

DEBUNKING THE ENEMY RELEASE HYPOTHESIS: DOES INTRODUCED NORWAY MAPLE LACK DEFENSES AGAINST NATIVE HERBIVORES?

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ABSTRACT

Norway maple (*Acer platanoides*) is an invasive plant species, which originated in Europe, and has become prominent in forests of eastern North America. The enemy release hypothesis (ERH) suggests that invasive species are successful because they have no natural predators in their new location. Our hypothesis was that if ERH is a factor explaining Norway maple success as an invader, there would be less damage from insect herbivores on Norway maple leaves than on leaves of two native species, American beech (*Fagus grandifolia*) and sugar maple (*Acer saccharum*). The study was conducted in the Drew Forest Preserve in Madison, New Jersey. Leaves from the three species of trees were sampled in the Preserve. Leaves were collected from sapling and mature trees so that the effect of age could be determined. Thus, we had six species-age categories. Norway maple had greater area of damage from herbivores but lower percent damage than the native species. The lower percent damage was limited to the sapling Norway maples. These findings do not support the enemy release hypothesis as an explanation for Norway maple invasion in eastern forests. However, the low percentage of area damage in Norway maple saplings indicates that this introduced species may be invasive because it possesses a competitive edge over native trees. The advantage of larger leaves in sapling Norway maple may mitigate the effects of higher insect herbivory in that the potential to gather energy through photosynthesis is not as affected by loss of leaf area as it is in native species. It is possible that Norway maple have not developed defenses to native herbivores because they did not coevolve.

INTRODUCTION

In the United States, over \$120 billion dollars worth of damage is caused annually by invasive species (1). An invasive species is one that has been displaced from its natural ecosystem and intentionally or unintentionally introduced into a foreign environment where it often outcompetes native species. While some invasive species were intentionally introduced for public benefit and have positive influences in society, such as the corn, wheat, rice, poultry, and livestock that make up more than 98% of the United States' food system, many species are destroying ecosystems, causing extinctions, or spreading diseases (1).

Factors that contribute to how invasive species interfere with other species and gain competitive advantages over these species include the characteristics of the ecosystem and the strengths and weaknesses of each invasive species (2). In the case of trees and other plants, some explanations for why an introduced species thrives include greater survival and reproduction due to diminished damage from natural enemies, higher rate of photosynthesis,

greater water use efficiency, more rapid germination and growth, and greater survival of seedlings, especially during the initial winter and first two growing seasons (3). The strongest, most influential trees are those which have reached the uppermost canopy level, as they are able to exploit resources such as sunlight, while impeding access to sunlight by other trees. This means that the sapling-age trees are potentially the most vulnerable trees in the forest (3).

At Drew University in Madison, New Jersey, the Norway maple (*Acer platanoides*) is a major invasive species in the college's forest preserve (2). Having the most extensive and far-reaching range in Europe, the Norway maple was initially introduced to the United States in 1756 as a decorative landscaping tree (4). Following an enormous outbreak of Dutch elm disease, the Norway maple has firmly established itself in American arboriculture and has replaced elm as an urban street tree due to its hearty characteristics. Norway maples are able to withstand moderate pollution, dust, dry soil, and difficult growth conditions, allowing the trees to thrive in such harsh environments as cracks in pavement (4). What enables the Norway maple to dominate in non-native forest ecosystems such as Drew's reserve on the other hand, also likely includes its lower winter mortality, earlier spring emergence, extended growth period, tolerance of shade and nutrient-poor soils, and larger, wind-dispersed seeds (2, 3). For example, Norway maples are capable of significantly impairing competitor trees once reaching canopy height (3). By creating high shade levels, this invasive species inhibits the growth of other trees, which cannot match the Norway maple's ability to grow in shaded areas. At the same time, Norway maple saplings flourish in this darker environment, gaining a competitive advantage over the other tree species and allowing for continued dominant propagation.

One of the most popular current theories for how an invasive species damages its new ecosystem is the enemy release hypothesis (ERH). The ERH states that the greater success rate shown by invasive species is due to their relative lack of predation compared to native species, which allows the invasive species to grow unchecked as opposed to their native counterparts which are heavily exploited by predators (4). In addition, generalist enemies in the area should prefer native species over non-native, because native predator and prey species coevolved together, so native predators would be more likely to prefer native species over exotic species (5). Those who wish to biologically control an invasive species according to the ERH sometimes introduce enemies from the plant's native range into the new ecosystem, or try to reduce its access to resources (6). In order to evaluate whether the ERH is applicable in an area, one must calculate leaf damage to determine whether herbivores favor the native plants' leaves and neglect the invaders, or whether the insects have no bias in their leaf consumption (4).

In a study conducted in 2009 at the New Jersey Governor's School of Sciences, the ERH failed to explain Norway maple's invasion in Drew University's forest reserve. However, the study was inconclusive partly because the team assessed the area of leaf damage using visual approximation instead of technology, rendering the data somewhat unreliable (7). In general, many factors could make the ERH inapplicable to certain species. For example, if the plant does not rely on short-term and frequent seed production for survival, the status of its enemies will have little impact on its proliferation in either its native or a foreign ecosystem (4). Other species have evolved to produce chemicals that deter enemies from attack or to tolerate the loss of biomass caused by predation (5). However, other researchers have found support for ERH as an explanation for why Norway maple is so successful in eastern forests (8).

We built upon the study conducted at the 2009 Governor's School in the sciences at Drew University. Instead of merely collecting our samples from Norway maple and sugar maple (*Acer saccharum*) leaves, we expanded the categories to include age (sapling vs. mature) as well as an entirely new native species, American beech (*Fagus grandifolia*). In addition, we collected leaves from both mature and sapling Norway maple, sugar maple, and American beech trees. We then tested the ERH by assessing the amount of leaf damage in the six categories based on species and age. Based on our hypothesis, we predicted that if ERH is an important factor in facilitating the maple invasion, then saplings and mature Norway maple would have less damage than the native sugar maple and American beech.

MATERIALS AND METHODS

Study Area

Leaves were collected from trees within the 18 ha. Drew University Forest Preserve (Madison, New Jersey, 40°46'N, 74°26'W). The Drew campus is located in a suburb within the New York Metropolitan area, around 27 miles from New York City. Although recently the preserve has been used primarily for scientific studies, it was originally a pasture, which began its growth into a forest around 1867 when the Drew Theological Seminary was established (2). The dominant tree species in the area are Norway maple, sugar maple, and American beech, with populations including large numbers of both sapling and mature trees of all these species. The forest preserve is also home to a large number of animal species and is heavily browsed by white-tailed deer (9).

Sample Collection

Leaves were collected from multiple sapling and mature Norway maple, sugar maple, and American beech. We defined sapling trees as those with a DBH (diameter at breast height) between 2-9 cm and mature trees as those with a DBH of over 11 cm. Using a tree clipper on a long pole to cut branches from different heights on a tree, we collected about 10-15 leaves without bias regarding damage. We analyzed between 275 and 310 leaves from each species-age group for a total of 1810 leaves.

Damage Assessment

We first separated the undamaged leaves from the damaged leaves. Only herbivorous insect damage was counted, whereas fungal damage such as brown spots was not considered. The damaged leaves were then taped to sheets of paper to prepare for scanning. If damage existed at the edge of the leaf, we drew in the outline of the missing edge in green pen based on the existing part of the leaf, allowing the image analysis program to register the approximate leaf outline as it was prior to sustaining insect damage. The leaves were then scanned and measured using Image J (10). Taking the total leaf area as well as the area of the absolute leaf damage and

the undamaged area of the leaf, we calculated the percent damage for each leaf. Undamaged leaves were recorded as having 0 mm² of damage and 0% damage.

Statistical Analysis

Statistical Package for the Social Sciences 17.0 (SPSS) was used to analyze the leaf area damage. We performed an ANOVA to test whether the total area of damage and the percent damage were significantly different among species-age groups. *Post-hoc* comparisons were made between groups using the Bonferonni test. This test was chosen because it corrects for the effects on the alpha level due to multiple comparisons. All tests were considered significant at P<0.05. The data were log-transformed prior to analysis to meet the assumptions of normality and equal variance.

RESULTS

Mean and standard deviation for different species and age categories of trees are summarized in Table 1. Because the data did not meet the assumptions of normality and equal variance inherent in the statistical tests, the data were log-transformed.

Table 1. Mean and standard deviation of leaf damage (absolute and percent) analyzed by species, age, and species-age categories.

Variable	Category	Mean	Standard Deviation
Absolute leaf area damaged (mm²)*	Norway maple	24.529	70.596
	Sugar maple	85.253	1427.487
	American Beech	25.843	83.124
	Mature	19.379	55.381
	Sapling	69.729	1.148
	Norway maple-mature	28.373	69.777
	Norway maple-sapling	20.594	71.329
	Sugar maple-mature	6.024	16.284
	Sugar maple-sapling	160.630	2002.135
	American beech-mature	22.973	60.547
	American beech-sapling	28.695	100.689
Percent leaf area damaged (%)*	Norway maple	0.253	0.802
	Sugar maple	0.648	3.854
	American Beech	0.570	1.928
	Mature	0.431	1.351
	Sapling	0.548	3.286
	Norway maple-mature	0.314	28.373
	Norway maple-sapling	0.189	0.616

	Sugar maple-mature	0.503	1.511
	Sugar maple-sapling	0.716	5.212
	American beech-mature	0.479	1.516
	American beech-sapling	0.661	2.262

*Zero-values included in calculations

The remaining statistics refer to the log-transformed values for absolute leaf area damaged and percent leaf area damaged. Absolute leaf damage and percent area damage varied with species type (Table 2 and Fig. 1 and 2). Norway maple had a higher absolute damage than sugar maple ($P < 0.001$; Fig. 1). However, sugar maple and beech had a higher percentage area damage than Norway maple ($P = 0.001$ and $P < 0.001$, respectively, and Fig. 2). Percentage leaf damage area varied with age of tree, with mature trees having more percent damage than the saplings ($P = 0.034$, Fig. 3). Absolute damage did not show significant differences between age groups ($P = 0.826$; Table 2). In addition, there was an interaction between species and age for absolute leaf area damage and percentage damage ($P < 0.001$ and $P = 0.001$, respectively, and Fig. 4 and 5). Mature Norway maples had a higher absolute damage than the other groups, except the sugar maple saplings ($P = 0.006$ for mature beech, $P < 0.001$ for others; Fig. 4). Mature sugar maples had a lower absolute damage than each of the other groups ($P = 0.007$ for Norway maple saplings, $P = 0.004$ for beech saplings, $P < 0.001$ for others; Fig. 4). Norway maple saplings had a lower percent damage than each of the other groups ($P = 0.001$ for mature Norway, $P < 0.001$ for others; Fig. 5). Norway maple saplings had a higher total leaf area (undamaged) than all other species/age groups ($P < 0.001$) except mature Norway maple.

Table 2. Results of ANOVAs testing whether species, age, and species*age affect the amount of damage sustained by insects.

Damage Measure	Factor	F	P
Absolute Damage* (mm ²)	Species	10.448	<0.001
	Age	0.048	0.826
	Species * Age	22.878	<0.001
Percent Area Damage*	Species	15.215	<0.001
	Age	4.509	0.034
	Species * Age	6.625	0.001

*Log-transformed

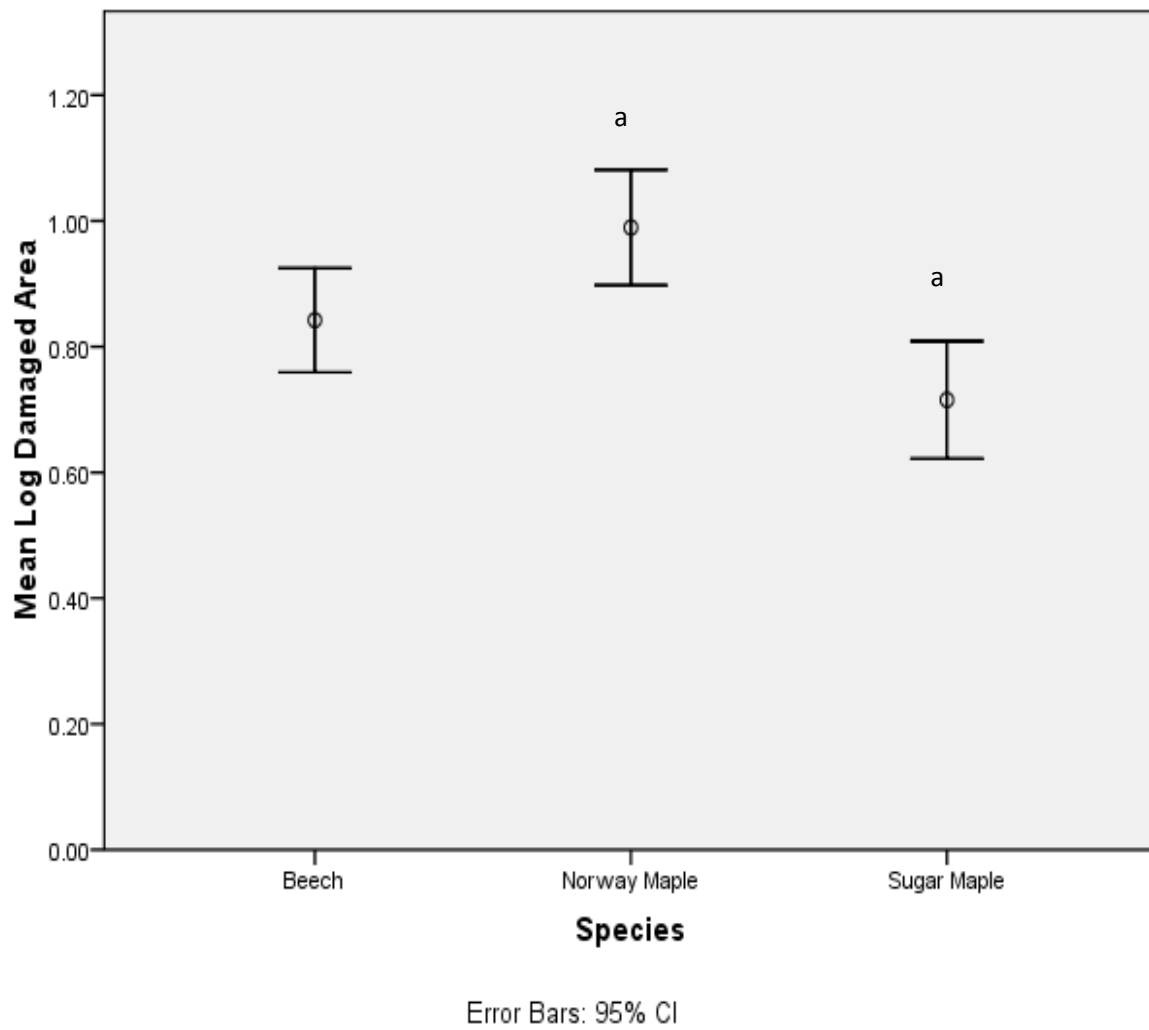


Figure 1. Mean absolute damaged area by species. This graph shows the mean absolute damaged area for the three species. The letter “a” indicates those species that are different ($P < 0.05$).

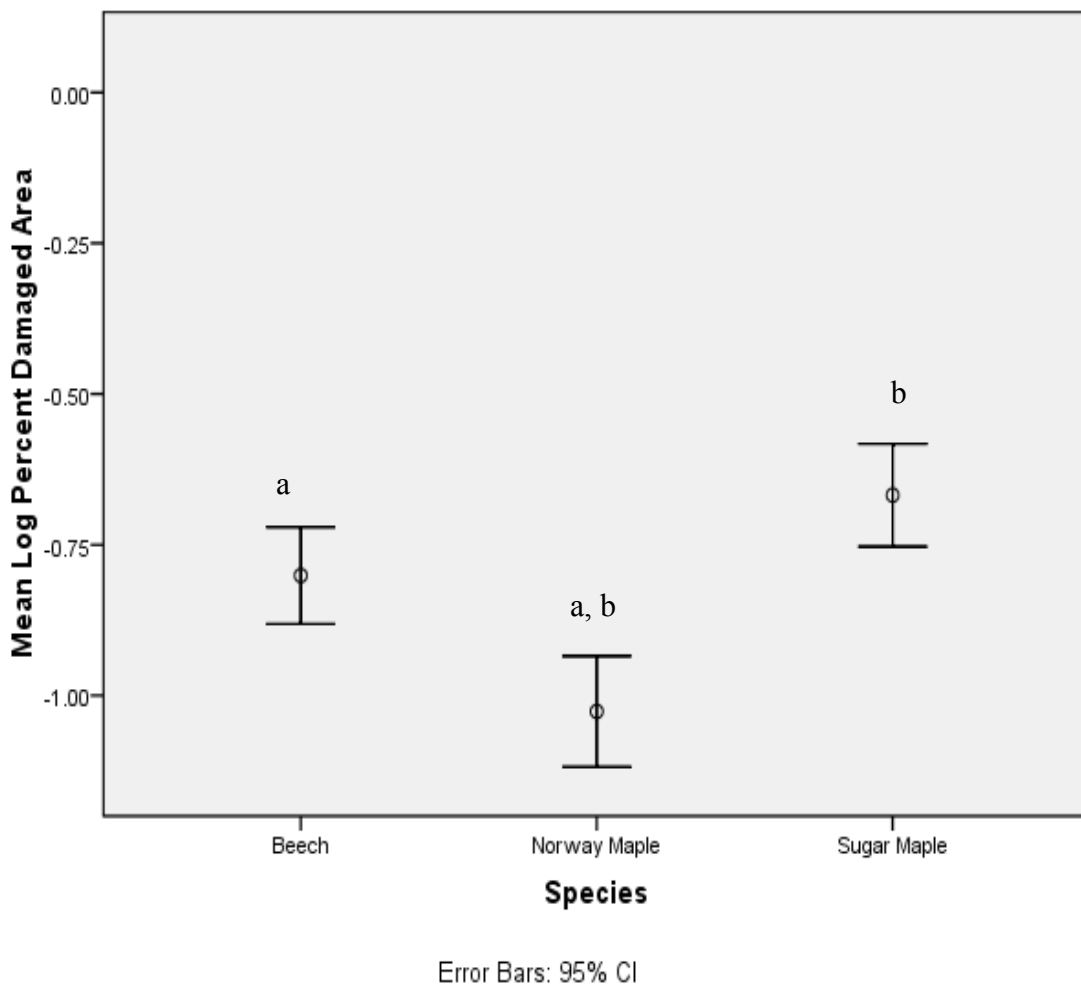


Figure 2. Mean percent damaged area by species. This figure shows the mean absolute percent damaged area for the three different species. The letters “a” and “b” indicate categories that are different ($P < 0.05$).

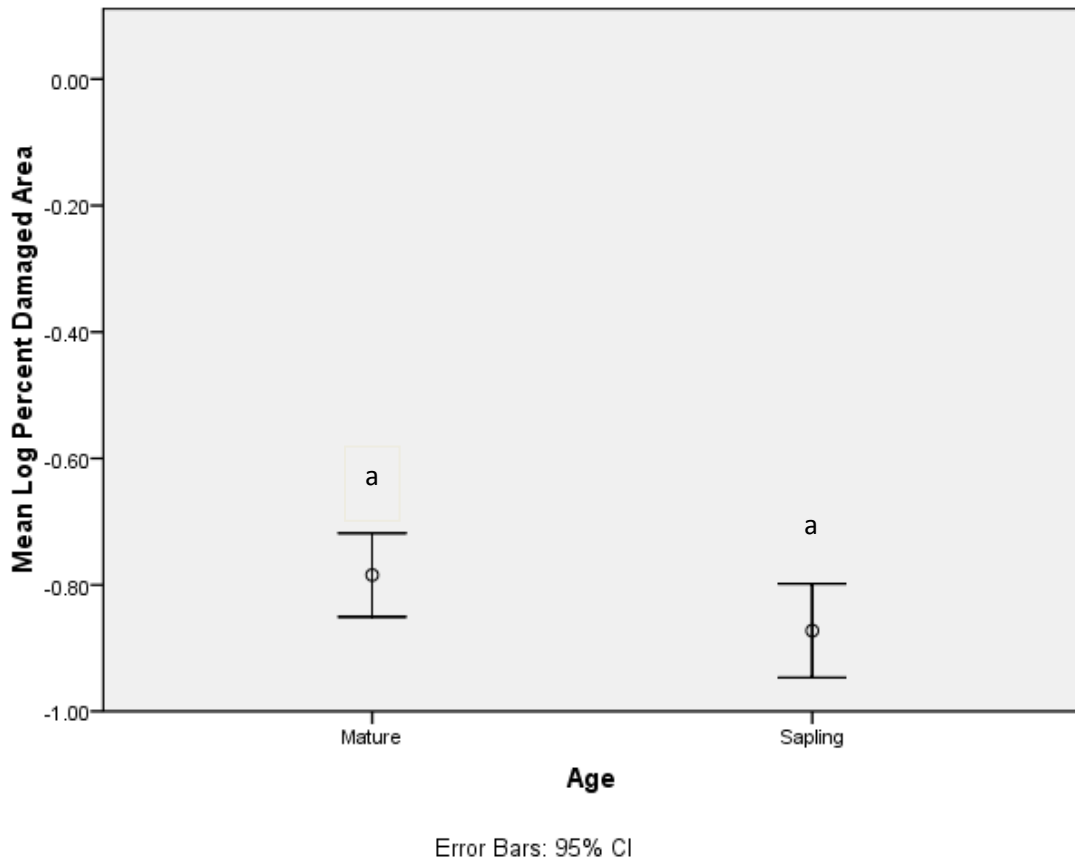


Figure 3. Mean percent damaged area by age. The figure shows the mean absolute percent damaged area by age group, with error bars displaying a 95% confidence interval. The letter “a” indicates categories that are different ($P < 0.05$).

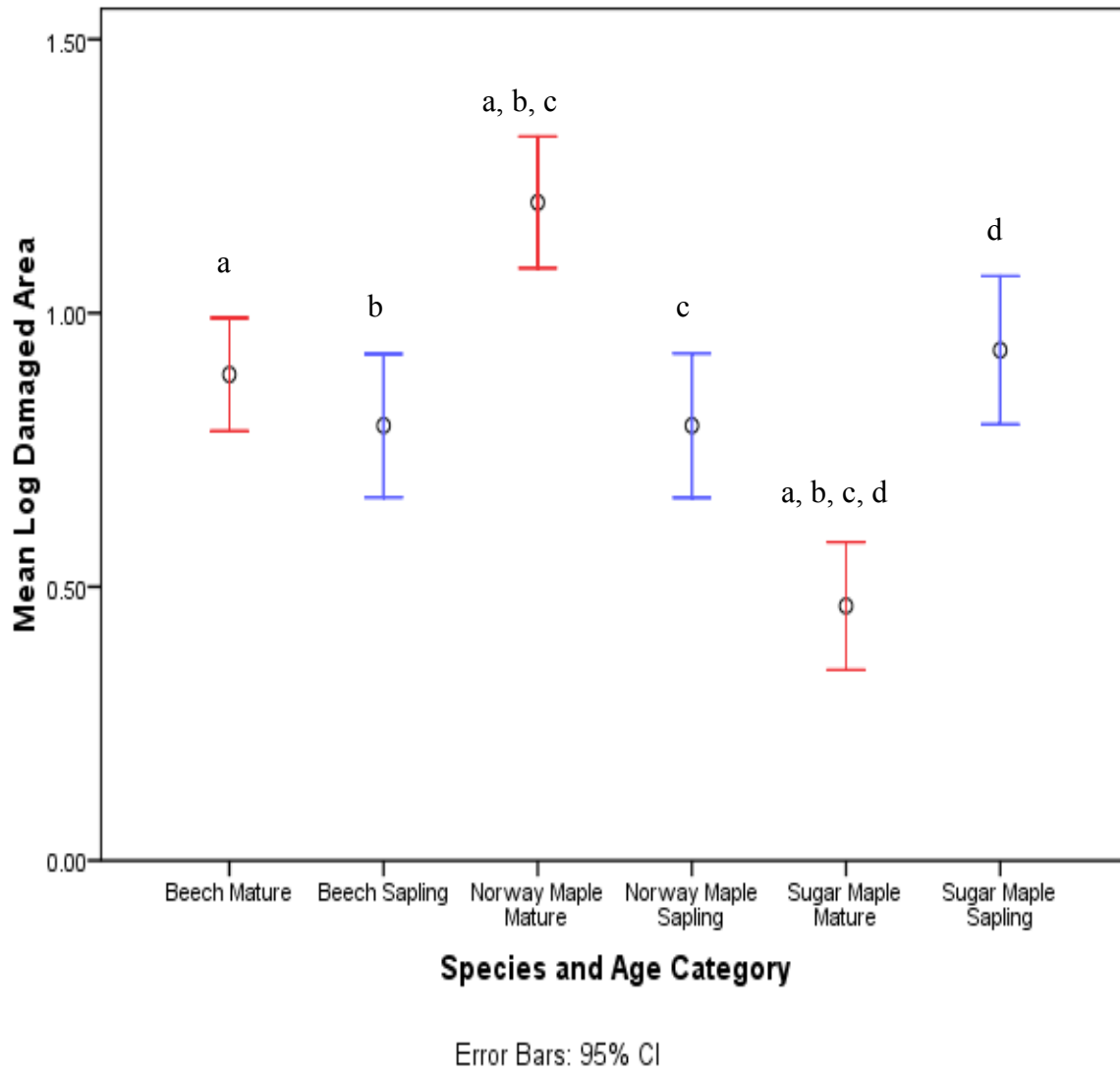


Figure 4. Mean damage and interaction between species and age. This figure shows the mean absolute damage area in relation to the age and species of the tree. The letters “a, b, c, d” indicate categories that are different ($P < 0.05$).

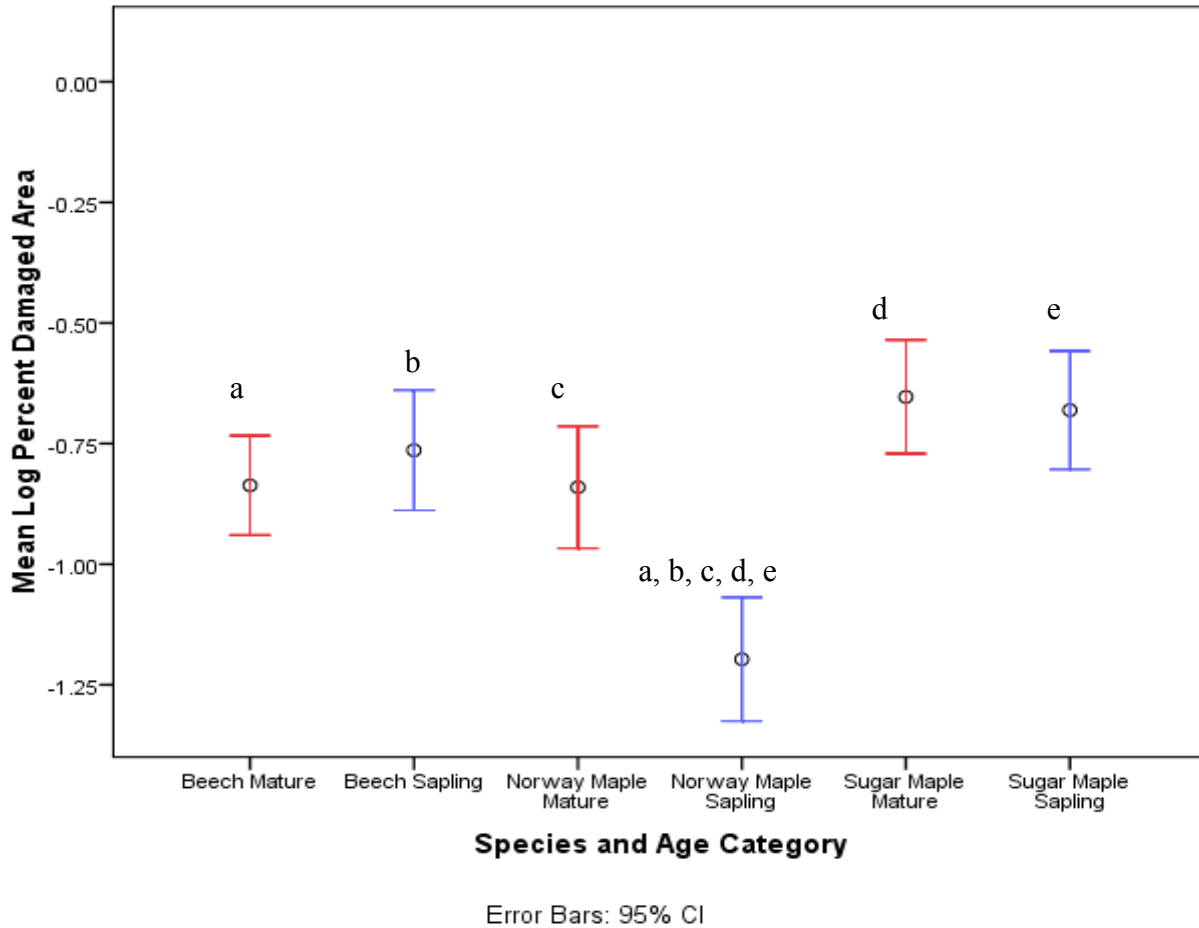


Figure 5. Mean percent damaged area and interaction between species and age. This figure shows the relationship between the percent damage area of the leaves broken into categories based on the tree species and age. The letters “a” through “e” indicate a difference between categories ($p < 0.05$). The figure shows that Norway maple saplings are the only tree species that show statistically significant differences from all other ages and species.

DISCUSSION

Our data did not support the Enemy Release Hypothesis as an explanation for why Norway maple has a strong competitive advantage over other species of tree in eastern forests. Instead, we found that Norway maple, not the native trees, was actually undergoing higher levels of herbivory. It is possible that our failure to support the ERH is due to human error including observer bias. During our leaf collection, we may have had bias in choosing leaves with more or less damage to enhance the outcome in favor of our hypothesis. In addition, our wait period between picking the leaves and scanning the leaves was about 5 days. Although the leaves were stored in an airtight bag, several leaves began to dry out. During storage, the leaves may have

suffered greater damage than they originally had, skewing our data to show that the leaves had a greater area of damage than in actuality. Later, while sorting the leaves, we had to differentiate between fungal and insect damage, which we may have misclassified. Furthermore, we used estimation to draw the outer edge of damaged leaves so that Image J could analyze the total leaf area. Our procedure was slightly flawed as well, as time constraints caused us to forego scanning the undamaged leaves. Although we accounted for their total lack of damage in the data, we were unable to include their area when calculating the averages, possibly skewing the data. Furthermore, our experiment cannot account for leaves completely eaten by pests, which would show 100% damage. This error could create inaccuracies in our final data results, and should be kept in mind when interpreting the results of our experiment.

While there are these potential sources of error, it is also likely that there are other ecological and evolutionary processes affecting Norway maple in North America and some factors may not favor the invasion of this species. Although one may have predicted that the Norway maple would have a greater chance of survival based on the ERH, another possibility is that in some cases it is the introduced species experiences more damage from herbivores. This is because the invasive species is exposed to predators for which it has not yet developed natural defenses through co-evolutionary processes. One study, which measured the defenses of annual plants against herbivores, showed that the plants were able to form more effective defenses against the herbivores following periods of extensive predation (11). Therefore, it would follow that the longer a type of plant is in contact with different species of herbivores, the greater the chances are that the plant will be able to defend itself against the predator.

In a similar study, various host species were exposed to novel species of parasites and to parasites with which their species had come in contact in the past. The study concluded that the “naïve” hosts were more susceptible to the parasites than the hosts that had encountered the parasite (12). The host species that had come in contact with the parasite species previously had the advantage of coevolving with the parasite; the host had adapted defenses against the parasite, the parasite had adapted to those defenses, and this cycle of adaptations continued. However, the host species experiencing that specific parasite species for the first time lacked adaptations to defend themselves against the parasite. This theory could also apply to the trees analyzed in this study. Norway maple is an exotic species, and therefore has not yet adapted to the predators in this region of the world. However, the sugar maple and American beech have developed resistance to the herbivores because they have evolved together. This would explain the greater amount of damage seen on Norway maple leaves than sugar maple and American beech leaves.

An additional explanation for the greater herbivory observed on Norway maple leaves is the Optimal Foraging Theory. This theory states that organisms forage in ways that maximize their energy intake (13). Thus, it is possible that herbivorous insects simply prefer to consume Norway maple leaves more than American beech or sugar maple leaves because the Norway maple leaves have a different chemical composition that may enable the insects to gain nutrients more efficiently.

Another possibility is that herbivores are having little or no effect because of their low densities and thus exerting negligible selective pressure on either native or exotic trees in the forest. In this scenario, predators are likely keeping the herbivore populations low and

preventing heavy selection pressure from occurring due to herbivory (14). This is a top-down approach as opposed to the bottom up approach suggested by ERH.

Another prevalent theory that could explain why Norway maple is so successful in North American forests is the resource availability hypothesis. This hypothesis states that an invasive plant species proliferates due to high resource availability either because the area is a resource-rich area or the competing species obtain resources less effectively (15). In some cases, the invasive species is better equipped to obtain the needed resources, sometimes diminishing the supply available to competitor species. For example, in a more stressful environment, trees that grow more slowly and thus have a lower metabolic requirement will out-compete those rapidly growing plants that are accustomed to resource-rich environments. On the other hand, in a resource rich environment, trees that have a rapid leaf turnover will have an adaptive advantage because they photosynthesize so much (16).

While our analysis of absolute leaf area suggests that Norway maple do not gain a competitive edge through lack of insect predators or via defenses against insect predators in their new environment, our findings related to percent leaf area damage do suggest a possible explanation for why Norway maples are so successful. Although the Norway maple had a larger total damaged area than the sugar maple, the results for percent damaged area based on the total leaf area were quite different. Norway maple saplings had a substantially lower percent damage than all other species-age categories, including leaves from mature Norway maple. This implies that while the Norway maple had equal or greater herbivorous damage, they were not as affected because they have a larger leaf area than both the sugar maple and the American beech. The large leaf size, especially in the saplings, which have fewer leaves and need the nutrients to grow, is a huge adaptive advantage for several reasons. Although there is no inherent defense against the predators by having these leaves, the larger leaves provide the tree with a greater ability to gather resources through photosynthesis despite higher absolute leaf loss to herbivory. This might be especially important because it is the sapling age trees that are most vulnerable and at risk of mortality. Thus, it is possible that the Norway maple are less impaired by insects than the sugar maple and the American beech because they are consumed in the same amounts but have a larger surface area to compensate. In addition, the larger leaf area provides them with a wider zone of shade tolerance and the ability to impede growth of competitors under their canopy.

A possible study that one could perform to test this idea would include surveying the herbivorous damage done to a community that consists of various invasive species of plants as well as native species of plants. The experiment would display whether introduced species are at a disadvantage when dealing with the native predators in an area because the nonnative species has not been able to adapt defenses against those predators. Therefore, the introduced species lacked the coevolved defenses that exist between the native species and their predators. In addition, studies of plant defense chemicals and feeding preferences of native insect herbivores could be conducted to assess whether the Norway maple do indeed lack chemical defenses against native herbivores. This should coincide with studies assessing the nutritional content of leaves and their effect on herbivores feeding on them to account for the effect of foraging preferences. Finally, we would want to test whether lower percent damage to Norway maple saplings confers a true survival/reproductive advantage given that total leaf damage is not that great.

ACKNOWLEDGEMENTS

We would like to thank several people who were involved in helping us accomplish our project. First of all, we would like to thank the New Jersey Governor's School in the Sciences and all its donors for making it possible for us to be here to conduct our research. We would also like to acknowledge all the guidance and support that NJGSS director Dr. David Miyamoto gave us to aid in designing and obtaining the needed equipment for an alternative project when the means necessary for our original plan went awry.

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