Appendix C

Result 3: Introduction and evaluation of Garlic Mustard biological control agents in Minnesota

LCCMR Final Report

Title: Monitoring garlic mustard (*Alliaria petiolata*) in anticipation of future biocontrol release

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EXECUTIVE SUMMARY

Garlic mustard (*Alliaria petiolata*) is an invasive forb that is native to Europe and has become abundant in forested regions in the US. Garlic mustard can form dense populations in the forest understory and crowd out native species. Garlic mustard also exudes allelopathic chemicals which can impede seed germination and reduce populations of native mycorrhizal soil fungi. Three *Ceutorhynchus* weevil species are being studied to determine their suitability as biological control agents for garlic mustard. In 2008, a proposal to approve the release of *Ceutorhynchus scrobicollis* was submitted to the USDA Technical Advisory Group. In anticipation of the future release of garlic mustard biological control agents, a garlic mustard population monitoring program was initiated in Minnesota in 2005.

Garlic mustard is a biennial and its population can vary widely from year to year. Several years of monitoring are necessary to provide an accurate assessment of the pre-release population and to understand normal levels of year to year population fluctuation. The populations can then be followed post-release to determine if the biological control agent had its intended effect of reducing garlic mustard. To monitor garlic mustard populations we used a nationally standardized protocol in which data is collected on garlic mustard population density and cover, garlic mustard plant heights and silique (seed pod) production, insect damage to garlic mustard, the cover of the associated plant community, and litter cover. Twenty permanent $0.5m^2$ monitoring plots were established at 12 sites throughout Minnesota. Data was collected each June and October from 2005 to 2007.

Three years of monitoring data show that garlic mustard is currently experiencing very little herbivory in Minnesota and that garlic mustard populations can vary considerably from year to year. Overall, garlic mustard plants had an average of 1.8% of their leaf area removed by herbivores. An introduced biological control agent could greatly increase the amount of damage garlic mustard is currently experiencing. At about half of the sites, population changes in garlic mustard from year to year are due to the biennial nature of garlic mustard. These sites tend to be dominated by either the 1st or 2nd year plants in any given year. For example, at Warner Nature Center, the density of adult garlic mustard cycled from 1 plant/m^2 in 2005 up to 85 in 2006 and down to 15 in 2007. The other sites had more stable or increasing garlic mustard populations. We also observed variation in garlic mustard adult plant height and silique production from year to year. It is expected that after biological control release, garlic mustard populations as a whole will decrease and shoot heights and silique production of individual plants will decrease as well.

We were also able to characterize the plant community in which garlic mustard is growing and estimate the potential for native species recovery should garlic mustard populations decrease. Sites with greater garlic mustard cover had lower native species richness and cover then sites with lower garlic mustard cover. After biological control agent release, we will be able to determine if garlic mustard is reduced, and if so, how native species populations respond. To determine which species are likely to germinate if garlic mustard populations are reduced, we collected soil samples to describe the composition of the seed bank at seven sites. We found that seeds of native species were more common than nonnative species. There are several sites that may need additional

restoration help because of a low proportion of native species in the seed bank and in the existing vegetation. However, other sites are likely to have better recovery as they have larger covers of existing native vegetation and a variety of native species present in the seed bank.

To further examine the impacts of garlic mustard on native species and the potential for native species recovery in the absence of garlic mustard, several greenhouse experiments were designed to explore the effects of garlic mustard's allelopathic root exudates. Activated carbon was used to apply treatments that negated the impact of the allelochemicals. We found that plants growing with garlic mustard were negatively impacted due to competition with garlic mustard; however, removing the allelopathic effect did not significantly improve plant performance. A second experiment determined that native plants did not have less above-ground biomass in soils in which garlic mustard had grown previously, indicating that there was no legacy effect of garlic mustard allelochemicals. While allelopathy did not have a direct impact on plants growing with garlic mustard or in garlic mustard soils, this does not exclude the potential for indirect effects due to negative impacts on the native mycorrhizal soil fungi upon which many native species are dependent. If native plants have difficulty reestablishing following a reduction in garlic mustard, it likely would be due to indirect impacts, not due to direct residual allelopathic impacts of garlic mustard.

Chapter 1

Garlic mustard (*Alliaria petiolata*) monitoring in anticipation of future biological control release

INTRODUCTION

Nonnative invasive species are one of the main threats to native species diversity (Williamson 1996, Schmitz et al. 1997, Wilcove et al. 1998). Nonnative species may displace native species as well as alter entire ecosystem processes (Mack et al. 2000, Mack and D'Antonio 1998). Garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande] is an invasive, nonnative species that is invading forested regions throughout the United States (Cavers et al. 1979, Meekins et al. 2001). Garlic mustard is able to invade high quality forests in addition to disturbed areas (Nuzzo 1999). Garlic mustard is a concern because of its ability to invade high quality forests, form dense populations, and decrease abundance of native species (Blossey et al. 2001).

Due to the number of negative impacts of garlic mustard, a program was initiated to develop biological control agents (Blossey et al. 2001). Ideal candidate insects to be a biological control will only feed and complete its life cycle on the target organism. In this way, the biological control agent can reduce the population of the invasive plant and allow native species populations to return to non-impact levels. Currently, three weevil species are being tested at the University of Minnesota quarantine facility to determine their host specificity and their suitability as biological control agents. The three species are the root-crown feeding weevil *Ceutorhnychus scrobicollis*, and the stem-mining weevils *Ceutorhnychus alliariae* and *Ceutorhnychus robertii* (Blossey et al. 2001, Katovich et al. 2005). A proposal to approve the release of *Ceutorhnychus scrobicollis* was submitted to the USDA-APHIS Technical Advisory Group committee in the first quarter of 2008.

To determine if the biological control agents are effective at reducing garlic mustard, it is necessary to monitor the plant communities into which the insects are released. The impact of the control agents and the response of the plant community can be assessed by comparing data gathered before and after the insects are released (Blossey 1999). Garlic mustard is a biennial species which can cause populations to fluctuate significantly from year to year (Meekins and McCarthy 2002). Due to the variability of garlic mustard populations it is preferable to have several years of plant monitoring data before the insects are released. A grant for implementing and monitoring biological control releases was proposed to the Legislative-Citizen Commission on Minnesota Resources (LCCMR) and was accepted (Skinner 2005).

Garlic mustard is part of a complex community and its role in that community is not clear. Nonnative earthworms have invaded many forests and denuded the litter layer, altered soil processes, and decreased native species abundance (Bohlen et al. 2004, Hale et al 2005). Bartuszevige et al. (2007) found that garlic mustard seedlings had the greatest establishment in plots with litter removed versus control or litter added plots. Garlic mustard is often found in areas with little to no litter layer, implying that garlic mustard may succeed in sites that have been invaded by earthworms (Blossey et al. 2005). The overpopulation of deer in many areas has put additional pressure on native plants. Garlic mustard seems to be grazed less than natives and so may do better than natives in sites with high deer population density (Blossey et al. 2005). Garlic mustard itself may change soil properties. Garlic mustard has allelopathic root exudates which can inhibit germination in some species (Prati and Bossdorf 2004). The root exudates have also been found to have a negative impact on mycorrhizal fungi (Stinson et al. 2006). Many late-successional native species are dependent on mycorrhizae, so the loss of mycorrhizae can negatively affect native species abundance.

By monitoring garlic mustard populations and the associated plant community and environmental conditions we can begin to answer questions about garlic mustard impacts and the effectiveness of biological control agents. We can assess the level of herbivory garlic mustard is experiencing in Minnesota in the absence of biological control insects. Low herbivory levels indicate that existing herbivores are not having a large impact on garlic mustard populations and that the biological control agents could have a large impact. Monitoring data also allows a characterization of the year to year fluctuations in garlic mustard cover, population density, heights, and siliques (seed pod) production so normal population variation can be separated from long-term impacts of biological control agents. By monitoring the plant population growing with garlic mustard we can gain a better understanding of the relationship and impacts of garlic mustard on native and nonnative plant species.

METHODS

The main goal of the garlic mustard monitoring project was to establish long-term monitoring sites to characterize garlic mustard populations in anticipation of biological control insect release. Twelve monitoring sites were selected based on the presence of a garlic mustard population of sufficient size and population density to accommodate the plots and a willingness of the owners to refrain from any management to reduce the garlic mustard during the course of the study. At each site, 20 permanent 1m x 0.5 m plots ($0.5m^2$ quadrats) were established. To monitor the plant communities, we used the protocol developed by the Ecology and Management of Invasive Plants Program in 2003 (available at http://www.invasiveplants.net). Monitoring data is collected in June and October. Following the protocol, data is collected on 1st year garlic mustard plants (cover and number of individuals), 2nd year garlic mustard plants (cover, number of individuals, height, and number of siliques), cover of other species present, amount and type of insect damage to garlic mustard, ground cover, and litter depth. This information will allow us to determine if the biological control agents have their desired effects of reducing cover of garlic mustard, decreasing seed set of garlic mustard, and increasing native species cover.

In June 2005, garlic mustard monitoring plots were established and data was collected at five sites. All 12 sites were established by the fall monitoring data collection period in October 2005 (Table 1). In 2006 and 2007, data was collected from all 12 sites in June and October. City and county information is provided for each site in Table 2. All sites are upland deciduous forests, except for Coon Rapids and Fort Snelling which are floodplain forests (although flooding is currently rare due to management of adjacent rivers). There is also variation from site to site in terms of dominant tree and herbaceous layer species composition.

Data is summarized by season and site. In all graphs, the error bars are standard errors. Garlic mustard is a biennial, so there are three terms used to describe garlic mustard life stages. In the first year of life, garlic mustard germinates from a seed and enters the "seedling" stage in the spring. By the fall of that year, the seedling will have grown into a basal "rosette". The rosette over-winters and then in the spring, the second year of life, a stem bolts from the rosette and forms the "adult" flowering stalk. Adult plants flower in the spring, set seed in the summer, and die by the fall. The spring data includes both the seedling and adult stages, while only the rosette stage is present in the fall. The adult plants can be further divided into those with siliques present and those with no siliques present.

In addition to collecting data on garlic mustard, data was also collected for the other plants growing within the garlic mustard plots. Other species within a plot were identified to species, when possible, and their percent cover in the plot recorded. The species were then categorized as native, nonnative, or unknown (species that cannot be identified to the taxonomic level where native or nonnative status can be determined). All native or nonnative determinations were based on the Minnesota Department of Natural Resources species list for Minnesota (http://files.dnr.state.mn.us/ecological_services/plant_list9-25-02.pdf). By collecting data on the other species present, we can track the changes of the plant community over time.

RESULTS: 2005 through 2007 monitoring period

1. Garlic mustard herbivory levels

While evidence of insect feeding was widespread (Table 2), the actual amount of leaf damage was low (Fig. 1). Across all sites, seasons and years the average amount of leaf area damaged due to insects was 1.8 ± 0.03%. Leaf damage did not vary widely from site to site. The lowest mean leaf removal was 0.95% at Pine Bend in 2006, while the highest was 4.4% at Fort Snelling (Fig. 1). Most of the damage was in the form of edge or internal hole feeding (Table 2). Leaf mining and windowpane feeding also occurred. While edge and hole feeding remained common in the fall, leaf mining was much less common, dropping from occurring in 31% of the plots in the spring to only 1% in the fall, indicating that most leaf mining was on adult plants. When biological control weevils are released it is expected that insect damage, especially windowpane feeding, will increase.

2. Fluctuations in garlic mustard populations over time

Garlic mustard's biennial life cycle drives some of the changes in garlic mustard cover and population density from year to year. At some sites, one life stage clearly dominates in each year. For example, a site may be dominated by adult flowering plants in spring 2005 and have few seedlings present. In the fall of 2005 there would be few rosettes. In the spring of 2006, the seedling stage would dominate and the site would have many seedling and very few adults. By fall 2006 there would be many rosettes. This pattern is demonstrated in Figure 2 with photos from Baker Park.

Of the 12 sites, six showed a pattern of one life stage dominating each year (Fig. 3). Over three years of monitoring, the rosette population density cycled from low to high to low in some sites and from high to low to high at others (Fig. 3). It is important to take these population cycles into account when analyzing the impacts of biological control insects. A decrease in adult plants from one year to the next may simply be a result in this natural oscillation in life stage dominance. It will take several years of data to separate out natural population cycles from long-term decreases in population.

Not all sites were dominated by one life stage in a given year. At many of the sites there were similar amounts of first and second year plants coexisting each year. Three sites showed little variation in rosette population density from year to year (Fig. 3). The final three sites showed either increasing or decreasing rosette population density over time. Of the six sites that didn't show life stage cycling, five showed a trend of an increase in rosettes over time. At these sites, it is likely that garlic mustard populations are still growing and expanding, causing an increase in rosettes over time. In addition to changes in rosette population density due to the cycling of life stages or a growing population, population density can also change in response to abiotic and biotic factors. For example, in years of low precipitation, we would expect to see lower cover of garlic mustard.

The springtime population densities of garlic mustard adults and seedlings varied from year to year (Fig. 4). Since only five sites were established in 2005, there are only five sites with three years of spring data. The patterns that were clear in the fall data are less visible in the spring as most sites have only two years of data. In most cases, the sites that showed strong year to year population cycling in the fall reflect strong year to year variation in the spring population densities of seedlings and adults (Fig. 4). For example, the population density of adult plants at Warner Nature Center fluctuated widely, going from 0.5 plants per $0.5m^2$ plot in 2005 to 42.6 plants/plot in 2006 and then down to 7.7 plants/plot in 2007 (Fig. 4). The low of 0.5 plants/plot (1 plant/m^2) and the high of 42.6 plants/plot (85.2 plants/m^2) at Warner Nature Center were the lowest and highest densities found across all sites and years. Seedling densities also ranged widely, from a low of 14.1 plants/plot (18.2 plants/m^2) to a high of 114.7 plants/plot (229.4 plants/m^2) (Fig. 4). Comparing the population density of seedlings in the spring with rosettes in the fall, it is clear that many seedlings die before reaching the rosette stage (Figs. 3 and 4).

In addition to monitoring garlic mustard population densities, we also monitored the percent cover of garlic mustard. At some sites, small numbers of plants were large and covered a large percentage of the plot, while at other sites, large numbers of very small plants covered only a small area. The percent cover of garlic mustard is another way to visualize the dominance of garlic mustard at a site and to track impacts of biological control agents. The biological control agents may stunt plants, causing them to be smaller in stature then before. The total cover of garlic mustard in the spring (adults plus seedlings) ranged from 20 to 70% (Fig. 5). Garlic mustard cover decreased in the fall because the adult plants have set seed and senesced, so only first year rosettes are present. Total garlic mustard cover did vary from year to year, although the range of garlic mustard cover was similar from year to year.

3. Fluctuations in garlic mustard plant height and reproductive output

Data was collected on garlic mustard plant height and number of siliques as measures of vigor and reproductive output of the plants. It is anticipated that the introduction of biological control insects will stress the plants and result in smaller plants which produce fewer siliques (Gerber et al. 2007). The year to year variation in garlic mustard average heights and numbers of siliques again underscores the importance of pre-release monitoring. Monitoring sites with and without biological control release will help us determine the impacts of biological control agents versus natural year to year variation.

Large natural fluctuations in garlic mustard plant height and numbers of siliques were detected as height and siliques production decreased from 2006 to 2007 (Fig. 6 and 7). The mean height of garlic mustard plants decreased at all twelve sites from 2006 to 2007 (Fig. 6). Mean heights ranged from 48 to 82 cm in 2006, but only 21 to 56 cm in 2007. Environmental factors, such as below normal precipitation in 2007, were the likely cause of decreased growth at all the sites. Smaller garlic mustard plants then produced fewer siliques (Fig. 7). In 2006, silique production was high with 67 to 444 siliques present in a $0.5m^2$ quadrat, but by 2007 there were only 43 to 240 siliques/$0.5m^2$ (Fig. 7A). The number of siliques per quadrat gives an estimate of seed output at a site. After biological control agent release, silique production on the site level should decrease. The number of siliques produced per quadrat will also vary from year to year because in some years the silique-producing adult plants will dominate, but the next year the non-reproductive seedling stage will dominate. The numbers of siliques per plant are likely to remain relatively constant from year to year in the absence of biological control or strong environmental stress (Fig. 7B). The mean number of siliques per stem is a measure of the fecundity of individual plants at a site. Since plants were generally smaller in 2007 than 2006, they correspondingly produced fewer siliques per plant with 5-14 siliques/stem in 2006 versus 2-11 siliques/stem in 2007 (Fig. 7B). When biological control is released, we expect that individual plants will produce fewer siliques.

To further characterize the population, adult stems were categorized as to stems with siliques or with no siliques. Almost all adult plants produced siliques; stems without siliques were rare (Fig. 8). At most sites, fewer than 5% of the adult stems did not produce siliques. With one exception, the other sites had fewer than 10% barren stems (Fig. 8). The high percentage of barren stems (26%) observed at Hilloway Park in 2006 was due to early season buckthorn (*Rhamnus cathartica*) control which resulted in herbicide drift onto garlic mustard plants. This caused reduced and delayed silique development in many plants. The low percentages of barren stems across sites indicate that most adult plants have the resources to complete their life cycle and produce seed. It is anticipated that the number of stems without siliques will increase with the introduction of biological control insects as the insects stress the plants.

4. Relationship between garlic mustard and native species

One of the impacts of garlic mustard is that it forms dense populations which negatively impact native species (Nuzzo 1999, Blossey et al. 2001). Sites with greater garlic mustard cover had lower native species richness and cover than those sites with less cover of garlic mustard (Fig. 9). The negative correlations were consistent in both 2006 and 2007 (Fig. 9). Sites varied in the amounts of native and nonnative species present. Native species richness ranged from a low of 1.8 species/$0.5m^2$ quadrat at Baker Park in 2005 to a high of 6.7 species/$0.5m^2$ at Willmar in 2007. Native species cover ranged from a low of 9% cover at Baker Park in 2005 to a high of 50% cover at Nerstrand in 2007. Nerstrand also had the lowest nonnative species richness and cover (no nonnative species present in the spring 2005-2007). The highest nonnative species richness was found at Baker Park in 2007 (1.6 nonnative species/0.5m^2) and the highest nonnative cover was found at Coon Rapids in 2006 (26.3%).

In addition to monitoring whether biological control insects will decrease garlic mustard populations, we can also monitor the response of the native vegetation. Ideally, native species cover and richness will increase as the populations of garlic mustard decrease. Monitoring data provides baseline information on native species cover and richness. By continuing to monitor after biological control release, we will be able to determine if native species remain stable or increase or if other nonnative species are increasing. This data will provide information on the response of the plant community to the release of biological control agents and indicate whether additional restoration work may be necessary.

5. Garlic mustard and leaf litter

When nonnative earthworms invade a forested site, they cause a dramatic decrease in the litter layer (Bohlen 2004, Hale et al. 2005). In Minnesota, litter layer depth decreased from 10 cm to 0 cm with the presence of earthworms (Hale et al. 2005). Blossey et al. (2005) suggested that garlic mustard invasion follows earthworm disturbance. Data on the percent cover of bare soil and the depth of the litter layer were collected to assess the impact of earthworms at the sites. We used low litter layer depth and high cover of bare soil as indicators of earthworm disturbance. All sites had very low litter depth with average spring litter depths ranging from 0.09 cm to 2.4 cm. There was no accumulated litter from previous years; the litter that was measured was recent leaf fall. The low variation in litter depths across sites made it difficult to detect any correlation between increased garlic mustard densities in sites with low litter depth (Fig. 10A). The percent cover of bare ground did vary widely across the sites (ranging from 0 to 84% of the ground cover in the spring). Even with a range of bare ground cover, there was no indication of increasing garlic mustard population density with increasing amounts of bare ground (Fig. 10B). Our data showed little evidence for increased garlic mustard with increasing impacts of earthworms. However, all sites were found to have significantly impacted litter layers, so there was no comparison with undamaged sites.

DISCUSSION

Garlic mustard in Minnesota is currently experiencing very little herbivory. On average, less than 2% of the leaf area was damaged by herbivores. The 1.8% leaf damage levels in Minnesota are similar to the 3.3% leaf damage levels reported in Michigan (Evans et al. 2007). This low level of damage may be one reason why garlic mustard has been such a successful invader. An introduced biological control insect has the potential to greatly increase insect damage from its present level.

Garlic mustard plant populations do vary considerably from year to year. Two to three years of pre-release monitoring data have given us a good understanding of the year to year fluctuations in populations. At some sites, they population fluctuations are due the changes in dominance between the seedling and adult stages. After biological control insects are released we expect to see decreases in garlic mustard populations (Davis et al.

2006, Gerber et al. 2007). With long-term data collection we can see long-term trends in garlic mustard populations. When biological control insects are available they will only be initially released at half of the monitoring sites. This will help in separating population changes due to biological control insects from changes due to environmental factors, such as years of low precipitation.

Through the monitoring data we will be able to see both the impact of the biological control insects on garlic mustard and the impact of changes in garlic mustard abundance on other plant species in the community. Individual species and functional groups have been found to vary in their responses to experimental removal of garlic mustard (McCarthy 1997, Stinson et al. 2007). Tree seedlings and native grasses especially susceptible to the presence of garlic mustard and are some of the first species to increase after garlic mustard removal (McCarthy 1997, Stinson et al. 2007). Through monitoring, we will find out if garlic mustard populations decline due to biological control and whether or not those declines allow native species to increase. We will be able to characterize the sites and determine if native species cover and species richness improve when garlic mustard populations are reduced. If other nonnative species increase as garlic mustard populations decrease then additional restoration work may be necessary. It will likely take several years of reduced garlic mustard populations before impacts on the forest understory can be observed (Hochstedler et al. 2007).

After biological control release, there is the potential for large differences in native plant community recovery among the different sites. Some sites have high levels of disturbance (low litter levels, high nonnative species cover) while others have a more robust native plant community. For example, Nerstrand had no nonnative species cover measured in the spring in all three years. If garlic mustard decreases, there is a large, diverse native species population ready to expand. In contrast, Baker Park had the lowest cover of native species and the highest diversity of nonnative species. It is not clear how the native plant community will respond to the reduction in garlic mustard. The twelve sites encompass a range of disturbance levels and their responses to biological control will help clarify the impacts of the biological control agents and whether those impacts are consistent across sites. Finally, with the monitoring program in place and with key pre-release release baseline data, we will be able to determine the benefits, or vegetative outcomes, of the garlic mustard biological control efforts.

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TABLES

Table 1. Garlic mustard monitoring sites in Minnesota. The ID column gives the abbreviation for the site found in the data summaries.

Site #	ID	Site Name	City	County
1	BP	Baker Park Preserve*	Maple Plain	Hennepin
2	CR	Coon Rapids Dam Regional Park	Coon Rapids	Anoka
3	CG	Cottage Grove Ravine Regional Park	Cottage Grove	Washington
4	FS	Fort Snelling State Park*	Saint Paul	Ramsey
5	HP	Hilloway Park	Minnetonka	Hennepin
6	LL	Luce Line	Long Lake	Hennepin
7	NE	Nerstrand State Park, Prairie Creek SNA*	Nerstrand	Rice
8	PB	Pine Bend Bluffs SNA*	Inver Grove Heights	Dakota
9	PL	Plainview – private land	Plainview	Winona
10	WN	Warner Nature Center*	Marine on St. Croix	Washington
11	WH	Westwood Hills Nature Center	St. Louis Park	Hennepin
12	WI	Willmar - private land	Willmar/New London	Kandiyohi

*=site was established in time for spring 2005 data collection

Table 2. Garlic mustard presence and types of insect feeding present. The percent of plots with garlic mustard present out of the 20 plots at each of 12 study sites in Minnesota over 3 years. Of the plots with garlic mustard present, the percentages of those plots with various types of leaf damage are listed.

Time	Garlic mustard present (% of all plots)	Edge feeding	Holes	Leaf miner	Windowpane feeding
		(% of plots with garlic mustard present that showed this type of damage)			
Spring 2005	100	96	98	31	4
Fall 2005	87	99	98	1	1
Spring 2006	98	96	97	31	9
Fall 2006	84	97	98	0.5	0.5
Spring 2007	99	100	100	33	0
Fall 2007	88	97	96	1	0

FIGURES

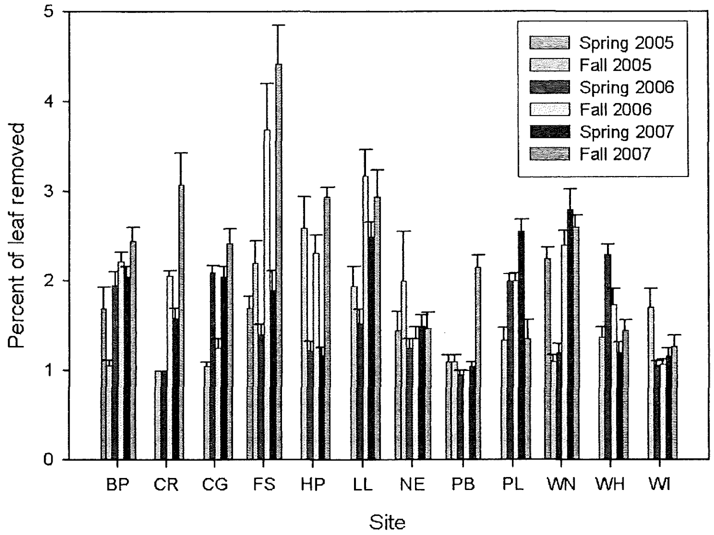

Figure 1. Levels of insect damage to garlic mustard leaves in the absence of biological control agents by site in Minnesota. Damage was quantified as visual mean percent of leaf removed. The percent of leaf removed per site is the average of 20 plots per site. Data was collected during the June and October of 2005 through 2007. Data was collected in the spring at 5 monitoring sites in 2005 (NE, WN, BP, FS, PB) and at all 12 monitoring sites in fall 2005 and spring and fall in 2006 and 2007.
BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar

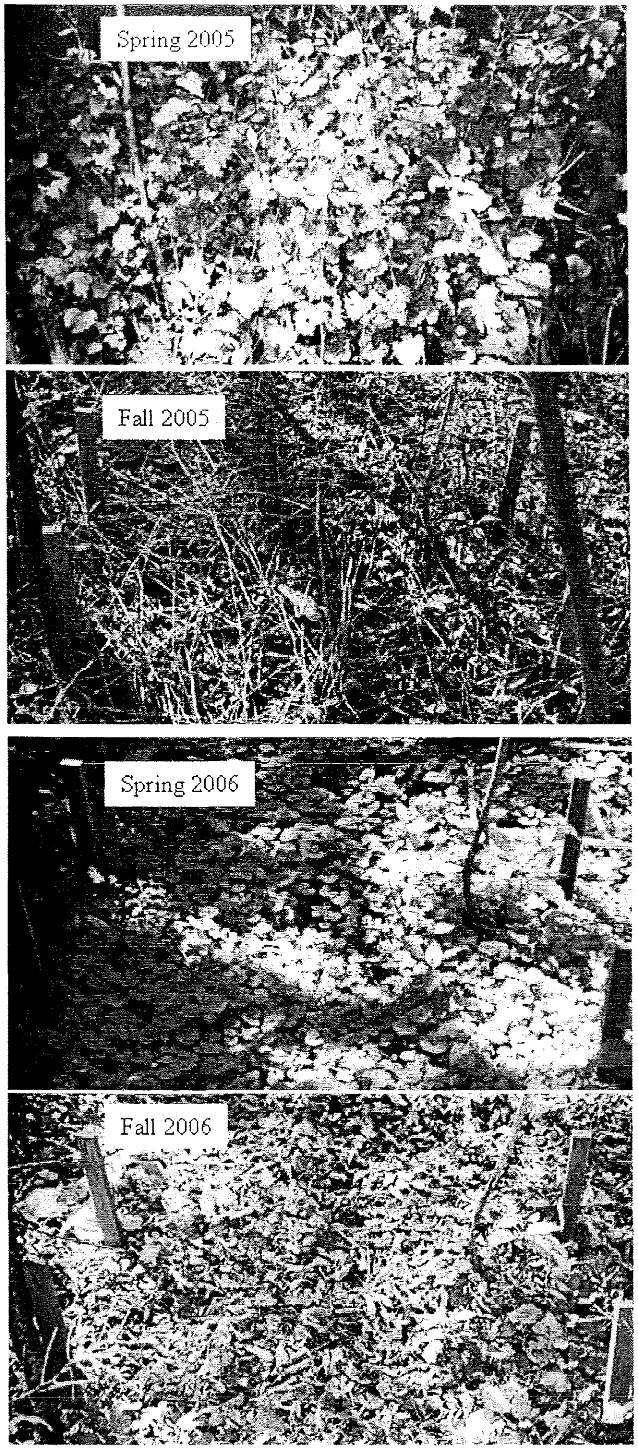

Figure 2. Photos of a single plot over time, showing the dominance of different garlic mustard life stages at Baker Park, MN 2005-2006. The adult flowering plants were dominant in the spring 2005 with few seedlings present. By fall 2005, the adults senesced and there was little other vegetation present. In spring 2006 there was a carpet of garlic mustard seedlings. By fall 2006 the surviving seedlings had grown into rosettes.

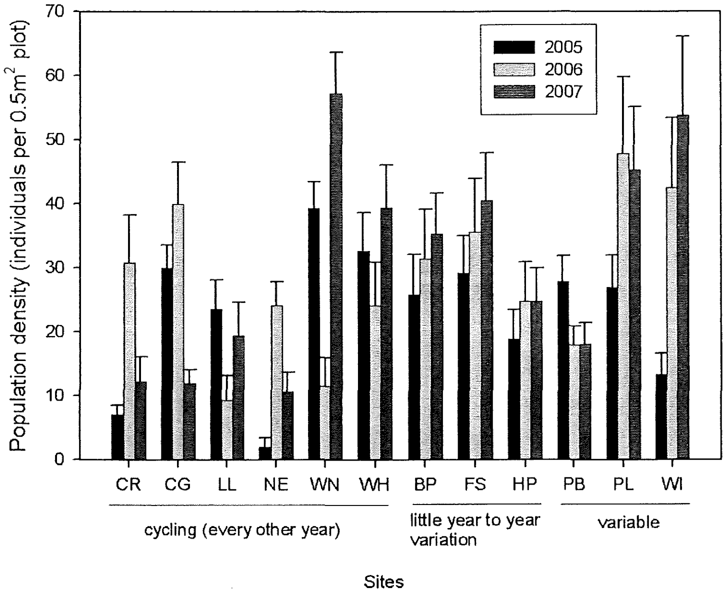

Figure 3. Population density of garlic mustard rosettes over time as measured in the fall at 12 monitoring sites in Minnesota, 2005-2007. Six sites show strong cycling (one life stage is dominant each year) with rosette densities peaking every other year. Three sites show little year to year variation in rosette population density (densities with standard error overlap from year to year). Three sites show variation over time with one site showing a decrease in rosette population density and two sites showing increases in rosette population density.

BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar

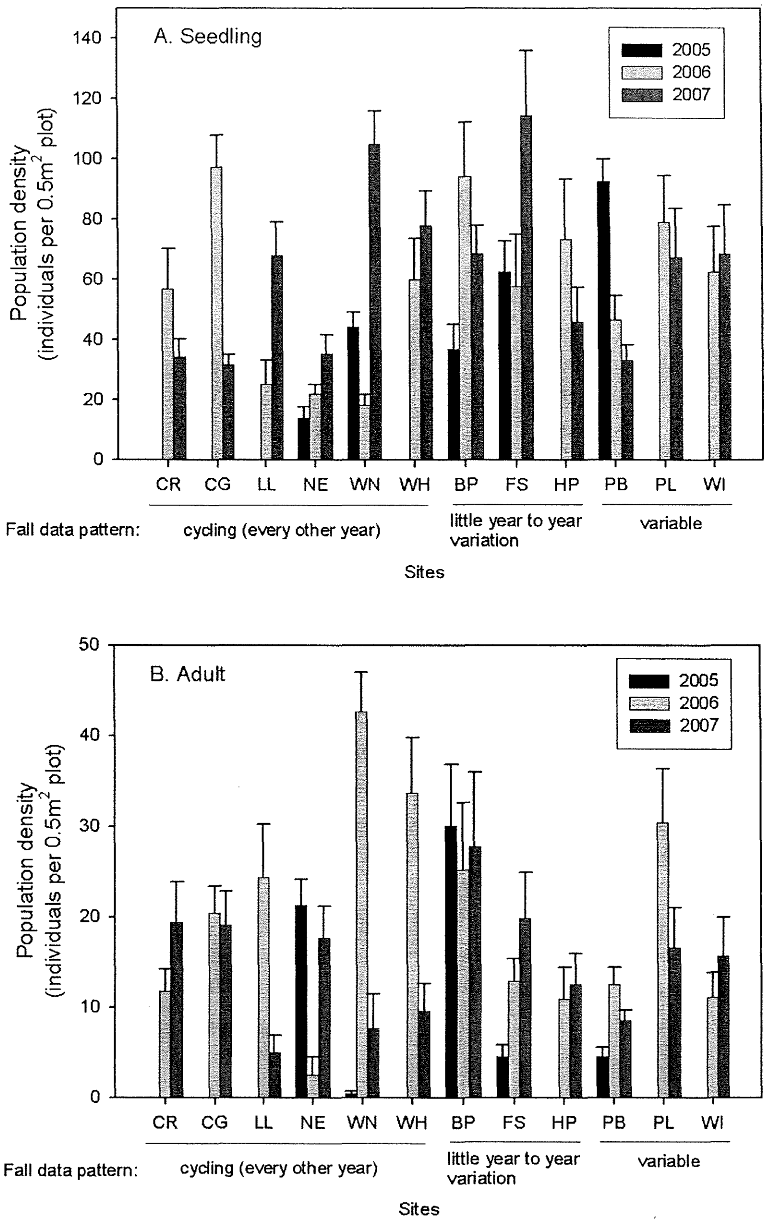

Figure 4. Population density of garlic mustard seedlings (A) and adults (B) over time. Data was collected in Minnesota in the spring at 5 monitoring sites in 2005 (NE, WN, BP, FS, PB) and at all 12 monitoring sites in 2006 and 2007. Sites are organized according the density patterns seen in the fall data. With only 2 years of data at many of the sites, it is difficult to see the same cycling patterns present in the fall data, although WN clearly shows an alteration between the seedling and adult stages over the three years with seedlings dominant in 2005 and 2007 and adults dominant in 2006.
BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar

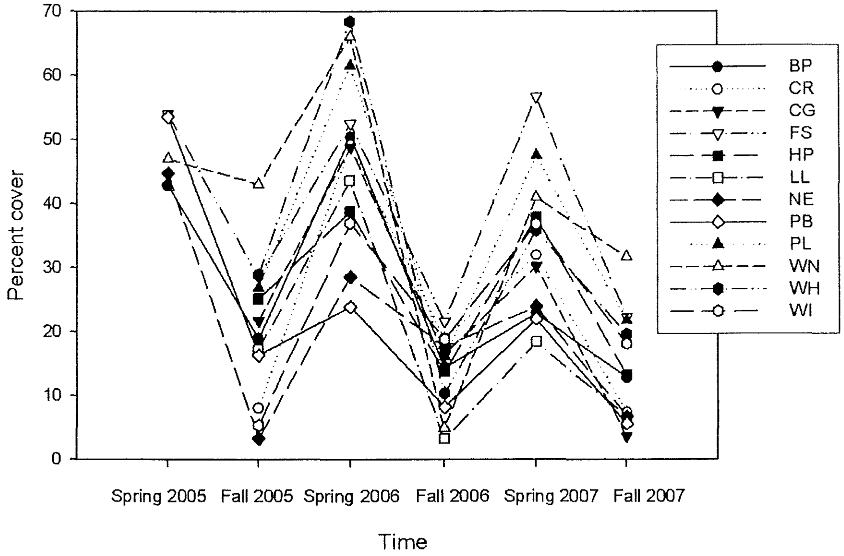

Figure 5. Visual total of the percent cover of garlic mustard over time. Spring garlic mustard cover is the total cover of adults and seedlings. Fall cover is cover of the rosettes. Data was collected in Minnesota in the spring at 5 monitoring sites in 2005 (BP, FS, NE, PB, WN) and at all 12 monitoring sites in 2006 and 2007.
BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar

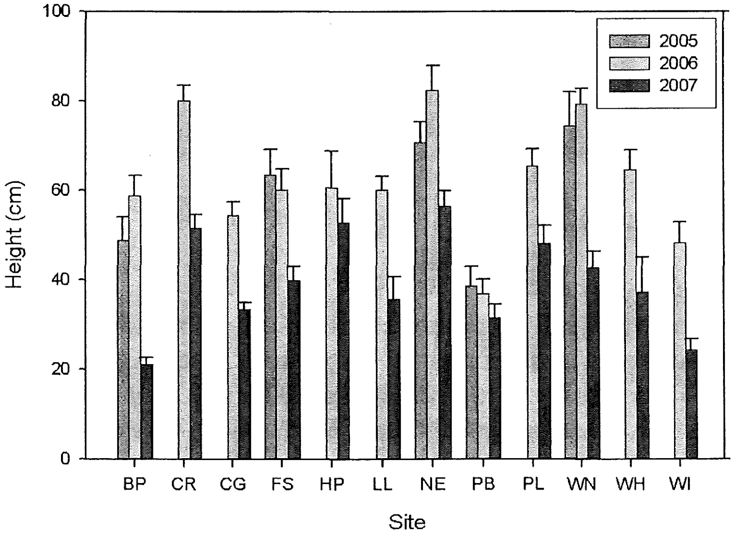

Figure 6. Mean garlic mustard plant height per quadrat averaged by site. Data was collected in Minnesota in the spring at 5 monitoring sites in 2005 (BP, FS, NE, PB, WN) and at all 12 monitoring sites in 2006 and 2007.

BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar

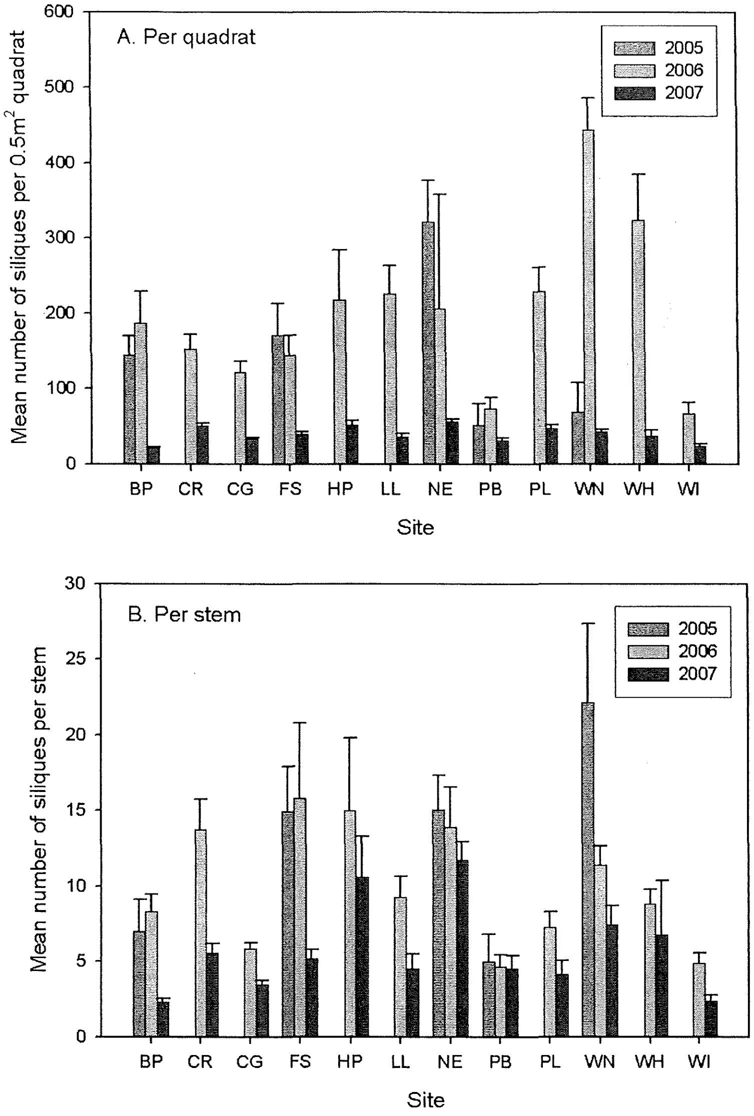

Figure 7. A: Mean number of siliques per quadrat (an estimate of seed output at a site). B: Mean number of siliques per stem (a measure of the fecundity of individual plants at a site). Data was collected in Minnesota in the spring at 5 monitoring sites in 2005 (BP, FS, NE, PB, WN) and at all 12 monitoring sites in 2006 and 2007.

BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar

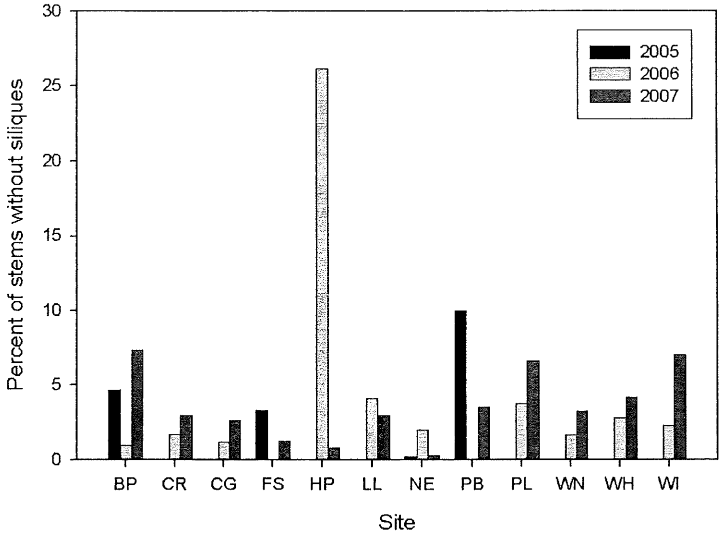

Figure 8. Percent of adult stems without siliques present. Note that the 2006 Hilloway Park (HP) percent barren plants is high because many garlic mustard plants were impacted by herbicide directed toward buckthorn. Data was collected in Minnesota in the spring at 5 monitoring sites in 2005 (BP, FS, NE, PB, WN) and at all 12 monitoring sites in 2006 and 2007.
BP=Baker Park, CR=Coon Rapids, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, PL=Plainview, WN=Warner Nature, WH=Westwood Hills, WI=Willmar

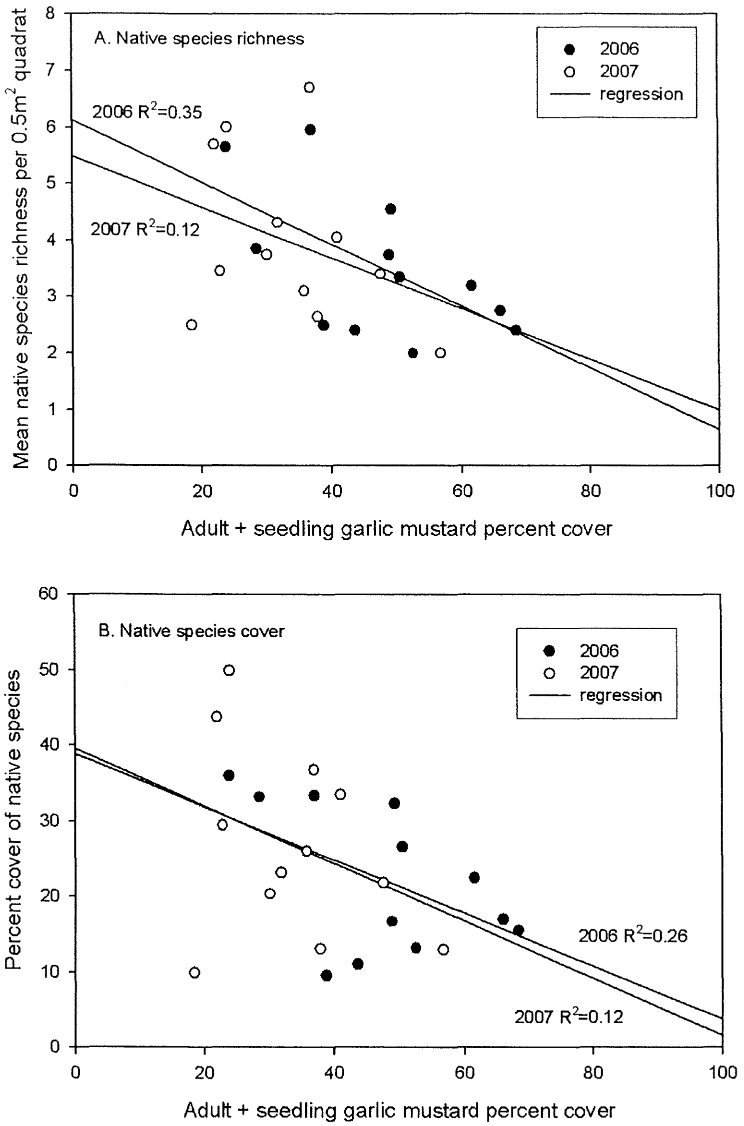

Figure 9. A: Regression of native species richness (mean richness per quadrat) by cover of adult + seedling garlic mustard in the spring. B: Regression of native species percent cover by cover of adult + seedling garlic mustard in the spring. Data points are the mean values for each of the 12 sites as determined from 20 quadrats at each site. Data was collected from 12 sites across Minnesota in 2006 and 2007.

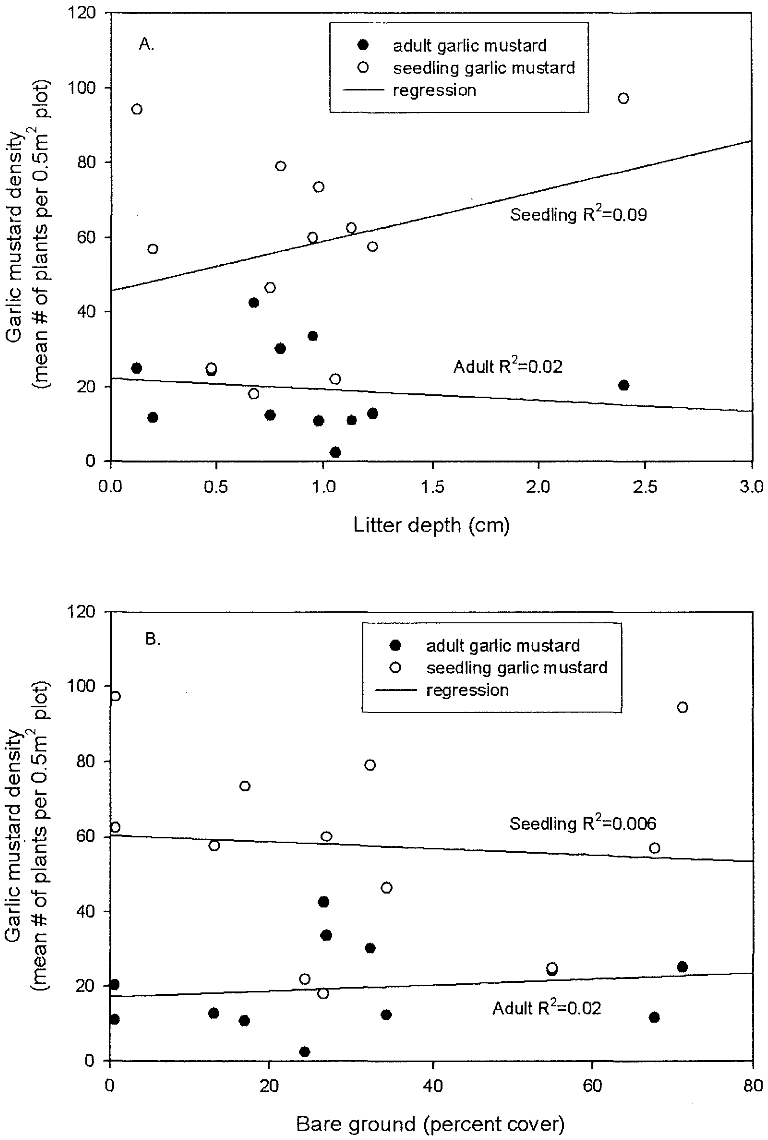

Figure 10. A: Regression of garlic mustard plant population density by litter depth (spring 2006). B: Regression of garlic mustard plant population density by percent cover of bare ground (spring 2006). Data points are the mean values for each of the 12 sites in Minnesota as determined from 20 quadrats at each site.

Chapter 2

Recovery potential of garlic mustard sites: germinable seeds in the soil seed banks

INTRODUCTION

Garlic mustard (*Alliaria petiolata*) is a nonnative, invasive species which can dominate forest understories in the US (Blossey et al. 2001). The main goal of the garlic mustard biological control program is to reduce garlic mustard populations. The intent is that once garlic mustard cover is reduced, native species will be able to recover. In order to assess the effects of the biological control agent, monitoring sites were established throughout Minnesota in 2005. At each site, data is collected on the species composition. If a decrease in garlic mustard opens up space in the forest floor, there are several sources for the plants that may come to occupy the space. Existing perennial plants and plants that reproduce vegetatively may expand to occupy the space. New seeds may be brought in by wind, water, or animals. Existing seeds in the soil seed bank may germinate and occupy the site. The plant composition data from the monitoring sites can be used to determine which species are already growing at the site. In order to determine what species are likely to germinate if garlic mustard is reduced, we collected soil samples to determine the plant species composition of the soil seed bank. It is important to have an adequate seed bank to reestablish native species once garlic mustard cover has decreased. By collecting seed bank data, we are also able to assess the extent of the garlic mustard seed bank. When there is little evidence of a native species seed bank then additional restorative efforts, such as seeding native species, should be considered.

The existing seed banks are an important component to management of the sites. Instead of assuming the existence of a viable native seed bank, it is sensible to determine whether or not it exists (van der Valk and Pederson 1989). If it is not found to exist, then preparation can be made for additional restoration efforts. Consequently, our main research question was: Is there a native species seed bank to re-colonize the site if garlic mustard is reduced by biological control insects? A second question was: What seeds of unwanted species are present? Unwanted species are nonnative invasive species that may expand their populations and impede native species recovery if garlic mustard cover is reduced. Garlic mustard itself can have a large seed bank that will take time to deplete.

Many of the sites had high garlic mustard cover and were generally not high-quality forest. It was likely that the native seed bank would be somewhat depleted. Garlic mustard is often associated with a nonnative plant community, either because it responds to disturbance as do other nonnatives or because garlic mustard creates an unfavorable site for native species. Soil seed bank data and continued monitoring of the sites will help estimate future plant communities at the monitoring sites.

METHODS

Soil samples were collected in September, 2005 from the seven Minnesota monitoring sites which had been established by that time (Table 1). A soil corer was used to collect soil cores 8 cm in diameter by 5 cm deep (surface area = 50 cm^2, soil volume = 251 cm^3). The monitoring plots were laid out along transects, so the soil samples were collected adjacent to those same transects. Along each transect, a soil sample was collected every 5 m. A total of 40 soil samples were collected at each site (total surface area = 2011 cm^2, total soil volume = 10,053 cm^3).

Upon collection, the soil samples were placed in a cold room (2 to 4 °C) for four months to stratify the seeds. The soil sieving method of Ter Heerdt et al. (1996) was then used to reduce the bulk of the soil. Soil was sieved through a coarse (4.0 mm) sieve to remove bulky material and through a fine sieve (0.212 mm) to retain the seeds and fine soil. The remaining soil and seed mixture was spread into flats in the greenhouse. The soil was spread thinly on top of sterilized potting soil and thin layer of sand. The trays were kept moist. As seedlings emerged their identity was recorded. Once a seedling was identified it was pulled. Unidentifiable seedlings were transplanted to additional pots and grown until identification could be made. Counts were made weekly until there was no additional germination for three weeks. At this point the seed bank soil was mixed to bring any additional seeds to the surface. The flats were then monitored until there was no additional germination for three weeks. Only germinable seeds were counted, there were no additional searches or counts of non-germinable seeds.

Data was summarized by site. The number of individuals of each species was totaled to give a seed bank profile for each site. The species were also categorized as native, nonnative, or unknown (species could not be positively identified). The richness and population density of native and nonnative species seed banks were determined.

RESULTS

There was a germinable seed bank at all 7 sites, but the number of seeds found from the 10,053 cm^3 of soil per site ranged from only 6 seeds at Luce Line to 85 seeds at Hilloway Park (Table 2). Luce Line, Baker Park, Fort Snelling, and Nerstrand all had very few seeds germinate from soil collected (12 or fewer individuals, Table 2). *Pilea* sp. was the most common species to germinate. It had the greatest number of seeds and was found at six of the seven sites (Table 2). *Pilea* was most abundant at Hilloway Park, where 68 individuals germinated (Table 2). It was also common at Pine Bend and Warner Nature Center.

Seeds of native species were more common than nonnative species, although *Pilea* accounted for most of the individuals (112 individuals, Table 2). Most of the other native species were site specific; eight of the native species only occurred in one site each, and the other three only occurred at two sites (Table 2). Adjusting the data to a seeds/m^2 scale, the native species seed bank ranged from 10 to 402 seeds/m^2 (Table 3). Four of the sites have more native species seeds/m^2 than nonnative species, but three of the sites have more nonnative species seeds than native species seeds (Table 3). Native species seed bank richness was low, ranging from 2 – 5 native species per site (Table 4).

Garlic mustard was the most abundant nonnative species recovered from the soil samples (Table 2). In total, there were 14 germinable garlic mustard seeds recovered from the 70,371 cm^3 of soil collected from the 7 sites. The next most common nonnative species were *Stellaria media* (8), *Leonurus cardiaca* (6), and *Medicago lupulina* (6) (Table 2). Garlic mustard was present in the seed bank in four of the seven sites (Table 2). *Chenopodium album* was also found in four sites. Other common nonnative species included *Medicago lupulina* (present at 3 sites), *Stellaria media* (2 sites), and *Taraxacum officinale* (2 sites) (Table 2). Pine Bend, Warner Nature Center, and Nerstrand had the highest numbers of nonnative seeds (Table 2). All of the sites, except for Hilloway Park and Luce Line, had greater nonnative species richness than native species richness, but this was generally only 1 additional species (Table 4).

DISCUSSION

A widely differing species composition between seed banks and standing vegetation has been found in forests (Leck et al. 1989). The seeds for many forest herbs are very rare in the seed bank (Leck et al. 1989). Forest seed banks often have seeds from earlier successional species as these species can have widespread seed production and dispersal (Leck et al. 1989). This was likely the case for many of the nonnative species found in the seed bank in our monitoring study. These early successional species produce large numbers of seed, some of which will fall in favorable locations. It was surprising that garlic mustard seeds weren't found in all of the sites, since garlic mustard is so abundant in all of the sites. A more intensive sampling method would likely increase the number of seeds recovered. Additionally, garlic mustard can be difficult to germinate in lab conditions (Baskin and Baskin 1992). This study used germination to determine the seed bank. This method gives an estimate of what is likely to germinate, but can underestimate the total seed bank if there are species that are difficult to germinate (Gross 1990).

There are several sites that may need additional restoration help because of a lack of native seeds in the seed bank. Baker Park, Fort Snelling, Luce Line, and Nerstrand all had low native seed density. However, Nerstrand had one of the higher covers of native species in the above ground vegetation (see monitoring summaries) and so may be able to compensate more quickly than the other three sites. It will be important to continue monitoring. If native species are not recruiting into spaces opened up by reduced presence of garlic mustard, additional restoration efforts may be necessary. It is also possible that there may be a flush of early successional nonnative species if garlic mustard populations are reduced. However, these species may be replaced over time by native species that do better in the forest environment than the more disturbance-oriented species. The nonnative species that were most commonly expressed in the seed bank were generally not species known to dominate forest understories.

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TABLES

Table 1. Identification and latitude and longitude of the garlic mustard monitoring sites from which soil samples were collected in Minnesota in September 2005.

ID	Site Name	City	County	N deg	N min	W deg	W min
BP	Baker Park Preserve	Maple Plain	Hennepin	45	19.356	94	59.667
FS	Fort Snelling State Park	Saint Paul	Ramsey	44	52.373	93	11.634
HP	Hilloway Park	Minnetonka	Hennepin	44	57.552	93	26.098
LL	Luce Line	Long Lake	Hennepin	44	58.441	93	35.137
NE	Nerstrand State Park, Prairie Creek SNA	Nerstrand	Rice	44	21.527	93	5.809
PB	Pine Bend Bluffs SNA	Inver Grove Heights	Dakota	44	47.076	93	1.732
WN	Warner Nature Center	Marine on St. Croix	Washington	45	10.583	92	49.641

Table 2. Number of seeds found per site by species (seeds per 0.2011 m^2 surface area). Soils were sampled at seven garlic mustard monitoring sites in Minnesota in September 2005.

Species	**BP**	**FS**	**HP**	**LL**	**NE**	**PB**	**WN**	**Total seeds**	**Site occurrences**
Native species:									
Desmodium sp.	0	0	0	0	0	0	1	**1**	**1**
Erechtites hieracifolia	0	0	1	0	0	0	0	**1**	**1**
Erythronium albidum	0	0	0	0	2	0	0	**2**	**1**
Eupatorium rugosum	0	1	0	0	0	0	0	**1**	**1**
Galium sp.	0	0	1	1	0	0	0	**2**	**2**
Parietaria pensylvanica	0	0	0	0	0	10	0	**10**	**1**
Pilea sp.	1	0	68	2	1	23	17	**112**	**6**
Pinus strobus	0	0	9	0	0	0	0	**9**	**1**
Ranunculus abortivus	0	0	0	0	0	1	0	**1**	**1**
Sambucus canadensis	0	0	0	1	0	1	0	**2**	**2**
Typha sp.	1	1	0	0	0	0	0	**2**	**2**
Viola sp.	0	0	2	0	0	0	0	**2**	**1**
Total native seeds	**2**	**2**	**81**	**4**	**3**	**35**	**18**	**145**	**7**
Nonnative species:									
Alliaria petiolata	0	0	1	0	6	5	2	**14**	**4**
Chenopodium album	0	1	0	1	2	1	0	**5**	**4**

Leonurus cardiaca	0	0	0	0	0	6	0	**6**	**1**
Medicago lupulina	2	3	0	1	0	0	0	**6**	**3**
Robinia pseudoacacia	0	0	1	0	0	0	0	**1**	**1**
Silene vulgaris	0	1	0	0	0	0	0	**1**	**1**
Solanum nigrum	0	0	0	0	0	2	0	**2**	**1**
Stellaria media	1	0	0	0	0	0	7	**8**	**2**
Taraxacum officinale	0	1	0	0	1	0	0	**2**	**2**
Urtica dioica	0	0	0	0	0	0	1	**1**	**1**
Verbascum thapsus	1	0	0	0	0	0	0	**1**	**1**
Total nonnative species	**4**	**6**	**2**	**2**	**9**	**14**	**10**	**47**	**7**
Unknown	**2**	**2**	**2**	**0**	**0**	**0**	**0**	**6**	**3**
Total seeds	**8**	**10**	**85**	**6**	**12**	**49**	**28**	**198**	

BP=Baker Park, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, WN=Warner Nature

Table 3. The number of native, nonnative, and unknown seeds per site on a seeds/m^2 scale. Soils were sampled at seven garlic mustard monitoring sites in Minnesota in September 2005.

Site	BP	FS	HP	LL	NE	PB	WN
Total native	10	10	402	20	15	174	90
Total nonnative	20	30	10	10	45	70	50
Total unknown	10	10	10	0	0	0	0
Total	40	50	423	30	60	244	139

BP=Baker Park, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, WN=Warner Nature

Table 4. Seed bank species richness per site. Soils were sampled at seven garlic mustard monitoring sites in Minnesota in September 2005.

Site	BP	FS	HP	LL	NE	PB	WN
Native richness	2	2	5	3	2	4	2
Nonnative richness	3	4	2	2	3	4	3

Unknown species richness	1	1	1	0	0	0	0
Total	6	7	8	5	5	8	5

BP=Baker Park, FS=Fort Snelling, HP=Hilloway Park, LL=Luce Line, NE=Nerstrand, PB=Pine Bend, WN=Warner Nature

Chapter 3

Competitive and allelopathic effects of garlic mustard

INTRODUCTION

The competitive relationships between native species and garlic mustard (*Alliaria petiolata*) are not well understood. Garlic mustard is able to invade and displace native vegetation, even in relatively undisturbed forests (McCarthy 1997). Garlic mustard's ability to be a strong competitor is a likely reason why native species are displaced when garlic mustard invades (Meekins and McCarthy 1999). In addition, garlic mustard roots exude allelochemicals which can negatively affect native species by decreasing germination rates (Prati and Bossdorf 2004). The active allelopathic compound in garlic mustard is allyl glucosinolate (sinigrin) (Vaugh and Burhow 1999). This study addresses the potential for competition between garlic mustard and native species as well as the role of allelopathy.

Previous studies have had conflicting results on the importance of allelopathic effects of garlic mustard. McCarthy and Hanson (1998) found little evidence that allelopathy impacted other species, but Vaughn and Berhow (1999) pointed out that in McCarthy and Hanson's method was potentially flawed. Prati and Bossdorf (2004) did find evidence of a negative impact on germination due to allelopathy. Previous studies have looked at the impact of garlic mustard allelopathy on standard assay plants (McCarthy and Hanson 1998, Vaughn and Berhow 1999) or *Geum* species (Prati and Bossdorf 2004). In this study we address the effects of allelopathy on species native to Minnesota.

Experiment 1 was designed to determine if the effect of garlic mustard on native species is due primarily to competition or allelopathy and to determine the relative influence of each on native species. Experiment 2 provides information on the recovery potential of sites invaded by garlic mustard. It addresses whether allelochemicals left behind by the garlic mustard could inhibit germination and growth of key native species even after the garlic mustard is removed. If there is little residual effect of garlic mustard in the soil, then it is unlikely that there will be long-term direct allelopathic effects on plant species that attempt to reestablish in sites that had been heavily invaded by garlic mustard.

METHODS

Experiment 1: Growing with garlic mustard

This experiment tests the hypothesis that growing with garlic mustard reduces native species biomass more than growing with a conspecific. We hypothesized that the allelochemicals produced by garlic mustard would give garlic mustard an advantage. To differentiate effects due to competition from effects due to allelopathy, activated carbon was used as a treatment to ameliorate the effect of the allelochemicals (Prati and Bossdorf 2004). Carbon itself has been found to have little effect on plant growth (Ridenour and

Callaway 2000, Inderjit and Callaway 2003). We predicted that native plant biomass would be greatest in pots without garlic mustard regardless of carbon presence/absence and would be the least in pots with garlic mustard and no carbon, since these would experience allelopathic effects (Fig. 1). If allelopathy was a strong mechanism, then carbon addition should counteract the effects, so plant biomass should be similar in pots without garlic mustard and pots with garlic mustard and carbon addition. This amelioration of allelopathic effects would be visible in interaction plots and in ANOVAs with an interaction term.

To test these hypotheses, pots with native species were established in a full factorial, completely randomized block design with the following treatments: 1) no carbon addition or activated carbon added at a 1:50 carbon:soil ratio (Ridenour and Callaway 2000, Siemens et al. 2002) and 2) no garlic mustard present (planted with a conspecific plant instead) or garlic mustard present. To test the impact of carbon addition on garlic mustard itself, pots with the treatment of either no carbon addition or carbon addition were established and garlic mustard sown into them. All pots contained a 2:1 MetroMix 200 potting soil: steamed soil mixture and 15-9-12 (NPK) fertilizer, applied at the label's medium rate of 3.6oz/ft^3 so nutrients would not be limiting.

The response to the treatments was measured by the aboveground biomass of the species of interest. Garlic mustard and five native species were seeded, but only garlic mustard, *Solidago flexicaulis* (zig-zag goldenrod, Asteraceae), and *Thalictrum dioicum* (early meadow rue, Ranunculaceae) germinated in sufficient numbers for data analysis. Plantings of *Geranium maculatum* (wild geranium, Geraniaceae), *Impatiens* capensis (spotted touch-me-not, Balsaminaceae), and *Erythronium albidum* (white trout lily, Liliaceae) were unsuccessful. Pots were overseeded to try to achieve the target number of replicates (10 per treatment). If more than one seed germinated in a pot, additional seedlings were removed. The target number of plants was not achieved, but enough germinated to justify analyzing the results (Table 1). The native species were acquired as seed from Prairie Moon Nursery. Seeds of garlic mustard were collected in July 2005 from Baker Park, Fort Snelling State Park, and Warner Nature Center. All seeds (garlic mustard and natives) were in cold storage (2 to 4 °C) from August 2005 to January 2006.

For each native species there were 2 carbon treatments x 2 garlic mustard treatments x 10 replicates = 40 pots. "Garlic mustard only" pots had 2 soil treatments x 10 replicates = 20 pots. Each pot was 13.5 cm x 13.5 cm x 13.5 cm. After planting, the pots were placed in a greenhouse in a randomized block design. The photoperiod in the greenhouse was 16 hours light/8 hours dark and the temperature ranged from 15 to 21 °C. Pots were watered with hoses every other day to maintain moist soil. The plants were allowed to grow for 11 weeks and then they were harvested. The plants were dried for 48 hours at 60 °C to determine the aboveground biomass of natives and garlic mustard.

ANOVAs were used to analyze the effect of garlic mustard and carbon (roles of competition and allelopathy). The model for the ANOVA was the effect of block, garlic mustard presence/absence, carbon presence/absence, and garlic mustard – carbon interaction on the biomass of the species in question. For each species, the biomass was compared among the treatments. For the pots that contained only garlic mustard, the final biomass of garlic mustard was compared among the soil treatments to determine if the carbon had an impact on garlic mustard growth.

Soils from the greenhouse pots and garlic mustard shoot tissue were analyzed to examine the effects of fertilizer, carbon, and garlic mustard plants on soil properties and the effects of fertilizer and carbon on garlic mustard shoot tissue. Soil was taken from pots that were not seeded to native plants or garlic mustard, but had either 1) no fertilizer and no carbon, 2) fertilizer only, 3)carbon only, or 4) both fertilizer and carbon. Four pots with garlic mustard were also analyzed; all four contained fertilizer, but only 2 had carbon added. The University of Minnesota Research Analytical Lab performed the analyses. Soils were tested to determine pH and macro and micronutrient levels (pH, P, K, Zn, Fe, Cu, and Mn are discussed in the results). Descriptions of soil analysis methods can be found at http://ral.cfans.umn.edu/soil.htm#1. Shoot tissue from garlic mustard plants grown with and without carbon were also analyzed by the University of Minnesota Research Analytical Lab to determine if there were differences in micro and macronutrients due to carbon addition. The lab used the elemental analysis by inductively coupled plasma (ICP) method - dry ashing method (485°C ashing temperature) to determine Al, B, Ca, Fe, K, Mg, Mn, Na, P, and Zn (methods at http://ral.cfans.umn.edu/plant.htm). Four plants grown in carbon and four plants grown without carbon were analyzed. These small samples of soil and shoot tissue were analyzed to alert us to any major impacts of carbon on macro and micronutrients. These analyses were not meant to be an exhaustive study of the effects of carbon, fertilizer, and garlic mustard on soil and shoot properties.

Experiment 2: Growing on soil conditioned by garlic mustard

This experiment addresses the recovery potential of native species seeded in to soils in which garlic mustard had been previously grown. Presence of garlic mustard can inhibit germination of some species through allelopathic chemicals from root exudates, although species tested differed in sensitivity (Prati and Bossdorf 2004). Our experiment addresses the hypothesis that native species will have less aboveground biomass in pots that contained garlic mustard due to allelochemicals. We hypothesized that the natives would grow larger in pots where allelochemicals from garlic mustard are minimized by the presence of activated carbon. Garlic mustard was also re-seeded into soils previously conditioned by garlic mustard to determine if new garlic mustard plants are impacted by soil conditioning of previous garlic mustard plants.

This experiment used a full factorial, randomized complete block design. The treatments were 1) no carbon addition or activated carbon added at a 1:50 carbon:soil ratio (Ridenour and Callaway 2000, Siemens et al. 2002) and 2) garlic mustard never present or garlic mustard previously grown in the pot. As in experiment 1, all pots were 13.5 cm x 13.5 cm x 13.5 cm and contained a 2:1 MetroMix 200 potting soil: steamed soil mixture and 15-9-12 (NPK) fertilizer, applied at the medium rate of 3.6oz/ft^3. The pots were placed in a greenhouse in a randomized block design. As in experiment 1, the photoperiod in the greenhouse was 16 hours light/8 hours dark and the temperature ranged from 15 to 21 °C and pots were watered with hoses every other day to maintain moist soil. In pots with the garlic mustard conditioning, the garlic mustard was allowed to grow for 3 months and then it was removed from the pots by cutting it below the crown. Pots were left un-watered for two weeks to kill any remaining parts of the garlic mustard plant. After this period seeds of native species or garlic mustard were added to the pots. Seeds of *Solidago flexicaulis, Thalictrum dioicum*, and garlic mustard established. Few *Allium canadense* (wild garlic, Liliaceae) bulbs and *Isopyrum biternaturm* (false rue anemone, Ranunculaceae) seedlings established, so those results are not reported. Plants were allowed to grow for 14 weeks after emergence and then shoots were harvested for aboveground biomass measurements (dried at 60 °C for 48 hours). For each species there were 2 soil treatments (carbon present or absent) x 2 garlic mustard treatments (garlic mustard conditioned soil or no garlic mustard ever present) x 10 replicates = 40 pots. There was enough germination to reach the expected 10 replicates for each treatment for *Solidago, Thalictrum,* and garlic mustard.

ANOVAs were used to analyze the effect of presence of garlic mustard and effect of carbon (potential presence of allelochemicals). As in experiment 1, the model for the ANOVA was the effect of block, garlic mustard presence/absence, carbon presence/absence, and garlic mustard – carbon interaction on the biomass of the species in question. For each species, the biomass response was compared among the treatments. A presence of garlic mustard by presence of allelochemicals interaction would indicate that the allelochemicals have an impact on native species and can affect the plant growth during the recolonization of garlic mustard sites.

RESULTS

Experiment 1: Growing with garlic mustard

None of the species had interaction plots (Fig. 2) similar to the pattern expected if allelopathy was driving the effects of garlic mustard on other species (Fig. 1). The interaction plots for *Thalictrum* showed that biomass varied little in the carbon addition pots with and without garlic mustard, but biomass was greater in the no-carbon pots with no garlic mustard than the no-carbon pots with garlic mustard (Fig. 2A, B). This pattern suggests that carbon addition had a negative impact on *Thalictrum* growth. However, in the ANOVA of *Thalictrum* biomass, there was no statistically significant effect of block ($F_{8,7}$=2.30, P=0.14), garlic mustard presence or absence ($F_{1,7}$=3.49, P=0.10), carbon presence or absence ($F_{1,7}$=0.60, P=0.37), or an interaction between the two ($F_{1,7}$=0.36, P=0.57). Only one *Thalictrum* plant was able to grow in a pot with garlic mustard and no carbon, so statistical tests lack the replication needed to determine significance (Table 1). While the ANOVA did not show statistically significant effects of garlic mustard competition or allelopathy, the fact that only 1 plant was actually able to grow in the garlic mustard/no carbon treatment (versus 4 in the garlic mustard/carbon treatment, and 6 and 8 in the treatments without garlic mustard, Table 1) indicates that allelopathy may hinder the ability of *Thalictrum* to grow with garlic mustard.

Solidago plants were affected by garlic mustard and carbon presence or absence, but the expected interaction (the presence of carbon counterbalancing the negative effect of garlic mustard) was not observed (Fig. 2C, D). In the ANOVA of *Solidago* biomass, garlic mustard presence or absence ($F_{1,25}$=5.14, P=0.03) and carbon presence or absence ($F_{1,25}$=8.66, P=0.007) were both statistically significant. However the interaction between the two ($F_{1,25}$=0.01, P=0.90) was not. There was also no statistically significant effect of block ($F_{9,25}$=1.01, P=0.46). *Solidago* biomass was greater in pots where garlic mustard was absent than when garlic mustard was present and when carbon was absent versus when carbon was present (Fig. 2C, D).

Garlic mustard growth was not hindered by carbon addition (Fig. 2E). The ANOVA showed no effect of block ($F_{4,2}$=0.38, P=0.82) or carbon ($F_{1,2}$=0.27, P=0.65). Data analysis was hindered by the small sample size. The lack of carbon effect supports the data that show that carbon does not affect plant growth. Results for all species are summarized in Table 2.

Soil analysis of pots with no plants present showed the degree to which fertilizer increased phosphorus (P), potassium (K) and manganese (Mn) (Fig. 3). Carbon presence resulted in lower levels of P, Mn, zinc (Zn), and copper (Cu) and higher levels of K. Of course, without replication, it cannot be determined if these values are all within the same range of values or if there is a statistically significant difference between them. Iron (Fe) did not vary greatly among pots without plants. Fertilizer tended to decrease pH and carbon did not change this effect. Pots with garlic mustard and fertilizer had similar amounts of P and K, regardless of the presence or absence of carbon (Fig. 3). There was variation in iron among pots with garlic mustard although the variation did not relate to the presence or absence of carbon (Fig. 3). Zn, Cu, and pH did not vary dramatically among the different treatments. The shoot tissue from garlic mustard plants grown without carbon was compared to those grown with carbon (Fig. 4). Sodium (Na) and boron (B) were slightly higher in plants grown with carbon, Mn was slightly lower with carbon, but for most elements there was little difference due to carbon (Fig. 4). Overall, there was no indication that carbon presence reduced any nutrient level so low that it was insufficient to meet the nutrient needs of the plant.

Experiment 2: Growing on soil conditioned by garlic mustard

For *Thalictrum*, there was an effect of carbon presence ($F_{1,27}$=4.97, P=0.03), but there was no effect of previous garlic mustard presence ($F_{1,27}$=0.06, P=0.80) or an interaction between previous garlic mustard presence and carbon ($F_{1,27}$=0.02, P=0.88) (Fig. 5A, B). *Thalictrum* biomass was higher in pots with no carbon. Similarly, for garlic mustard, there was an effect of carbon presence ($F_{1,27}$=14.56, P=0.0007), but there was no effect of previous garlic mustard presence ($F_{1,27}$=0.20, P=0.65) or an interaction between the previous garlic mustard presence and carbon ($F_{1,27}$=0.44, P=0.51) (Fig. 5 E,F). For *Solidago* there was no effect of previous garlic mustard presence ($F_{1,27}$=0.11, P=0.74), carbon presence ($F_{1,27}$=2.5, P=0.12), or an interaction between the two ($F_{1,27}$=0.05, P=0.83) (Fig. 5C, D). Although not statistically significant, *Solidago* biomass tended to be greater in pots with no carbon. Unlike *Solidago* and *Thalictrum*, garlic mustard plants had higher biomass in pots that had carbon (Fig. 5E, F). For all three species, previous soil conditioning by garlic mustard had no impact. Since there was no legacy effect of garlic mustard, there was no interaction with carbon (no amelioration of garlic mustard's impact). Results for all species are summarized in Table 2.

DISCUSSION

Strength of competition with garlic mustard

When plants were grown with garlic mustard, the impact of garlic mustard on those plants were likely due to its impact as a competitor and not from allelopathy. Garlic mustard and carbon treatments had direct effects, but with no interaction of the two treatments, there is little evidence for an allelopathic effect (Table 2). Garlic mustard showed a trend toward decreasing *Thalictrum* biomass. Garlic mustard is likely having an effect on *Thalictrum* through competition, although the increased germination in carbon soils means that allelopathy may potentially play a role. Garlic mustard did decrease *Solidago* biomass, but carbon did not ameliorate that effect, so in this case the effect of garlic mustard on *Solidago* was due solely to competition and not to allelopathy. Competition with garlic mustard is likely having a stronger impact on native plants than any direct impacts due to allelopathy. This, however, does not exclude the potential for garlic mustard to have strong indirect effects due to allelopathy. Garlic mustard's allelopathic exudates have been found to be detrimental to mycorrhizal fungi in the soil (Stinson et al. 2006, Callaway et al. 2008). Many native species are dependent on mycorrhizae and grow poorly when mycorrhizae populations are suppressed. The importance of allelopathy in garlic mustard is likely through this indirect mechanism of altering soil biota as to be unfavorable for native species (Stinson et al. 2006, Callaway et al. 2008).

Effects of garlic mustard soil conditioning

Growing in soil conditioned by garlic mustard did not cause a decrease in *Thalictrum, Solidago,* or garlic mustard biomass (Table 2). There was little evidence that allelochemicals left behind by garlic mustard had any impact on the growth of the target species. This bodes well for restoration attempts in soils which had garlic mustard. Barring other changes to soil chemistry and soil biota, native plants should be able to grown on soils where the previous presence of garlic mustard may have released allelchemicals. Again, direct allelopathic effects on native plants is likely less important than potential negative impact of allelochemicals on mycorrhizae. If allelochemicals from garlic mustard have degraded the native soil biota then native species dependent upon that soil biota may have difficulty reestablishing in sites with garlic mustard infestations (Stinson et al. 2006, Callaway et al. 2008). Our study used greenhouse soils and was not designed to test this indirect mechanism of impact on native species.

Effects of carbon addition

Carbon had a greater effect than anticipated. It was anticipated that carbon addition alone would have little impact. In several cases, carbon addition caused a decrease in biomass, for some it had no effect, and in one case carbon increased biomass (Table 2). It is not clear why this occurred. The limited data collected on soil nutrients indicate that carbon additions weren't dramatically changing the soil nutrients, but the small differences in nutrients such as Mn and P may have had an impact. Carbon may also have altered soil moisture which resulted in the varying impacts on different species and studies (Inderjit and Callaway 2003).

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TABLES

Table 1. The number of replicates of each treatment for each species in experiment 1 (growing with garlic mustard or a conspecific). The goal was 10 replicates per treatment. Treatments varied in the presence or absence of garlic mustard (GM) and carbon (C).

	GM absent C absent	GM absent C present	GM present C absent	GM present C present
Thalictrum	6	8	1	4
Solidago	9	9	10	10
Garlic mustard	NA	NA	6	2

Table 2. Summary of effects of the garlic mustard and carbon treatments on the test species in experiments 1 and 2. Garlic mustard presence caused a decrease in *Solidago* biomass, but did not affect *Thalictrum* in experiment 1. Conditioning soil with garlic mustard did not have an impact any of the species in experiment 2. Carbon presence tended to decrease biomass of *Solidago* in both experiments. Carbon presence decreased *Thalictrum* biomass, but increased garlic mustard biomass in experiment 2. There was no evidence for a garlic mustard – carbon interaction in either experiment.

	Garlic mustard effect	**Carbon effect**	**Garlic mustard - carbon interaction**
Experiment 1: Growing with garlic mustard			
Thalictrum	decrease biomass (trend in graph)	none	none
Solidago	decrease biomass*	decrease biomass*	none
Garlic mustard	NA	none	none
Experiment 2: Growing on soil conditioned by garlic mustard			
Thalictrum	None	decrease biomass*	none
Solidago	None	decrease biomass (trend in graph)	none
Garlic mustard	None	increase biomass*	none

*= P value <0.05 in ANOVA

FIGURES

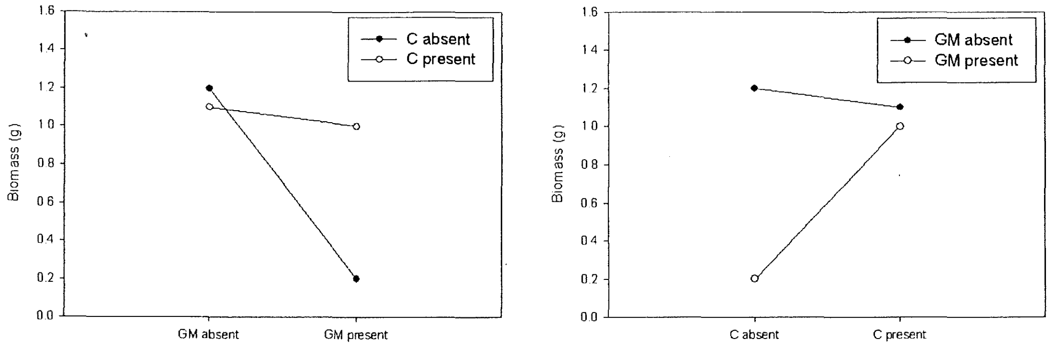

Figure 1. Hypothetical interaction plots if garlic mustard has an allelopathic effect on test plant biomass that is ameliorated by the addition of carbon. In the absence of carbon, plants have lower biomass when grown with garlic mustard. By examining the biomass of the test plant in the presence of carbon it can be determined whether this is due to either competitive or allelopathic effects. If the biomass of the test plant when grown with garlic mustard is greater when carbon is added (as pictured in the figure), then this indicates that the carbon is ameliorating the effect of the garlic mustard allelopathic chemicals. If carbon had little impact on test plant biomass, and biomass was less in the presence of garlic mustard irrespective of carbon, then that would indicate that the impact of garlic mustard is more heavily due to competition and not allelopathy. The pictured interaction plot is a hypothetical example of results that would support an allelopathic effect of garlic mustard. The numbers are made-up to demonstrate the pattern and to show that some variation in biomass is expected (for example, biomass would not be *exactly* the same between the garlic mustard absent pots with and without carbon due to normal variations in plant growth among the replicates).

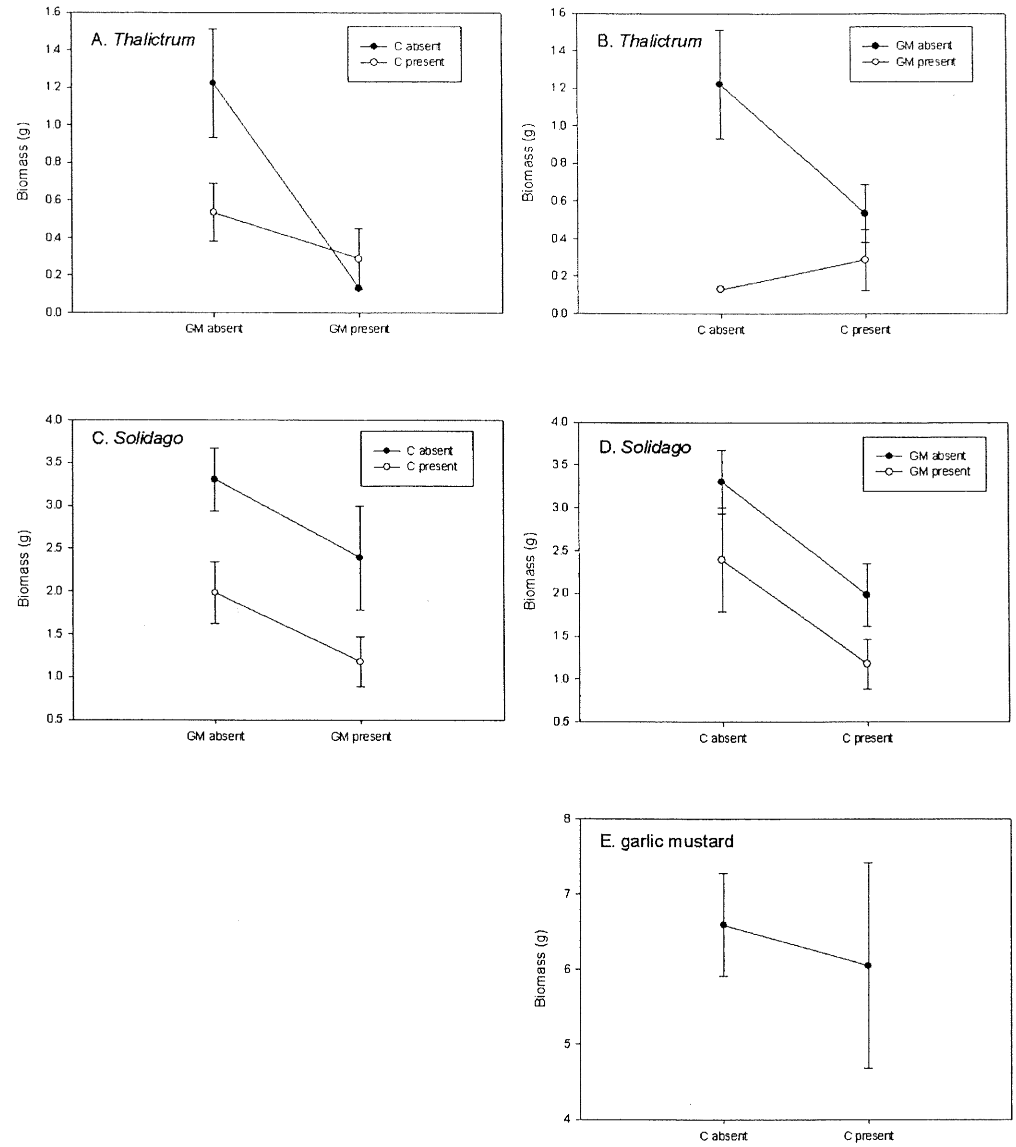

Figure 2. Experiment 1 (growing with garlic mustard) results: Interaction plots for *Thalictrum dioicum* (A,B), *Solidago flexicaulis* (C,D), and garlic mustard (E). Test plants were grown in the greenhouse for 11 weeks, growing with or without garlic mustard (GM) and with or without carbon (C). Plants were then harvested and the dry weight of their aboveground biomass was determined. Mean biomass of the shoots are graphed along with their standard error.

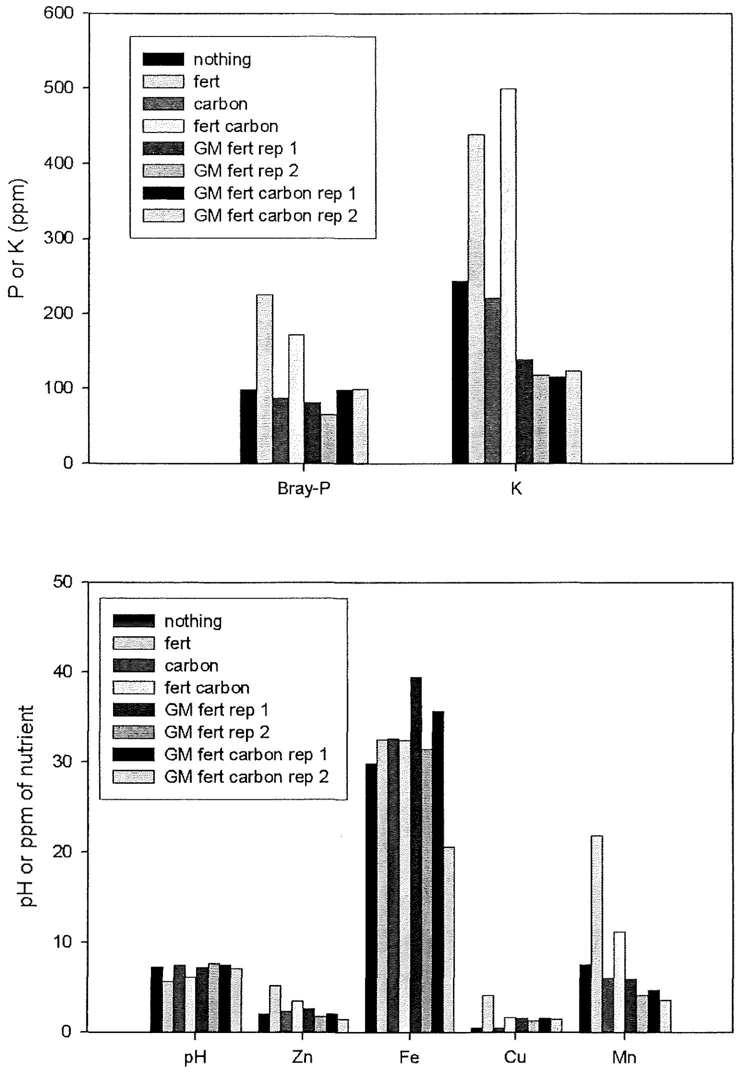

Figure 3. Properties of soils from pots in garlic mustard greenhouse experiments. The first four soils were from pots with no plants growing in them: 1) nothing additional added, 2) fertilizer added (fert), 3) carbon added, and 4) fertilizer and carbon added. The second four soils were from pots in which garlic mustard (GM) had grown for 3 months. There were two replicates of garlic mustard soils with fertilizer only and two replicates of garlic mustard soils with fertilizer and carbon present. All soils had been watered every other day for 3 months. After 3 months the soils were collected and the analyses performed.

P=phosphorus, K=potassium, pH, Zn=zinc, Fe=iron, Cu=copper, Mn=manganese

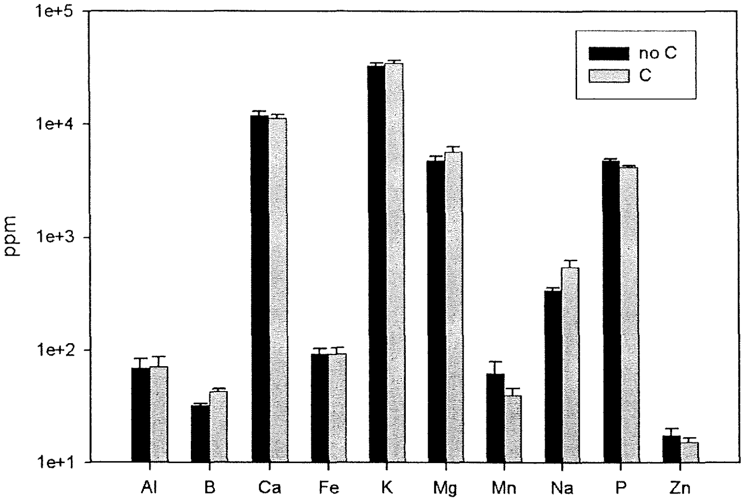

Figure 4. Analysis of garlic mustard shoot tissue. Garlic mustard plants had been grown in pots either with or without carbon (C) present. Plants were grown in the greenhouse for three months. Shoot tissue was analyzed to determine nutrient levels (note the log scale).

Al=aluminum, B=boron, Ca=calcium, Fe=iron, K=potassium, Mg=magnesium, Mn=manganese, Na=sodium, P=phosphorus, Zn=zinc

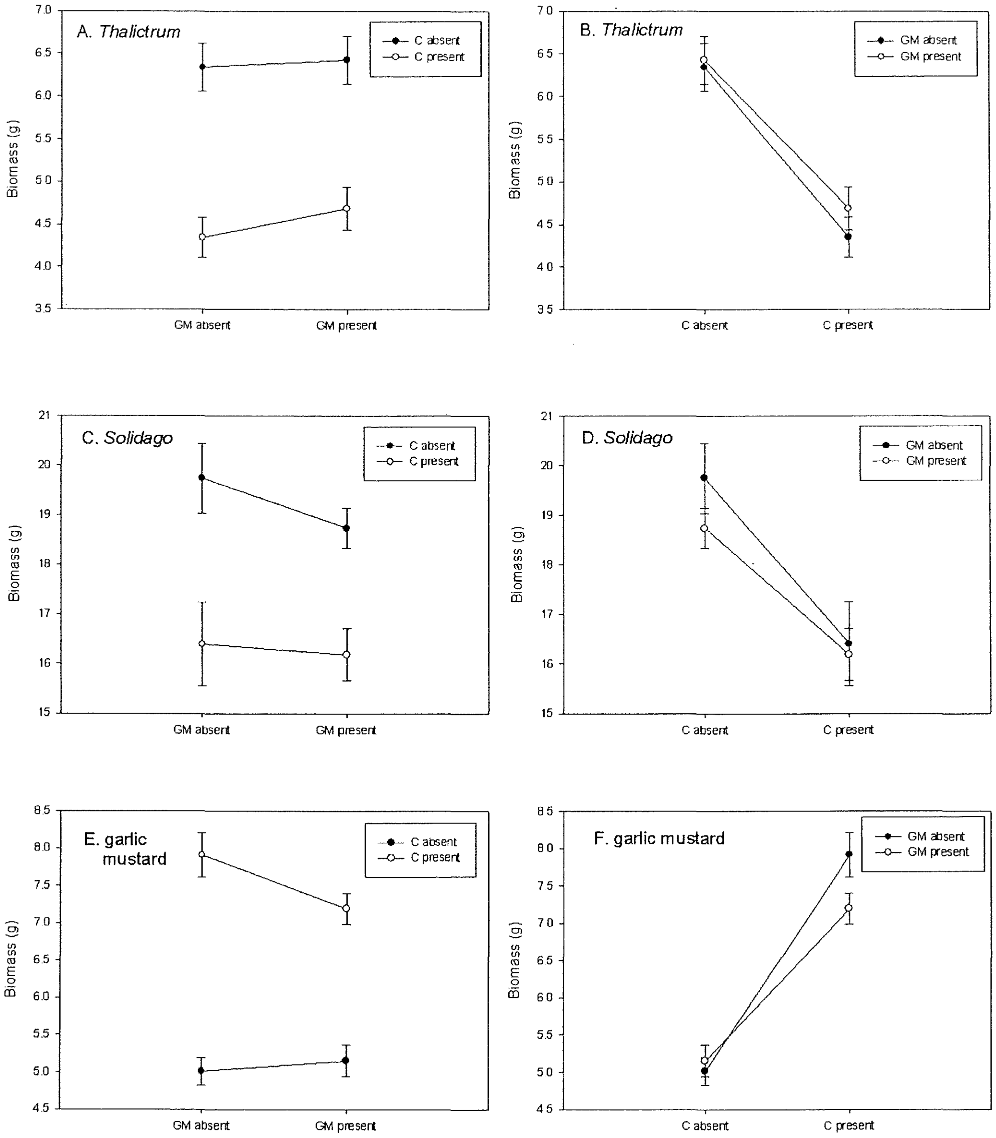

Figure 5. Experiment 2 (growing on soil conditioned by garlic mustard): Interaction plots for *Thalictrum dioicum* (A,B), *Solidago flexicaulis* (C,D), and garlic mustard (E, F). Half of the soils were conditioned by having garlic mustard grow in them for 3 months (GM present) and the other half of soils received the same amount of water, light, etc. for three months, but had no garlic mustard (GM absent). For each garlic mustard treatment, half of the pots had carbon (C) present and in half carbon was absent. After 3 months, garlic mustard was removed and the test plants (*Thalictrum*, *Solidago*, and garlic mustard) were seeded in to the soils with the 4 treatments. Test plants were grown in the greenhouse for 14 weeks. They were then harvested and the dry weight of their aboveground biomass was determined. Mean biomass of the shoots are graphed along with their standard error.