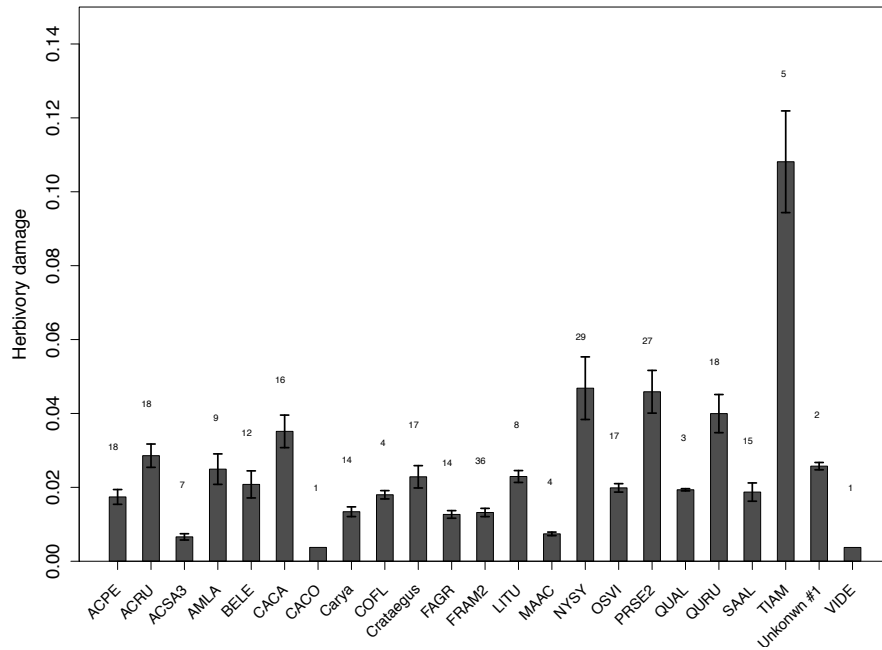
**Fig.1** Powdermill Nature Reserve map and vegetation survey plots distribution, PA.



**Fig.2** Plot setting demonstration

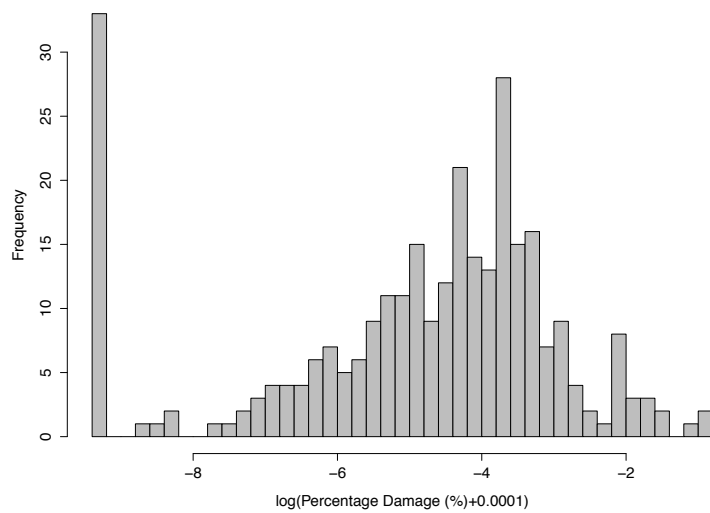


**Figure.3 Barplot of different species**



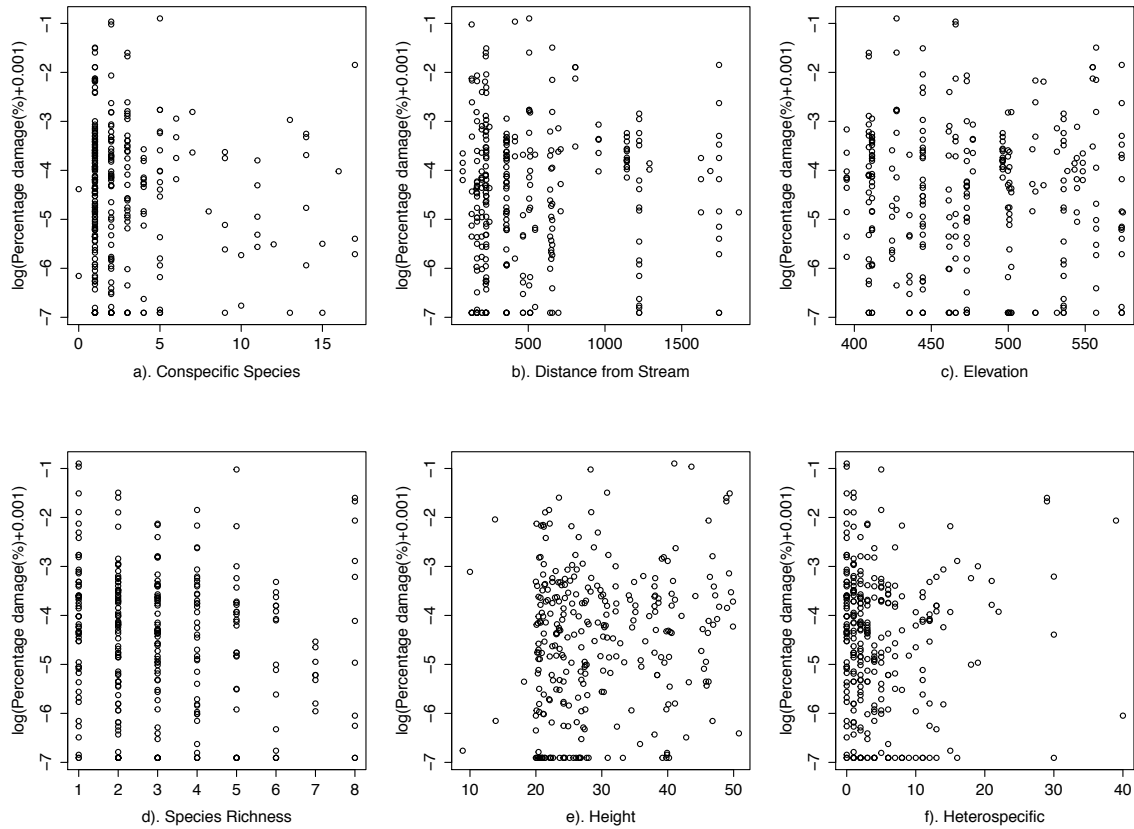
\*Scientific names: *Acer Pennsylvania* (ACPE), *Acer rubrum* (ACRU), *Acer Saccharum* (ACSA3), *Amelanchier laevis* (AMLA), *Betule lenta* (BELE), *Carpinus caroliniana* (CACA), *Carya cordiformes* (CACO), *Carya ovate* (Carya), *Cornus florida* (COFL), *Crataegus spp* (Crataegus), *Fagus grandifolia* (FAGR), *Fraxinus Americana* (FRAM2), *Liriodendron tulipifera* (LITU), *Magnolia acuminata* (MAAC), *Nyssa sylvatica* (NYSY), *Ostrya virginiana* (OSVI), *Prunus serotina* (PRSE), *Quercus alba* (QUAL), *Quercus rubra* (QURU), *Sassafras albium* (SAAL), *Talia Americana* (TIAM), and *Viburnum dentatum* (VIDE).

**Fig.4 Histogram of herbivore damage**



\* Transform data to logistic scale and add 0.0001 to original herbivore damage (%) to avoid errors caused by log(0).

**Fig.5 Herbivore damage rate of each tested factors.**



**Table.1 Linear regression model of multiple factors of herbivore damage.**

Variables	Coefficient	Standard Error	t-value	P-value
<b>Constant</b>	-4.002	1.392	-2.875	<b>0.0044</b>
<b>Heterospecific Species density</b>	0.040	0.028	1.438	0.152
<b>Height</b>	0.038	0.014	2.828	<b>0.005</b>
<b>Species Richness</b>	-0.245	0.099	-2.486	<b>0.014</b>
<b>Conspecific</b>	-0.005	0.035	-0.133	0.894
<b>Distance from stream</b>	$5.312 \times 10^{-4}$	$3.347 \times 10^{-4}$	1.587	0.114
<b>Elevation</b>	$-3.67 \times 10^{-3}$	$2.81 \times 10^{-3}$	-1.304	0.193
<b>R<sup>2</sup></b>	0.0577			

**Table.2 General information of herbivore damage (HD) in different species.**

Species Names	Mean HD	Standard error HD	Number of individuals (n)
<i>Acer pennsylvania</i>	0.0174	0.0230	18
<i>Acer rubrum</i>	0.0286	0.0363	18
<i>Acer Saccharum</i>	0.00660	0.00980	7
<i>Amelanchier laevis</i>	0.0249	0.0474	9
<i>Betule lenta</i>	0.0208	0.421	12
<i>Carpinus caroliniana</i>	0.0352	0.0507	16

<i>Carya cordiformes</i>	0.00374	NA	1
<i>Carya ovate</i>	0.0134	0.0151	14
<i>Cornus florida</i>	0.0180	0.0129	4
<i>Crataegus spp</i>	0.0229	0.0347	17
<i>Fagus grandifolia</i>	0.0127	0.0119	14
<i>Franxinus Americana</i>	0.0132	0.0128	36
<i>Liriodendron tulipifera</i>	0.0229	0.0185	8
<i>Magnolia acuminata</i>	0.00741	0.00552	4
<i>Nyssa sylvatica</i>	0.0468	0.0975	29
<i>Ostrya virginiana</i>	0.0199	0.0131	17
<i>Prunus serotina</i>	0.0459	0.0665	27
<i>Quercus alba</i>	0.0193	0.00344	3
<i>Quercus rubra</i>	0.0400	0.0594	18
<i>Sassafras albidum</i>	0.0187	0.0284	15
<i>Talia americana</i>	0.108	0.158	5
<i>Viburnum dentatum</i>	0.00373	NA	1
<b>Total</b>			298
<b>Average</b>	0.0250		

\* NA: No standard error, since only one individual.

**Table.3 Multiple linear regression Model for the largest 6 numbers of Species**

**a. *Acer Pennsylvanica***

	Variables	Coefficient	Standard Error	z-Value	P-value
<i>Acer Pennsylvania</i> (Strip Maple)	Constant	0.355	14.49	0.025	0.981
	Distance from stream	$1.7 \times 10^{-4}$	$1.47 \times 10^{-3}$	0.116	0.91
	Elevation	$-1.12 \times 10^{-2}$	0.025	-0.456	0.658
	Height	0.0283	$7.43 \times 10^{-2}$	0.382	0.71
	Species Richness	0.196	0.77	0.254	0.804
	Heterospecific Species density	-0.125	0.260	-0.481	0.640
	Conspecific Species Density	-0.514	0.529	-0.971	0.352
	R <sup>2</sup>			0.2299	

**b. *Nyssa Sylvatica***

	Variables	Coefficient	Standard Error	z-Value	P-value
<i>Nyssa Sylvatica</i> (Black gum)	Constant	-2.855	5.124	-0.557	0.583
	Distance from stream	$1.7 \times 10^{-4}$	$1.47 \times 10^{-3}$	1.271	0.271
	Elevation	$-1.23 \times 10^{-3}$	$9.91 \times 10^{-3}$	-1.247	0.226
	Height	$9.47 \times 10^{-3}$	$6.62 \times 10^{-3}$	1.416	0.171
	Species Richness	1.22	1.02	1.198	0.244
	Heterospecific Species density	-0.953	0.578	-1.649	0.113
	Conspecific Species Density	-0.205	0.334	-0.614	0.545
	R <sup>2</sup>			0.2384	

**c. *Franxinus Americana***

	Variables	Coefficient	Standard Error	z-Value	P-value
<i>Franxinus Americana</i> (White Ash)	Constant	-4.00	6.81	-0.588	0.561
	Distance from stream	$7.4 \times 10^{-4}$	$4.7 \times 10^{-3}$	0.156	0.877

<b>Elevation</b>	$-3.5 \times 10^{-3}$	$1.2 \times 10^{-2}$	-0.285	0.778
<b>Height</b>	$5.7 \times 10^{-2}$	$4.87 \times 10^{-2}$	1.161	0.255
<b>Species Richness</b>	-0.374	0.757	-0.494	0.625
<b>Heterospecific Species density</b>	-0.141	0.363	-0.388	0.701
<b>Conspecific Species Density</b>	$1.73 \times 10^{-2}$	$8.51 \times 10^{-2}$	0.203	0.841
<b>R<sup>2</sup></b>	0.3034			

**d. *Prunus Serotina***

	<b>Variables</b>	<b>Coefficient</b>	<b>Standard Error</b>	<b>z-Value</b>	<b>P-value</b>
<b><i>Prunus Serotina</i> (Black Cherry)</b>	<b>Constant</b>	-9.85	7.30	-1.35	0.194
	<b>Distance from stream</b>	$-1.73 \times 10^{-3}$	$1.83 \times 10^{-3}$	-0.949	0.355
	<b>Elevation</b>	$4.52 \times 10^{-3}$	$1.40 \times 10^{-2}$	0.322	0.751
	<b>Height</b>	$8.71 \times 10^{-2}$	$5.07 \times 10^{-2}$	1.717	0.103
	<b>Species Richness</b>	0.101	0.325	0.310	0.760
	<b>Heterospecific Species density</b>	$5.92 \times 10^{-2}$	$6.14 \times 10^{-2}$	0.966	0.347
	<b>Conspecific Species Density</b>	$9.27 \times 10^{-2}$	0.124	0.745	0.466
	<b>R<sup>2</sup></b>	0.3704			

**e. *Quercus Rubra***

	<b>Variables</b>	<b>Coefficient</b>	<b>Standard Error</b>	<b>z-Value</b>	<b>P-value</b>
<b><i>Quercus Rubra</i> (Red oak)</b>	<b>Constant</b>	-6.39	10.47	-0.610	0.554
	<b>Distance from stream</b>	$-1.51 \times 10^{-2}$	$2.71 \times 10^{-3}$	-0.559	0.587
	<b>Elevation</b>	$9.62 \times 10^{-3}$	$2.27 \times 10^{-2}$	0.424	0.68
	<b>Height</b>	$-2.28 \times 10^{-2}$	0.102	-0.223	0.828
	<b>Species Richness</b>	-0.904	1.159	-0.78	0.452
	<b>Heterospecific Species density</b>	0.193	0.691	0.279	0.785
	<b>Conspecific Species Density</b>	0.146	0.984	0.148	0.885
	<b>R<sup>2</sup></b>	0.1064			

**f. *Acer Rubrum***

	<b>Variables</b>	<b>Coefficient</b>	<b>Standard Error</b>	<b>z-Value</b>	<b>P-value</b>
<b><i>Acer Rubrum</i> (Red Maple)</b>	<b>Constant</b>	-5.135	4.138	-1.241	0.240
	<b>Distance from stream</b>	$-3.99 \times 10^{-4}$	$9.81 \times 10^{-4}$	-0.406	0.692
	<b>Elevation</b>	$3.88 \times 10^{-3}$	$7.13 \times 10^{-3}$	0.544	0.597
	<b>Height</b>	$-7.30 \times 10^{-3}$	$6.75 \times 10^{-3}$	-0.108	-0.916
	<b>Species Richness</b>	-0.253	0.423	-0.599	0.561
	<b>Heterospecific Species density</b>	$-1.06 \times 10^{-2}$	0.123	-0.086	0.933
	<b>Conspecific Species Density</b>	$3.67 \times 10^{-2}$	$9.82 \times 10^{-2}$	0.373	0.716
	<b>R<sup>2</sup></b>	0.1125			

\*Relationship between abiotic and biotic factors, and herbivore damage percentage (a) *Acer Pennsylvania* (Strip Maple), n=18, (b) *Nyssa Sylvania* (Black gum), n=29, (c) *Franxinus Amreicana* (White Ash), n=36, (d) *Prunus Serotina* (Black Cherry), n=27, (e) *Quercus Rubra* (Red Oak), n=18, (f) *Acer Rubrum* (Red Maple), n=18.

**Table.4 Spearman Rank Correlation**

	<b>P-value</b>	<b>Rho</b>
<b>Percentage damage (%) VS Richness</b>	<b>0.04026</b>	<b>-0.1197</b>

<b>Percentage damage (%) VS Height</b>	<b>0.03036</b>	<b>0.1259</b>
<b>Percentage damage (%) VS Conspecific species density</b>	0.5264	-0.0371
<b>Percentage damage (%) VS Heterospecific species density</b>	0.06253	-0.1088
<b>Percentage damage (%) VS Elevation</b>	0.9173	-0.00605
<b>Percentage damage (%) VS Distance from stream</b>	0.135	0.0871

**Table.5 ANOVA table Herbivore damage and Species**

	<b>Degree of Freedom</b>	<b>Sum of Sq</b>	<b>F-value</b>	<b>P-Value</b>
<b>Species</b>	22	4.639	1.14	0.304
<b>Residuals</b>	273	1111.0		