# *Alliaria petiolata* and its Invasive Effects, Both Above- and Below-Ground

by

Carissa Sanchez

An Undergraduate Thesis
Submitted in Partial Fulfillment for the Requirements of

Bachelor of Arts
in
Environmental Science: Conservation and Ecology

Carthage College
Kenosha, Wisconsin

May 2014

**Abstract**

*Alliaria petiolata*, known commonly as garlic mustard, is a high-profile invasive herbaceous species that has made appearances in over 40 Wisconsin counties. This invasive species, found primarily in deciduous forests, has been shown through various studies to decrease the growth and reproductive success of species native to the invaded ecosystems. In addition, garlic mustard disrupts underground colonization of arbuscular mycorrhizal fungi, which many plants depend on for nutrient gain. In this multi-dimensional study, garlic mustard's effects on arbuscular mycorrhizal fungi colonization and native herbaceous species growth were observed in a Wisconsin forest. Garlic mustard was treated both through removal and cutting. Soil samples were taken weekly, intended for analysis on roots and fungi colonization on those roots. The cover, abundance, and height of garlic mustard and native species was observed over the course of the study. The soil samples were not able to be analyzed. Other data did not show any significant differences in plant height or size between plots in which garlic mustard was present, removed, absent, or cut.

Keywords: invasive species; *Alliaria petiolata*; arbuscular mycorrhizal fungi

**Introduction**

America's forests are facing many difficulties right now, and few are as threatening to a forest's health and biodiversity as invasive species. These organisms are ones that are not native to an area, yet occupy and gradually cause severe damage to the growth and spread of native species. Invasives have the potential to form monocultures by outcompeting native species and tolerating a wider range of environmental conditions. Invasive species have been impacting various ecosystems for centuries. These non-native plants establish themselves in both disturbed and undisturbed forest communities, and can have disastrous effects on both the physical and biochemical characteristics of native species. Effects of invasive species on native plants include outcompeting species for nutrients, space, or sunlight (Vila, 2011); prohibiting native species from growing due to physical attributes (Ren, 2009); and inhibiting myccorhizal fungi from forming a symbiotic mutualism with plants that depend on those colonies for nutrients (Stinson, 2006). The growth and spread of invasive species can cause serious ecological and economic damage to an environment that can be hard to rehabilitate and costly to manage (Pimentel, 2004). With climate change potentially causing temperatures to rise and rainfall to increase, some invasive species may have an even greater advantage, and may spread further than they have (Dukes, 1999). One such invasive species, and the main focus of this study, is *Alliaria petiolata*, commonly known as garlic mustard. Garlic mustard is now commonly found in the eastern and Midwestern regions of the United States. Previous studies conducted on the East Coast have shown that garlic mustard suppresses the germination of vital mycorrhizal fungi on the roots of trees native to that region; in addition, it severely decreases the biodiversity of many forests by forming monocultures where native herbaceous species have difficulty competing. The purpose of this experiment is to examine whether or not garlic mustard exhibits those same invasive properties in Wisconsin forests, and to determine whether or not the removal of garlic mustard would benefit the health of forests in the Midwest.

## Literature Review

### *Alliaria petiolata*

Garlic mustard is an invasive species that originated from Europe. The first documented sighting of a garlic mustard plant in North American was in 1868, on the east coast (Shartell et. al., 2013). It is believed to have been intentionally transported over by early pioneers for use in erosion control and medicine (Shartell et. al, 2013). The genetic similarity between garlic mustard plants in Europe and ones in the United States, along with a general lack of genetic diversity among the American plants, suggests multiple introductions of garlic mustard to the United States, and that these plants probably originated from the British Isles and northern and central Europe (Durka, 2005). The species first became established in New York, then spread across the east coast. Garlic mustard is now found in many Midwestern states, and has spread as far west as Oregon (Kleinstein, 2001). In southern Wisconsin, it is becoming more abundant in the southern counties, and has been sighted in 40 counties (Forest Invasive Plants Resource Center (FIPRC)).

It is easy to identify garlic mustard in the field. The stem of a mature plant is hairy, and can grow as tall as 1.5 meters (Cavers et. al., 1978). Its leaves are coarsely toothed and are typically 3-8 centimeters long. Garlic mustard is a flowering plant; the petals of the small flower, of which there are many, are white and usually no larger than 6 mm long (Cavers et. al., 1978). This plant is also identifiable by the strong smell of garlic that it emits.

Seeds of this biennial species will begin to grow in early spring, though they can remain in the soil and still be able to sprout for a period of up to 10 years. Garlic mustard grows slowly during the first year of its life, but then reaches a shoot growth rate of 1.9 centimeters a day during its second spring (Anderson, 1996). At this point, it is able to start flowering. Garlic mustard can reproduce by both cross- and self-pollination, though the latter is probably more common (Anderson, 1996). After pollination, seed dispersal begins in July and continues until late October (Anderson, 1996). Seeds can lay dormant in the soil until the following spring, when they begin to grow.

Garlic mustard is very successful as an invasive species (Meekins, 1999). Though it prefers slight shade, this species is able to grow in both full shade and full sunlight. This species is also able to tolerate a wide variety of soils, ranging from damp and shaded to dry and exposed (Cavers et. al., 1978). In addition, garlic mustard has been found in both coniferous and deciduous environment, further exemplifying its versatility (Cavers et. al., 1978). It is able to grow and sprout quickly in its second year, and grows in abundance.

Garlic mustard also dispenses many seeds at one time; according to Anderson et. al. (1996), one square meter of garlic mustard can produce anywhere from 9,500 to 100,000 seeds. This method and rate of growth often prevents native herbaceous species from receiving proper sunlight, thus inhibiting their growth (FIPRC). Garlic mustard is capable of growing in various climates and sees the greatest success in areas that have been recently disturbed (FIPRC). Adult garlic mustard plants are so effective at forming monocultures and displacing other plants that they often outcompete and increase mortality in garlic mustard juveniles as well (Pardini et. al., 2009).

*Mycorrhizal Fungi*

Fungi undoubtedly play an important role in ecosystems. These organisms aid in such important processes as decomposition and nutrient mineralization (Dighton, 2003). Mycorrhizal fungi are types of fungi that greatly aid in nutrient mineralization for plants. These types of fungi, whose name literally translates to ‘fungal root’, grow and colonize on the roots of plants. However, mycorrhizal fungi do not harm the plants on which they grow. Instead, they form a symbiotic relationship in which the plant and fungi both receive vital nutrients.

Plants require certain nutrients from the soil in order to carry out certain functions. However, some of these minerals are bound in forms that make them poorly available for plant intake, such as parent rock material (Dighton, 2003). Mycorrhizal fungi are capable of mineralizing these minerals: they decompose the material in which the nutrients are bound and release them in a soluble form, as inorganic ions (Dighton, 2003). The most important nutrients that mycorrhizal fungi make available to plants are nitrogen, magnesium, and phosphorous. While the fungi make these nutrients accessible for plants, the fungi benefits from this relationship by receiving increased amounts of carbon from the plants (Dighton, 2003). There are three types of mycorrhizal fungi, but this study will focus on one type: arbuscular mycorrhizals.

Arbuscular mycorrhizal fungi are so named because of the arbuscule that they form within the roots of the host plant. First, the fungus will form an appressorium, from which a hypha emerges and penetrates the parenchyma cortex of a root (Gianinazzi-Pearson, 1996). The hyphae then culminates into an arbuscule, which will reach full development after a few days (Gianinazzi-Pearson, 1996). Arbuscules increase the surface area of the fungi inside the cell, therefore increasing the nutrient exchange. Arbuscular fungi exchange phosphorous with the plant, while receiving carbon in return (Dighton, 2003). Due to this relationship with arbuscular mycorrhiza, plants are able to obtain 500 to 600 times more phosphorous from the soil than plants of the same species can obtain without arbuscular mycorrhiza (Bolan, 1991). This relationship between plants and arbuscular mycorrhizal fungi is important in the survival and success of young trees (Burke, 2008).

A plant’s ability to compete with other plants increases when it is in a symbiotic relationship with arbuscular mycorrhiza, due to this increased nutrient intake (Gianinazzi-

Pearson, 1996). It is therefore clear that plants benefit greatly from this relationship. However, there are species of plants that can hinder the growth and colonization of arbuscular mycorrhizal fungi on plant roots of other species.

*Garlic Mustard and AMF*

One such harmful plant is garlic mustard, which can have a negative influence on arbuscular mycorrhizal fungi (AMF) colonies forming within the soil and on the roots of plants (Roberts, 2001). This is done through allelopathic suppression, which entails the production of a chemical that prevents other organisms from growing or developing. There are several chemicals found within garlic mustard that have an allelopathic effect on surrounding organisms. Vaughn and Berhow (1999) identified three glucosinolate chemicals that had a negative effect on other plant species within their experiment, including isothiocyanates and oxazolidinethiones. These chemicals prevent mycorrhizal fungi from thriving on plant roots by severely decreasing and hindering the germination rates of AM spores (Stinson et. al, 2006). Direct emission of the chemicals does not need to occur for garlic mustard to harm AM; they may infiltrate the soil through damaged root tissue or even leaf litter (Stinson et. al., 2006). A reduction in arbuscular mycorrhizae colonization on the roots of plants means that the plant will be unable to obtain as many nutrients, such as phosphorous, as it was previously able to. Decreasing the nutrient intake of the surrounding plants can result in decreased growth and development of potential competitors, which in turn leads to the successful invasion of forests by garlic mustard.

The previously referenced study, conducted by Stinson et. al. in 2005, examined the effect that garlic mustard has on the growth of red maple, sugar maple, and white ash seedlings. Soil samples were taken from both invaded and non-invaded forests in which these three tree species were dominant. For the first part of their experiment, they planted seedlings in pots (one per container) in the two different soil types. The second part of the experiment involved growing garlic mustard and each of the three tree species individually in non-invaded soil, in order to condition the soil to the plant types. After three months, the plants were removed from the pots in their entirety, and one seedling of each tree species was added to each pot, with a fifth pot of fresh non-invaded soil serving as the control group.

Their first experiment showed that the seedlings of all three dominant tree species showed significantly less AMF colonization when grown from the soil that had seen a history of garlic mustard invasion, to the point where AMF colonies were nearly undetectable (Stinson et. al., 2006). The seedlings that were planted in the invaded soil also showed slower growth than those planted in soil without a history of garlic mustard invasion. The results from this experiment show that garlic mustard does not need to directly release allelopathic chemicals in the presence of native species in order to inhibit AMF colonization. These results also supply evidence for the negative impacts that garlic mustard can have on native species, even woody ones.

The second part of their experiment sought to show that it was garlic mustard that was directly responsible for these AMF reductions. In the pots containing soil conditioned to garlic mustard, all three native tree species experienced significant decreases in their AMF colonization than in any of the other soils (conditioned or controlled). Again, the growth of the native tree species was significantly slower in garlic mustard-conditioned soil than in any of the other soil types used in this experiment.

Roberts and Anderson (2001) developed an experiment designed to evaluate the effects of garlic mustard extracts on AMF, and to observe what those effects may do to both root and plant growth. In order to determine this effects, they placed spores of an AMF on the surface of a plant culture medium that was prepared either with or without a garlic mustard leachate. This leachate was created through soaking the plant in sterile distilled water for 24 hours, sieving away any large particulate matter, and disinfecting. Two types of medium were used for this part of the experiment – one that contained only the garlic mustard leachate, and one that contained only water.

None of the AMF spores within the containers of garlic mustard leachate showed any germination. However, 7 out of 10 containers showed germination that contained only water. A second trial showed very similar results, except that even more germination occurred in the control containers.

Another part of Roberts's and Anderson's experiment was observing the colonization of AMF on the roots of a plant with high affiliation with the fungi. Sterilized tomato seeds were grown in a greenhouse with different mediums. After they had grown, the spores were examined for germination, and roots were examined for length and AMF colonization.

Again, no AMF germination occurred where the garlic mustard leachate was present, while 7 of 10 tomato plant seedlings were colonized by AMF. In addition, those plants who had grown without garlic mustard leachate showed significantly longer root lengths than those grown in the treatment containers.

From these experiments, Roberts and Anderson concluded that the release of glucosinolate chemicals from garlic mustard plants did inhibit the germination of AMF spores, and may even have a negative effect on the germination of seeds of plants that engage in relationships with AMF. Plants with shorter roots are less likely to acquire as many nutrients, and are also less likely to be colonized by AMF. These negative effects can harm the growth of native plants who form symbiotic relationships with these fungi, and encourage the growth of garlic mustard and other non-mycorrhizal species.

A way in which garlic mustard can harm the mycorrhizal fungi colonies in an ecosystem is by reducing the mycorrhizal inoculum potential, or MIP, of the soil (Roberts and Anderson, 2001; Anderson, 2010). AMF spores and hyphae (both in roots and in the soil) factor in to produce the MIP of a soil, due to their ability to initiate further colonization. Reducing the potential for AMF to form new colonies  can negatively impact the growth, reproduction, and competitiveness of native species. Thus, an abundance of garlic mustard will lead to a decline in the numbers of native species, which in turn means that the root mass of AMF host plants will be reduced (Anderson et. al., 2010). In addition, areas where the allelopathic chemical produced by garlic mustard has entered the soil will take a while to successfully recover.

Roberts and Anderson also included MIP experimentation in their study. Their study site included a white oak- and hickory- dominated forest in McLean County, IL, and Indiana Dunes National Lakeshore, in Porter, Indiana, where red oak and red maple are dominant.  30-meter transects were laid inside the forest; at 3-meter intervals, a quadrat was placed. 4 soil samples were taken from each quadrat and used to grow a corn root bioassay. Their results showed a statistically significant negative correlation between garlic mustard density and the soil's MIP.

Anderson et. al. (2010) conducted a study in Normal, IL in which they also observed the effects of garlic mustard removal on the MIP of the soil in the area where the plant had been abundant. A total of 120 plots were set up in this forest, half from which garlic mustard was removed. From 2005 to 2007, they removed the garlic mustard plants during late May. Plant cover (native plants, specific species, bare ground)  in those plots didn't significantly change during those three years between plots with garlic mustard and without garlic mustard.

In 2008, however, removal occurred during mid-April. After this early removal, spring dominant native species showed a significant increase in abundance and plant cover between removal and control plots, as opposed to no difference.

Soil samples were collected from randomly chosen plots in May 2009, and analyzed for MIP using corn roots as a bioassay. What the conductors of this experiment found was that after garlic mustard's continuous removal, especially when done early, the MIP of the soil in removal plots was greater than in control plots, where garlic mustard was not treated. Therefore, the removal of the invasive species was shown to have allowed for the soil in treated plots to recover over time. This study also shows how the presence of garlic mustard can decrease the number of native species and their success.

*Garlic Mustard Management Techniques*

Because garlic mustard is a hardy invasive species, it is hard to completely eradicate from many areas. However, it is possible to control the quantity and spread of the existing plants within a given site. There are few methods that can be utilized for controlling garlic mustard, many of which are common techniques for any invasive species removal.

Mechanical removal of the plant involves the use of tools to cut the stem of the plant at ground level. Clippers, scissors, or weed-whackers are common tools used in this removal method. Cutting is typically done during the second year of growth, when plants are at their tallest, and is most effective when the plant is already under stress (NRCS). When using larger tools such as lawnmowers or weed-whackers, great caution should be taken to avoid harming desirable native species.

Burning is often used in areas experiencing large infestations of garlic mustard. A hand torch or other device is used to start a controlled prescribed burn to a specific part of the land. This method has a few specific guidelines, however. According to the NRCS, burning of garlic mustard stands should take place before the plants grow rosettes and are able to distribute seeds. In addition, treatment by burning should be done every 3-4 years to eliminate both plants and dormant seeds lying in the seed bank. This method also poses a risk to native plants, if it is not performed before they grow in. Causing such disturbance to the landscape, should the fire not be hot enough to totally remove all life, could instead provide the perfect habitat in which new garlic mustard plants can grow and flourish.

Hand-pulling of garlic mustard is effective in small areas of garlic mustard growth. When a garlic mustard plant is pulled, the entire root system must be removed, as new plants can grow from roots that remain in the ground (NRCS). However, disturbing the soil by removing multiple plants may cause unintentional damage to the soil, or encourage seeds in the seed bank to sprout. Removing only the flowers and seed-heads is a widely-used alternative, but this would have to be performed multiple times during the growing season.

Chemical treatment has proven to be an effective control of garlic mustard. A solution containing 5% glyphosate or less can be applied to the rosettes, which kills off the rest of the plant. However, because it is an herbicide, it will harm most grasses growing around the treated plant (NRCS).

While all of these methods are effective at temporarily treating small areas, there are a few problems regarding the control of garlic mustard. These treatments are considered temporary unless applied every year for at least 4 years. New garlic mustard plants will sprout every spring, given an adequate number of seeds in the seed bank; a one-time treatment of adult plants will not be effective at long-term control of an invasion. Treatment occurring over many years will prevent new seeds from entering the seed bank while slowly depleting the bank of any garlic mustard seeds. Often, there are not enough resources or labor for treatment to occur over a long period of time. Mayer et. al (2011) report that control methods do not offer any long-term control over large areas.

Most of these treatment methods are also only doable in small areas. Pulling and cutting, for example, are time-and-labor-consuming, and are implausible to execute over large infestations, especially if the garlic mustard plant has formed dense stands (Mayer et. al., 2011). Because chemical treatment must be applied only on the rosettes of the plants, this method would also be difficult to implement in large stands. Often, there are not enough laborers or time to treat large areas with this method.

The one method that could be applied over a large area of infestation is burning, as long as the fire is controlled. However, burning must also occur year after year, and the flame must be hot enough. If the fire is too cool, or the area is not burned thoroughly, it could instead provide the kind of open, disturbed environment in which garlic mustard flourishes. In fact, all of these removal practices create large and open areas (Mayer et. al., 2011).

Herold et. al. (2011) conducted an experiment to observe the effect of both early and late removal of juvenile (first-year) and adult (second-year) garlic mustard plants in a deciduous forest. They intended to examine "competition between garlic mustard and native spring-dominant species including spring ephemerals, and between first-and-second year garlic mustard." Adult garlic mustard was removed to see the effects of the removal on both native herbaceous species and juvenile garlic mustard plants. Removal was done in both spring and summer, to compare the effects of early vs late treatment (referring to the growing season). First-year garlic mustard and native plants were significantly affected by the removal of adult plants; however, the treatment actually had a greater effect on the juvenile garlic mustard than on native plants. Neither treatment or site location affected second-year garlic mustard cover. In addition, treatment didn't significantly affect dominant spring and summer native species.

The effects of treatment on juvenile garlic mustard supports the hypothesis that adult garlic mustard plants are important and prominent competitors of the younger plants. This study, which removed adult plants from the landscape, suggests that juvenile plants suffer due to the shade caused by adult plants, and the space that they take up in the soil. Another implication of this is that the extensive management of adult garlic mustard only will encourage the survival of juvenile garlic mustard plants. Despite this, the authors suggest that treatment of garlic mustard should focus on adult plants and repeated over years to ensure a decrease of garlic mustard's presence in the seed bank. In areas with substantial cover, hand removal is recommended by the authors over herbicide use. However, as mentioned earlier, this is often impractical given the labor and time that would be required.

Another study on removal was done in 2013 by Shartell et. al. Instead of focusing on one removal method, this study implemented hand pulling, herbicide use, and burning, along with different combinations of those three methods. The experiment, conducted in Michigan, took place in a deciduous forest with rich soil and high productivity. Five different treatments were implemented overall:  hand pulling, herbicide, pulling/herbicide, scorching, and pulling/scorching. The control plots were left untreated. Hand pulling involved removing the entirety of adult garlic mustard plants. The glyphosate herbicide was applied evenly over the area with a hand-held sprayer. Scorching was done using a propane torch. In combination treatments, pulling was done first, then immediately followed by herbicide application or scorching.

Before treatment, the abundance of garlic mustard plants did not really differ from plot to plot. As expected, the control plot remained mostly unchanged over time in terms of plant abundance.

Unlike control plots, treatment plots underwent various degrees of change in garlic mustard abundance. A month after removing garlic mustard plants by hand, garlic mustard seedling abundance was higher than in control plots, though there was no statistically significant difference. Adults were also still present after one month; additionally, there was no significant difference in adult garlic mustard abundance from the control plots to the hand pulling plots.

In plots where herbicide was used as treatment, the abundance of garlic mustard seedlings was significantly lower than in control plots, one month after treatment. In addition, after one month, no garlic mustard adults were present in the plot. That reduction in garlic mustard abundance did not last, however- one year after treatment, the abundance of garlic mustard seedlings was significantly higher in herbicide plots than in control plots.

When pulling and scorching were combined, juvenile abundance was lower than in control plots. After one month, garlic mustard adults had a very small presence in plots.

Combining pulling and herbicide led to a decrease in the abundance of juveniles, significantly more so than in control plots. Herbicide use again proved itself to be an effective treatment only temporarily; after a year, the abundance of juveniles was significantly higher than in controls plots.

Though prevalent in some plots, juvenile garlic mustard abundance did not significantly differ after a month between control, hand pulling, scorching, and pulling/scorching treatment plots. One year after treatment, all treatment plots except hand pulling had significantly lower abundances of adult garlic mustard plants compared to the control plots.

Diversity and richness declined in herbicide, pulling/herbicide, scorching, and pulling/scorching treatments when compared to controls. After a year, however, richness was only significantly lower in the herbicide plots. This demonstrates that treatments in this study, meant to suppress the growth and abundance of garlic mustard, were not effective following a year after implementation. Adults generally saw a decrease in abundance one month after treatment, but it is unlikely that it would last.

Broad-range treatments involving fires and chemicals posed greater problems than others. In plots that underwent herbicide or scorching treatments (including combination plots), juvenile garlic mustard abundance actually increased one year following treatment. This may be due to 'recruitment' from the seed bank of plants outside the treatment area; the effect treatments had on native plants; and/or garlic mustard's rapid growth and seed production and germination (Shartell et. al., 2013). The non-target nature of herbicide application and burning plants caused species richness to decrease almost immediately. Herbicide use, then, could have a greater effect on native understory plants than other treatments (though fall herbicide application may reduce decreases in understory diversity).

Overall, single treatments were shown not to be sufficient in controlling garlic mustard infestations. Instead, they may increase garlic mustard's success for the exact reasons stated earlier. The authors concluded that treatment techniques that don't completely remove cohort, juveniles, or adults of garlic mustard most likely will not be effective over time. It is recommended that treatment should extent for a multiple-year period, and treatment should be avoided completely when a multiple-year treatment is not planned or feasible. As these prior studies show, there is no good long-term solution to the infestation of garlic mustard over large areas. Over time, garlic mustard will continue to negatively impact the environment in which it is growing, eventually forming dense, uncontrollable monocultures.

Biological control has recently been considered a potential treatment option for garlic mustard. This method could provide a solution for the lack of practical long-term control that the other treatment options present. There are only a few insects that have been recognized for biological control of garlic mustard; out of six species, five are weevils and one is a beetle. The weevils all belong to the genus *Ceutorhynchus. C. alliariae* and *C. roberti* adults eat leaves; their larvae eat the stems and leaf petioles, while *C. scrobicollis* mines the roots. These three species have been determined the most promising. Since these species are not native to North America, they had to be brought in from out of the country and were only released from quarantine in 2012. Experimentation on their effectiveness at controlling garlic mustard is still being executed.

**Experiment Plan and Hypothesis**

For my experiment, I intend to focus on the effect that garlic mustard has on the mycorrhizal fungi of woody species growing in Wisconsin. As this invasive species is growing and thriving in southern Wisconsin counties, it is important to understand the extent to which garlic mustard might affect the nutrient intake of those tree species that are abundant and prolific in those areas of the state. I also intend to see if physical properties of garlic mustard, such as its abundance, height, and leaf size / span hinder or even prevent the growth of native herbaceous species due to lack of space or sunlight. It is important to understand the extent to which garlic mustard interferes with the successful growth of native species.

My approach shifts focus from garlic mustard's effects on few species, to its effects on an ecosystem as a whole. The intensity of this species' impact on Wisconsin forest ecosystems may require increased and long-term removal and managing to prevent harm or impaired growth to native species. This experiment is designed to be a multi-dimensional analysis of garlic mustard's invasive effects in a well-established, moderately disturbed Wisconsin forest.

The objectives for the experiment are to:

- Determine if garlic mustard hinders the growth and activity of myccorhizal fungi associated with native woody trees.
- Determine if the spread and growth of garlic mustard significantly decreases the growth and abundance of native herbaceous species.

Using data collected during my experiment, I will be able to evaluate the below-and-aboveground effects of garlic mustard on species in the Arboretum. In addition, I will observe the effectiveness of the removal of garlic mustard in areas where it had previously been found in abundance.

Based on my research, I predict that the colonization of mycorrhizal fungi on the roots of native woody plant species will be reduced in areas where garlic mustard is thriving and abundant. garlic mustard in these areas is expected to emit the same allelochemicals that were found to be present in other studies. If this is so, then the spore germination and colonization of arbuscular mycorrhizal fungi on plant roots in these areas should be negatively affected.

I also predict that 2) the presence, abundance, and leaf spread of garlic mustard will hinder the growth and development of native herbaceous species.

## Methods

*Study Site*

This experiment took place in the Arboretum belonging to Carthage College in Kenosha, Wisconsin. This arboretum serves as a good model for a disturbed deciduous forest in the Midwest. Over the past couple of years, it has seen an increase in garlic mustard growth, and in many areas of the arboretum, it has already begun to form a monoculture. Trees common in this arboretum include oak, maple, and ash trees. Herbaceous species found in the area include wildflowers such as aster and goldenrod. Data were collected starting October 16, 2013, and samples were collected every week until November 17.

*Alliaria petiolata and Mycorrhizal Fungi*

For the first portion of my experiment, I set up six plots in different areas of the park. Plots 1 and 2 contained no garlic mustard, and were used as control groups. Plots 3 and 4 had an abundance of garlic mustard. Plots 5 and 6 initially contained garlic mustard, but each plant, including roots, was removed by hand. These plots were established as close to each other as possible, but the plots each contained oak, sugar maple, and ash trees. Each plot was 8 x 8 meters; the plots were dense enough to include at least 3 species of trees, but not too big as to render experimentation difficult. This meant that plots were established as close to one another as possible, though plots that contained no garlic mustard were farther away from the other plots.

Each tree in the plot was identified prior to any data sampling. The number of trees of each species were counted, the diameter at breast height (dbh) was measured for each tree using a diameter measuring tape, and the number of seedlings in each plot were counted.. The abundance of garlic mustard and the height of each plant was recorded. For one of the plots containing garlic mustard,, the abundance of garlic mustard plants and the height of each plant was recorded before removal occurred (this data for the second plot that underwent removal was not able to be obtained before the garlic mustard plants were removed).

Five soil samples were taken from each plot near the roots of the woody plants and *A. petiolata.* All of the soil samples will be tested for pH, nitrogen, and phosphorous levels. These will be tested for in order to determine the condition of the soil in each plot; if soil quality differs from site to site, that may have an effect on the growth of mycorrhizal fungi that is unrelated to garlic mustard.

*Aboveground vs Belowground Effects*

For the second part of my experiment, I set up six 1x1 meter plots in the Arboretum. These particular plots were not placed within my other plots, but rather in sections of the Arboretum with a more open canopy. I chose these plots by tossing rock over my shoulder, then setting up my plot, with the landing site of the rock serving as the placement for the top right corner of the plot. Plots 1 and 2 were areas in which garlic mustard was growing in abundance and will serve as control groups.  To determine correct height, experimentation with cutting garlic mustard was conducted prior to treatment of garlic mustard in these two plots. Plots 3 and 4 also contained garlic mustard, but the plants were cut at heights until plants were between 10 and 15 centimeters. These heights still allow for the plants to grow but remove flowers and leaves. Research has shown that this specific height is any height that leaves at least 10 centimeters of stem remaining above ground, enough for the plant to potentially recover from (Nuzzo, 1990).

Finally, plots 5 and 6 also originally contained garlic mustard, but the invasive species plants, including roots, were removed by hand. The plots contained two of the same native herbaceous species, aster and goldenrod.

The abundance of garlic mustard plants was recorded prior to treatment. For Plots 1 and 2, the height of each *A. petiolata* plant was measured. Average leaf length was meant to be measured during the entirety of the time, but due to time restrictions and labor, this measurement was taken for only two weeks, from November 2 to November 17. For plots 3 and 4, in which *A. petiolata* had been cut, the height of the stems after cutting was measured. Approximate leaf cover of all garlic mustard plants was calculated in each plot. The height of each native herbaceous species was measured, along with its abundance and total leaf cover of each species itself.

The removal and cutting of garlic mustard hold significance. Cutting of the invasive species at a point where most leaves and flowers are removed, yet it is still able to grow, could help determine if overcrowding and shade are the problems that this invasive species presents to native plants. Pulling 100% of the garlic mustard plants in one of the plots will allow for a control group in which the native plants are able to grow without potential interference from the invasive species; the plants must be there originally before pulling because it allows for observations to be made about the growth, spread, and species abundance / richness of native plants after the plot recovers from the removal of the invasive species.

*Data Analysis*

All data will be analyzed statistically, using *ANOVA*. For the first part of the experiment, the presence of garlic mustard was the treatment variable; AMF colonization and tree diameter were the response variables. The second part of my experiment used the same treatment variable as the first; for this part, the response variables included native plant abundance and height. The results from these tests indicate whether or not there is a statistically significant difference between the different plot types. Averages of tree count and dbh, garlic mustard and its height, and native herbaceous species abundance and height will be recorded.

## Results

*Garlic Mustard and Mycorrhizal Fungi*

Testing of the soil samples (pH, nutrients, root dyeing) was not able to occur. Therefore, no data can be provided about mycorrhizal fungi colonization within my plots.

Garlic mustard showed great abundance in Plots 1, 2, 3, and 4. In these plots, garlic mustard also provided much leaf cover among herbaceous species - 55% in Plot 1, 70% in Plot 2, 60% in Plot 3, and 80% in Plot 4. Removing garlic mustard from Plots 3 and 4, then, opened much of the understory in these areas, though no observable changes in the understory occurred during experimentation.

Oak, ash, and maple species were present in all six plots. Overall, oak was more present than other species in these plots (Fig. 1). The average diameter of trees in each plot type was averaged, and shown by plot type in Figure 2. There was no significant difference, in any of the tree species, of size between plots with garlic mustard and plots without garlic mustard. The *ANOVA* tests produced a p-value of 0.12 for oaks, 0.17 for ash, and 0.25 for maple. These values show that there is no significance in the difference of diameters between plots with garlic mustard, with garlic mustard removed, and without garlic mustard.

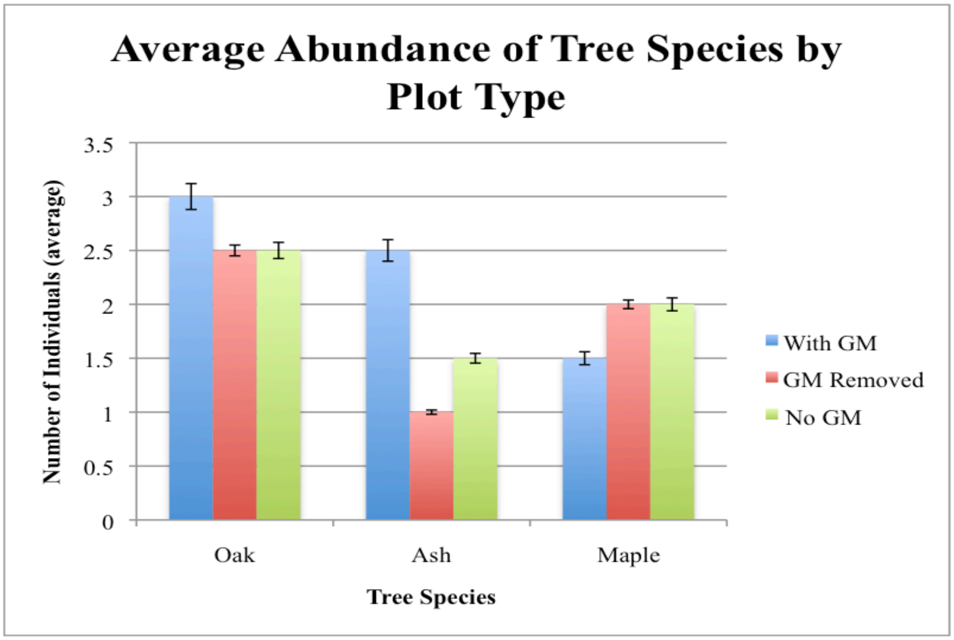


**Figure 1:** The abundance of tree species by plot. Plots 1 and 2 contained garlic mustard; Plots 3 and 4 had garlic mustard removed; Plots 5 and 6 contained no garlic mustard.

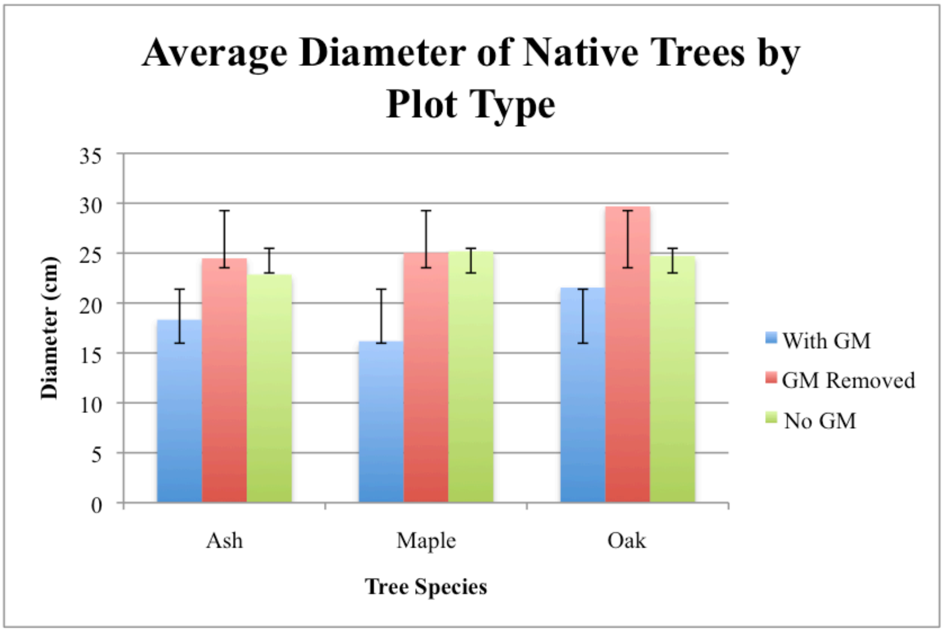


**Figure 2:** This chart shows the average diameter of native tree species, based on plot type.

*Garlic Mustard and Native Herbaceous Species*

For this part of my experiment, six 1x1 meter plots were set up in the Arboretum. In all six of these plots, garlic mustard was by far the most abundant species (Fig. 3). Aster, goldenrod, and nettle were present in all three plots, though their numbers were fewer. Because of this difference in abundance, the leaf cover of garlic mustard plants in all three plots was greater than leaf cover provided by the native species.

Over the course of my experiment, there was little, if any, change in the heights and abundance of both the garlic mustard plants and individuals of the three native herbaceous species studied. Figure 4 shows the average height of the species in each plot type - for the plots where garlic mustard was cut or removed, the average height was calculated before the treatment of these plants. Any increase or decrease in height that occurred in this experiment did not occur in more than 10 individuals total, even after the invasive species was removed or cut, and the changes in height were not significant enough to alter the averages.

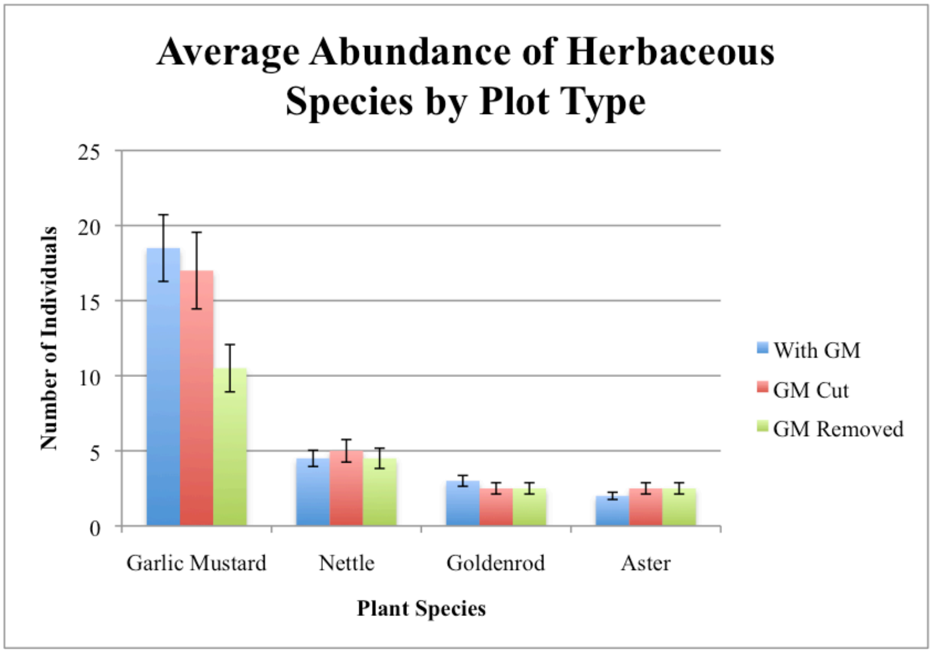


**Figure 3:** The abundance of herbaceous species by plot. Garlic mustard is the most abundant in all six plots.

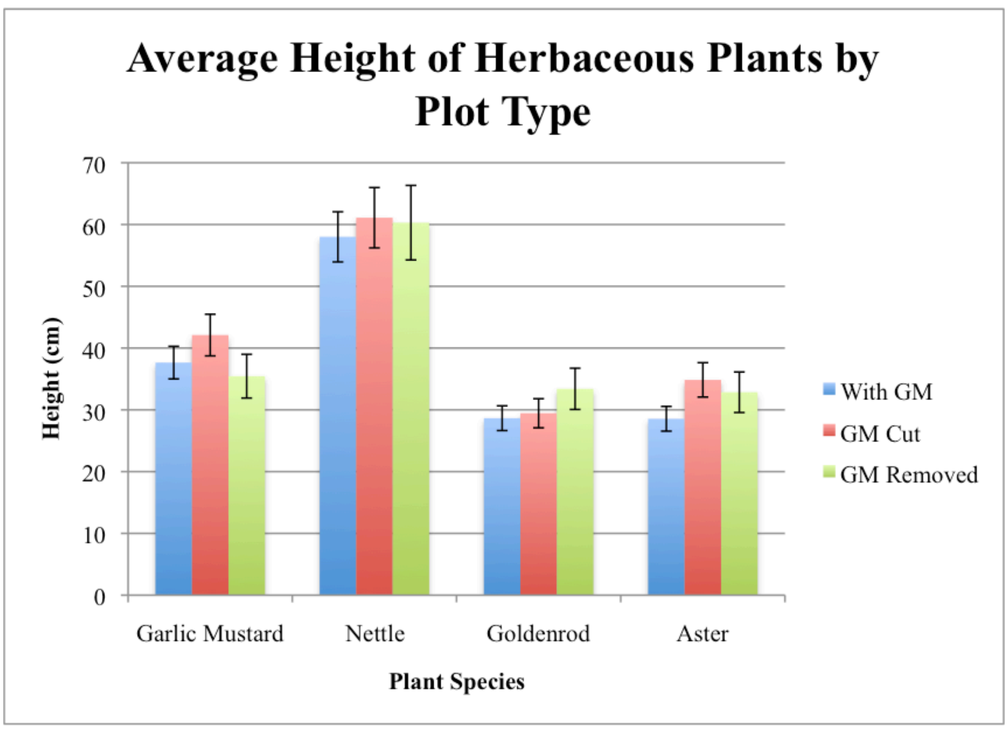


**Figure 4:** The average height of herbaceous plants by plot. The height data for garlic mustard in Plots 3, 4, 5, and 6 was retrieved prior to treatment. There were no significant changes in plant height over the course of the experiment.

**Discussion**

My first hypothesis addressed the relationship between mycorrhizal fungi and garlic mustard, stating that the presence of the invasive species would have led to less mycorrhizal fungi colonies on plant roots. Unfortunately, no data from the soil samples were able to be collected. Time constraints and difficulties obtaining a suitable dye prevented root dyeing from occurring in time.

My original methods included removing all roots from my soil samples and staining them for identification of mycorrhizal fungi colonies. I intended to count the mycorrhizal fungi colonies on the root samples, and observe their size, shape, and abundance on the roots. This would have been done using the Treseder methods, which includes: rinsing the roots in deionized water; heating roots in 2.5% KOH for 10 to 30 minutes; re-rinsing the roots; creating an acidic glycerol/trypan blue and an acidic glycerol dye; soaking the roots in various chemicals and allowing the solutions to sit (Koske, 1989). The trypan blue dye is standard for mycorrhizal fungi root staining, as it allows for one to see the arbuscules from the fungi within the root itself.

Based on my research, I would have expected to see less colonies of arbuscular mycorrhizal fungi present in plots where garlic mustard was abundant, and believe that further studies would support this. I also would expect to see higher levels of nitrogen and phosphorus in the soil samples taken from plots with garlic mustard than control plots. This is due to garlic mustard's ability to increase the availability of these nutrients in soil (Rodgers et. al., 2008). Removing the garlic mustard may lead to changes in these levels over time; however, the short time span in which experimentation occurred would likely not be enough time to see what effects removal has.

With a lack of data regarding the roots of plants from the plots, no conclusions can be drawn about the first hypothesis of my experiment. However, the data collected about native tree species, garlic mustard abundance, and the average diameters of each species does not indicate that the presence of garlic mustard has affected the growth of these trees. Between the three plots, there was no statistically significant difference in diameter at breast height (dbh) between individuals of the same species. Trees of all species growing in plots with garlic mustard showed no true differences between those growing in the two plots that were free of garlic mustard. Because garlic mustard's negative effects on mycorrhizal fungi have shown to decrease the competitive ability of native species, I was expecting the trees that had been growing in garlic mustard plots to be smaller, given that garlic mustard has been present in the Arboretum for a few years. This expectation was based on the overall abundance of garlic mustard in my plots - so much garlic mustard plant litter, along with years of infestation, may have had an effect on the mycorrhizal fungi colonies of these trees, reducing their ability to compete over the years. However, this was not supported by my results. It can therefore be concluded that neither the abundance nor presence of garlic mustard correlated with the diameters of trees in the Arboretum.

Data from this experiment does not support the hypothesis that the presence, abundance, and leaf spread of *A. petiolata* will hinder the growth and development of native herbaceous species. However, the height and growth of the three native species - stinging nettles, goldenrod, and white aster - did not show any significant change at all during the course of the study. Any increase in growth that occurred was by a single millimeter per plant. This was expected in the plot that contained garlic mustard, as the invasive species may have prevented any positive native plant growth. However, in plots where garlic mustard was cut or removed, the native plants did not increase in height. This could be due to multiple factors. Sampling occurred for only a few weeks, starting in late fall. Any growth that would have occurred during this seasonal transition would be very minimal. Had sampling began earlier, and/or occurred in the spring and summer, there may have been a larger change in plant size over time. It is also possible that cutting garlic mustard plants down, or removing them from the plots, had no effect on the growth of native plants at all. Given the short time span and season in which sampling occurred, however, I feel that this cannot adequately be concluded without further sampling. Regardless, the data that was collected during this experiment did not indicate that removing or cutting garlic mustard will improve the growth of nettle, goldenrod, or white aster.

Should this experiment be repeated in the future, a few changes would be made to the experimental procedure. For example, I was not able to collect my own data for the first two weeks of experimentation. This is because I broke my foot at the beginning of October, leaving me on crutches for a month. Accessing my plots in a well-grown forest on crutches was not possible, and so I received help from two friends. These friends took all of the samples for the first week of my experiment with my instruction. Though I showed them how to use the materials and instructed them on how exactly they were to sample, there were still slight errors in data collection. For example, most diameters were measured at the same height (1.5 meters), but some measurements were made at heights below or above the specified value. In addition, they did not measure leaf length of understory plants due to a lack of adequate time. The dbh measurements are not expected to significantly affect data; however, there is not enough leaf length data to accurately draw a conclusion about change over time. I was able to start collecting data myself after the second week; because of this, there may be slightly more consistency between samples collected after this point than samples collected in the first two weeks of experimentation.

In addition, data might have shown stronger patterns if the sampling had been done in a different season. In my original experimental design, the first samples were supposed to be taken at the beginning of *A. petiolata*'s growing season in early July. Samples would then have been taken every other week; this would have given enough time for any changes to occur while still allowing for a good amount of experimental repetition. The last samples would have been collected in early October, which marks the end of the growing season for *A. petiolata* and various other species of plants. If data collecting was done in this manner, it would have allowed for observations to be made on how arbuscular mycorrhizae and herbaceous plants are affected as *A. petiolata* grows and develops. In addition, comparisons could be made within the plot itself, and not from plot to plot; a single plot's patterns could be observed from when *A. petiolata* was present but had just began growing, to when *A. petiolata* had grown and spread across the study site. However, I was out of the country from the beginning of July until the end of August, and therefore could not begin sampling when I had first planned to. Adjustments in thesis and methods further delayed sampling, and as stated before, I broke my foot the weekend before I was set to begin sampling.

Data for the first part of my experiment was originally collected for nine plots instead of six. However, a significant part of that part of my procedure was that each plot contain at least three species of trees, so that data could be collected for a larger range of species. This was designed with the intention of sampling at a larger site than my actual one, with more species of plants growing in the area. In my site at the Arboretum, limited space meant that finding an 8 x 8 meter plot with at least three different tree species was more difficult than originally anticipated; it was because of this that I increased the area of my plots. I was only able to successfully find six plots; in two of the nine plots, one tree species was only represented by one individual, which would not have provided enough data to compare among other sites. The last plot only had two species of trees represented. Because of this, the data from these plots was ultimately removed from the statistical analyses.

In order to fully and more accurately draw conclusions about garlic mustard's effects on the growth of fungal colonies and understory plant species, future experiments should take place starting in mid-spring and continue until the end of fall. An ideal timeline would be from May or June until the last week of October. This allows for samples to be taken as all species of trees and plants are growing and changing in the ecosystem. Sampling that takes place over multiple years, if possible, would be ideal as to ensure that the trends observed are fairly regular. The sampling site should be in an infested forest area in which plots larger than 8 x 8 meters could be constructed, which could enable more root and soil samples to be taken, should labor and time permit. In addition, it would be beneficial for future studies to focus on seedlings in these plots, as opposed to much larger trees.

Understanding the effects that garlic mustard has on organisms in Wisconsin forests can aid in the consideration of control and management of this invasive species. Though I was not able to provide evidence through my own experiment, other studies do prove a very strong correlation between the presence of garlic mustard and the reduction in arbuscular mycorrhizal fungi colonization. A reduction in arbuscular mycorrhizae fungi can be problematic for many species of plants, including native woody species such as maple, ash, and oak. Through their relationship with the fungi, these tree species are able to obtain more essential nutrients such as nitrogen and phosphorous than those trees that do not form relationships with fungi. A greater access to nutrients means that the trees are able to outcompete other trees. Should these arbuscular mycorrhizae colonies shrink or are lost altogether, then those individuals that share a relationship with the fungi will see a reduction in their ability to compete within their ecosystem. This can lead to smaller tree sizes and diminished populations (Vaughn and Berlow, 1999; Stinson et. al., 2006). If these tree species are growing in areas where they experience much competition with other species, and garlic mustard is introduced to the area, the populations of the species with arbuscular mycorrhizae will not be able to thrive as well as their competitors.

In addition, native herbaceous species not only were unaffected by the cutting of garlic mustard, but they also did not show an increase in growth once the leaves and more spacious parts of the invasive species were removed. This may potentially indicate that garlic mustard not only affects the growth of native plants by overcrowding them, but may also compete with them underground for resources. Therefore, simply cutting the plant is not an effective method of controlling the spread of this plant.

It is clear that simply letting garlic mustard grow in any area is not an option that should be pursued if the health and diversity of the ecosystem are meant to be preserved. Cutting the plant may remove the flowers and leaves, but that will not be effective in preventing garlic mustard from negatively affecting the growth of species around it. Pulling the entire plant ensures that the root system is removed as well. However, as shown in various studies such as the one done by Vaughn et. al., the allelopathic chemicals emitted by the plant that harm fungi colonies do not only enter the soil through the plant's roots. Any litter and organic matter left behind by garlic mustard is capable of releasing those same chemicals (Vaughn and Berlow, 1999). In addition, hand removal is only a temporary solution and would not be effective in limiting garlic mustard in the long term. An area as large as the arboretum would prevent hand removal from being effective without adequate labor and time. Therefore, further long-term management techniques may need to be considered in order to ensure that garlic mustard does not cause further harm to ecosystems.

Future studies may include the implementation of various management techniques to see if the colonization of mycorrhizal fungi improves with those methods in place. Methods may include the ones applied in prior studies, or different combinations of treatments.

Garlic mustard is an invasive species that is still being spread in Wisconsin, and it is showing westward expansion as well. Wisconsin counties that are beginning to experience garlic mustard infestations should understand the effects that this invasive species can have in Wisconsin forests, and make proactive decisions in preventing the infestation from spreading too far before it becomes difficult to control.

**Acknowledgements**

I would like to thank Dr. Tracy Gartner for helping me find a topic that I was interested in and turn an idea into a paper. I would also like to thank Dr. Sarah Rubinfeld for her continued feedback and support during the past year, and for always steering me in the right direction.

Many thanks are due to the seniors in the Environmental Science program. Without such a kind, intelligent, and caring group of people, this thesis process would have been much more difficult. Thanks for the good times and the words of encouragement during the hard times.

I would also like to thank my professors for the past four years, for being understanding and helping me find what it really is that I am passionate about.

Of course, thanks are due to be given to those two students who aided in collecting data for me while I was stuck on crutches – without them, this thesis would not have been complete.

Lastly, I would like to thank both of my families – biological and fraternal – for offering their support and love when things got difficult.

**References:**

Anderson, Roger C, Shivcharn S Dhillion, and Timothy M Kelly. 1996. *Aspects of the Ecology of an Invasive Plant, Garlic Mustard (Alliaria petiolata), in Central Illinois*. Restoration Ecology. 4.2: 181-191. Print.

Cavers, Paul B., Heagy, Munrnr I., Kokron, Robert F. 1979. *The Biology of Canadian Weeds.* 35. *Alliaria petiolata* (M. Bieb.) Cavara and Grande. Can. J. *Plant Sci*. 59:.217-229.

Dighton, John. *Fungi in Ecosystem Processes*. New York: Marcel Dekker Inc., 2003.

Durka, W., Bossdorf, O., Prati, D., & Auge, H. 2005. *Molecular evidence for multiple introductions of garlic mustard (Alliaria petiolata, Brassicaceae) to North America.* Molecular Ecology, 14(6), 1697-1706. doi:10.1111/j.1365-

Forest Invasive Plants Resource Center (FIPRC). *Garlic Mustard (Alliaria petiolata)*. Retreived from http://na.fs.fed.us/spfo/invasiveplants/factsheets/pdf/garlic-mustard.pdf.

Gianinazzi-Pearson, Vivienne. 1996. *Plant Cell Responses to Arbuscular Mycorrhizal Fungi: Getting to the Roots of the Symbiosis.* The Plant Cell 8.1871-1883

Herold, J., Anderson, M., Bauer, J. T., Borowicz, V., & Anderson, R. C. (2011). *Comparison of the Effect of Early and Late Removal of Second-Year Garlic Mustard (Alliaria petiolata) on First-Year Plants and Deciduous Forest Spring and Summer Dominant Herbaceous Groundlayer Species in Central Illinois, USA*. Ecological Restoration, *29*(3), 225-233.

Hodge, A., Campbell, C.D., & Fitter, A.H. 2001. *An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material*. Nature, 413, 297-299.

Kleinstein, D. 2001. Garlic Mustard (Alliaria petiolata). *Introduced Species Summary Project*. Retrieved March 11, 2013.

Koske, R.E., and Gemma, J.N., 1989. Mycological Research 92(4):468-488.

Mayer, M., Hudson, W., & Robbins, G. 2011. *Annual Report on Garlic Mustard, Alliaria petiolata, an Alien Invader of New Jersey's Deciduous Forests.* New Jersey Department of Agriculture.

Meekins, J.F. and McCarthy, B.C. 1999. *Competitive Ability of Alliaria petiolata (Garlic Mustard, Brassicaceae), an Invasive, Nonindigenous Forest Herb.* International Journal of Plant Sciences , Vol. 160, No. 4 (July 1999), pp. 743-752.

Nuzzo, V. In press. 1990. *Experimental control of garlic mustard (Alliaria petiolata (Bieb.) Cavara & Grande) in northern Illinois using fire, herbicide, and cutting.* The Natural Areas Journal.

Pimentel, D., Zuniga, R., Morrison, D. 2006. *Update on the environmental and economic costs associated with alien-invasive species in the United States.* Ecological Economics, Volume 52, Issue 3, 15 February 2005, Pages 273-288, ISSN 0921-8009.

Ren, M. X., & Zhang, Q. G. 2009. *The relative generality of plant invasion mechanisms and predicting future invasive plants.* Weed Research, 49(5), 449-460.

Roberts, K.J., and R.C. Anderson. 2001. *Effect of Garlic Mustard Extracts on Plants and Arbuscular Mycorrhizal (AM) Fungi.* American Midland Naturalist. 146(1): 146-152

Rodgers, V.L., Wolfe, B.E., Werden, L.K. & A.C. Finzi. 2008. *The invasive species* Alliaria petiolata *(garlic mustard) increases soil nutrient availability in northern hardwood-conifer forests.* Oecologia, 157: 459-471.

Shartell, L.M., Nagel, L.M., and Storer, A.J. 2013. *Efficacy of Garlic Mustard (Alliaria petiolata) and Effects on Forest Understory Plant Diversity.* Forests, Volume 3, 3 August 2013, Pages 605-613, ISSN 1999-4907.

Stinson, K.A., Campbell, S.A., Powell, J.R., Wolfe, B.E., Callaway, R.M., et al. 2006. *Invasive Plant Suppresses the Growth of Native Tree Seedlings by Disrupting Belowground Mutualisms.* PLoS Biol 4(5): e140. doi:10.1371/journal.pbio.0040140

Vaughn, S.F., and M.A. Berhow. 1999. *Allelochemicals Isolated from Tissue of the Invasive Weed Garlic Mustard. Journal of Chemical Ecology.* 25(11): 2495-2504

Vilà, M., Espinar, J., Hejda, M., Hulme, P., Jarošík, V., Maron, J., & … Pyšek, P. 2011. *Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems.* Ecology Letters, 14(7), 702-708.