Mapping and Testing a Possible Control Method for Garlic Mustard (*Alliaria petiolata)*

Jonathan Finger and Dr. Laurie Eberhardt

Summer 2007

Pierce Cedar Creek Institute

Biology Dept. - Valparaiso University

Valparaiso, Indiana

Abstract

Garlic mustard (*Alliaria petiolata)* is an invasive allelopathic weed. Its alleopathy is suggested to be one of the key factors in its ability to out-compete other understory vegetation. Activated carbon has been shown to adsorb garlic mustard phytotoxins in the lab. Can activated carbon be used in the wild as a possible control mechanism? First, mapping at Pierce Cedar Creek Environmental Institute (Barry County Michigan) for locations of newly invading Garlic Mustard stands was done. After wild Garlic mustard was located, a field test was done at Pierce Cedar Creek to determine if activated carbon could reduce the allelopathic damage done to native Columbine flowers. A follow-up study was done using soil from the locations at Pierce Cedar Creek, which was placed in pots, to test if activated carbon could reduce Garlic Mustard's alleopathic effect on Columbine germination. Mapping of the Pierce Cedar Creek institute showed significant Garlic Mustard invasion, while a different survey (6 years ago) failed to find any. For the field experiments, even though statistical significance was hampered by wild animal damage; there was a positive statistical significance to the effect of activated carbon on Columbine seedlings growing in areas containing the Garlic Mustard's phytotoxins. Even though the seed germination test failed to yield any statistical significance, the overall trend in the graph closely mirrored the results of the field seedling growth experiment.

Introduction

Garlic mustard (*Alliaria petiolata* [Bieb.]) is an exotic invasive plant native to Europe and Asia belonging to the Brassicaceae family. Garlic mustard was first recorded in North America in 1868 on Long Island, New York. By 1990, Garlic mustard had been recorded in 27 states (Nuzzo, 1993). Once established, *A. petiolata* has the ability to invade mature second-growth forests, usually relatively resistant to invasive species, but also displace the understory vegetation in these invaded areas (McCarthy, 1997) and create monospecific stands. . Garlic mustard requires some degree of disturbance before it can colonize a site (Jonstone, 1986) which is why it usually progresses along disturbed areas such as the sides of roads or areas adjacent to trails.

Forested areas experimentally cleansed of the invasive show an increase of understory richness, including tree seedlings (McCarthy, 1997). Therefore, *A. petiolata* is likely to cause community interference by allelopathy or competition. Research findings regarding the allelopathic activity of garlic mustard have varied. McCarthy and Hanson (1998) found little evidence of allelopathic effects of garlic mustard extract on germination and biomass. However, Kelly and Anderson (1990) isolated phytotoxic compounds from garlic mustard extract which stunted germination.

Allelopathy can be defined as the effect of one plant on the growth and distribution of other plants through the release of chemical compounds into the environment (Rice, 1984). This definition also includes indirect effects, such as the the phytotoxic effects on the availability of nutrients. Garlic mustard produces glucosinolates (VanEtten and Tookey, 1983) which inhibit the germination of the arbuscular mycorrhizal (AM) fungus. This is of particular interest since *A. petiolata* is nonmycorrhizal, which suggests this may have an effect on other native species which are while remaining immune to the glucosinolates.

Zackrisson and Nilsson (1992) found that activated carbon added to water extracts in laboratory experiments and to the soil surface in field experiments reduced allelopathic effects by adsorbing the phytotoxins. Carbon produced by natural forest fires may play a key role in natural phytotoxic adsorption under natural conditions, but this is merely postulation (Hille and den Ouden 2005).

Our objective was to determine if applications of activated carbon would increase the growth of native species being invaded by *A. petiolata*. We hypothesized that native species planted in the presence of activated carbon and *A. petiolata* would grow larger than individuals grown without activated carbon.

Columbine (*Aquilegia canadensis* L.) is an indigenous, native plant that can be found in Pierce Cedar Creek and the surrounding Barry County. Columbine is inexpensive and easy to obtain, since it produces a pretty flower desired by most gardeners. The plant’s seeds ripen very quickly after dispersal which is ideal for germination analysis. The germination of the seed is relatively short at four to six weeks.

Garlic Mustard seems to have a reduced competitive ability when invading a new ecosystem (Prati and Bossdorf 2004). This means the role of allelopathy and how much of an advantage Garlic Mustard receives from it may be larger than previously thought. It is after this idea our hypothesis was formulated: If the alleleopathy is the primary competitive edge for Garlic Mustard, then can the allelopathy be reduced by the addition of activated carbon?

Methods

This project focused on two species: Garlic mustard (*Alliaria petiolata*) and Columbine (*Aquilegia canadensis*). Before starting either of our actual research experiments current invasive stands of wild Garlic Mustard were mapped. Then, in our first actual experiment, the seedling experiment, we tested the effect of activated carbon on reducing the allelopathic effect of Garlic Mustard on pre-germinated Columbine seedlings in the field. The second experiment, the germination experiment, tested the effect of activated carbon against the allelopathic effect of Garlic Mustard on germination and early seedling growth of Columbine. Wild Garlic Mustard obtained from invasive stands at Pierce Cedar Creek (Hastings, Michigan) was used for both experiments.

Mapping current stands of Garlic Mustard at Pierce Cedar Creek Institute: When arriving on location, earlier GIS maps were checked for any listing of Garlic Mustard. Then a systematic walking of the land was done in order to find current stands of Garlic Mustard. First, each and every one of the trails were walked twice (in opposite directions) on two consecutive days. Garlic Mustard favors disturbed areas for invasion, so trails, roads, and paths were the most likely to harbor Garlic Mustard. After all of the trails were walked and marked using GPS, the road (Cloverdale Road bisects the site, east to west) was walked twice (in opposite directions) on two consecutive days. After sites located on the roads were added via GPS, the staff and land managers were contacted for any further stands that might have been unknown. All of the research, walking, and contacting were done from May 18, 2007 – May 24, 2007.

The effect of activated carbon on columbine seedlings (pre-germinated) in sites with *garlic mustard and without-* For the seedling experiment, Columbine seedlings (plugs) were bought from J. F. New Inc. (Walkerton, Indiana) and transported to our research site. 10 sites across the property containing stands of garlic mustard were selected (Figure 1) based on Garlic Mustard density, amount of first and second year plants, and amount of sun; each site had both first and second year garlic mustard, relatively high Garlic Mustard density, and partial sun for at least half of the day. Four equal 1.5m x 1.5m square-plots were located within each site. Half of the plots at each of the ten sites had garlic mustard in the boundaries, while the other two plots per site were located nearby to Garlic Mustard plots but did not have any wild Garlic Mustard in them. A circle (20cm radius) was cleared in the middle of each plot; the circle was cleared of all plants including garlic mustard. Of the two plots at each site containing garlic mustard, one of the sites was randomly assigned to receive the activated carbon treatment. Similarly, one of the two non-Garlic Mustard plots at each site was randomly assigned a charcoal treatment as well. For each plot, three columbine seedlings were planted equal distance from each other in the cleared circle. For plots designated to receive the charcoal treatment, ~14g (1 tbsp) of activated carbon was planted in the bottom of the hole with each seedling, then soil was placed to fill in the rest of the hole. This was done with all three plants at the treatment plot, resulting in a plot (1.5m x1.5m) with garlic mustard plants surrounding a cleared circle (20cm radius) containing three columbine seedlings with or without ~14g of activated carbon.

The plots were allowed to grow for six weeks (June 13, 2007 – July 25, 2007). Every week, each stem of each plant was measured from soil surface to tallest leaf to see the difference in growth from week to week. At the end of two weeks, 1/3 of the plants (one randomly from each plot) was pulled, dried, and weighed. At the end of four weeks, another 1/3 (one from each plot) will be pulled, dried, and weighed with their above and below-ground biomass being recorded. At the end of the experiment, sixth week, the last third of plants will be pulled, dried, and weighed with their respective biomasses being recorded in similar fashion to the earlier weeks. Repeated measure analysis of variance statistical tests was performed on all data. During the entirety of the project and due to the dry summer season, each plot received ~0.6L of water every other day (rainstorms counted as a watering day).

The effect of activated carbon on Columbine seed germination and initial growth with or without Garlic Mustard present- For the second experiment, the germination test, soil samples were placed in pots from each plot of each site. In total there were 40 pots representing each of the four plots from each of the 10 sites (Figure 1). If wild Garlic Mustard was present in a plot (half of them had Garlic Mustard) then a single Garlic Mustard first year rosette was planted in the middle. 10 Columbine seeds were planted in each pot. Columbine seeds were collected from adult columbine plants located at Pierce Cedar Creek Institute butterfly-garden in Hastings, Michigan. Similar to the seedling experiment, of the four pots representing each of the four plots, if the plot was also designated to receive a charcoal treatment in the seedling experiment, then in the seed experiment, it also received charcoal during the seed planting process. This resulted in ¼ of the pots having Garlic Mustard and charcoal, ¼ having Garlic Mustard and no charcoal, ¼ having no Garlic Mustard but having a charcoal treatment, and ¼ having neither Garlic Mustard nor receiving the charcoal treatment. The pots were left to grow for seven weeks (July 18, 2007-September 10, 2007). For the first two weeks the pots were kept in flats (9 pots randomly assigned per flat) outside of the Pierce Cedar Creek Wet-Lab and watered three times per day with distilled water to maintain soil wetness. After those two weeks, the pots were transferred to the Valparaiso University greenhouse and watered under the same regimen. After the seven weeks were completed, the height of the tallest Columbine seedling per pot was recorded. Then all of the columbine seedlings were removed, dried, and weighed to obtain the average dry mass of a seedling per treatment across 10 sites. Univariate analysis of variance was used on the dry weight and height data.

Results

Mapping Invasive Stands: Figure 1 shows the current location of invasive stands of Garlic Mustard found during May 2007. It is important to note that these areas were last recorded as Garlic Mustard-free (Slaughter & Skean 2001).

Seedling Experiment: Figure 2 illustrates the average stem elongation for each treatment. Only data collected until week 4 was considered due to extensive animal damage. While data was collected after that, there was not enough data to properly analyze. Plots without Garlic Mustard and without charcoal had the highest stem elongation. Sites without Garlic Mustard but with the charcoal treatment had the lowest average stem growth. While sites that had Garlic Mustard and were treated with charcoal had a higher average stem elongation than sites with Garlic Mustard and did not receive the charcoal treatment. Repeated measures ANOVA showed a statistical significance ($F(1,6) = 11.23$, $p = .015$) between Garlic Mustard and presence/absence of charcoal.

Figure 3 depicts the relationship of average seedling mass per treatment after 2 weeks. While data was collected up to six weeks, animal damage depleted most of the dry mass sample size and could not be properly analyzed and only data from week 2 could yield any statistical data. Similar to Figure 2, sites without charcoal and without Garlic Mustard had the highest average dry mass. Sites without Garlic Mustard that received the charcoal treatment had the lowest average weight. While the Garlic Mustard sites with the charcoal treatment had a higher average mass than the garlic mustard sites without charcoal.

Seed Germination Experiment: Figure 4 shows the average maximum height of a seedling in each treatment. This data were collected after seven weeks since planting. Seedlings grown in pots without Garlic Mustard and without charcoal grew the most. Treatments of Garlic Mustard but with charcoal had the lowest average maximum height, and pots containing Garlic Mustard and no charcoal had the least maximum growth. Figure 5 shows the average seedling mass per treatment in the germination experiment after 7 weeks. The univariate ANOVA did not show a statistical significance between Garlic Mustard and charcoal, $F(1, 6)= 0.244$; $P=0.639$. The overall trend on the graph differed slightly from the other graphs found in this and the previous experiment. Most importantly, the charcoal treatments seemed to have a detrimental effect on the seedlings in the presence of Garlic Mustard while the other sets of data did show the charcoal helping.

It is important to note a few unpredictable circumstances that related to modifications in the data collection. First, a large portion of our sample size was destroyed from animal predation. The most obvious predators were wild white-tailed deer. By the end of the six weeks of the experiment 40% of our Columbine seedlings had been uprooted and eaten, chewed beyond recognition, or disappeared entirely. This is the primary factor in why the data analysis occurred for week 4 of the stem elongation data and week 2 of the dry weight data. If we had used later data points, there would be too many gaps to perform any kind of statistics. However, despite the short growing period, for both tests, statistical difference could be seen. Secondly, Michigan was under drought advisory for almost the entirety of the six week test period in June-July 2007. Even though Michigan was under drought advisory, the Columbine seedlings still grew, albeit a smaller amount than a normal Columbine seedling grown for 2-4-6 weeks.

Discussion

Mapping: Pierce Cedar Creek has significant Garlic Mustard stands invading on the western border. Six years ago the Pierce Cedar Creek property was free of Garlic Mustard, now there are significant amounts of Garlic Mustard density. The invasion is moving from the west along trails and Cloverdale Road. There are a few possible ways to help stop the advancement of Garlic Mustard. One would be to install shoe brushes at the beginning and end of each trail. Brushing off your shoes would help stop the spread of Garlic Mustard seed from one trail to another. Second, there must be large volunteer efforts to hand-pull the Garlic Mustard that is there. Unfortunately, Garlic Mustard is highly resistant to most herbicides and other methods of stopping invasive species, hand-pulling the entire plant (including the entire root-system) is the most effective way to stop the invasion.

Seedling Experiment: Of the three different interactions (Garlic Mustard, Charcoal, and Garlic Mustard x Charcoal) only the Garlic Mustard x Charcoal showed any statistical significance (Figure 2). This was a positive effect as the Garlic Mustard-plots with the charcoal treatment had more stem elongation than the Garlic Mustard-plots that did not receive the treatment. While the data should have showed a significant difference in the stem elongation of plants that did and did not have Garlic Mustard due to alleopathy, our data had a relatively small sample size due to animal damage. Since our data on stem elongation was only taken from the fourth week, if we had enough data from the sixth week there might have been a statistical significance since we can see a different in the graphs. Figure 3 shows a similar trend even though none of the data showed a statistical significance. Again, a portion of the data from weeks four and six were lost due to animal damage, so only the dry mass of week two was analyzed. If the data from week six had been analyzed, the plants would have had more time to react to the phytotoxins and it might have shown a greater statistical significance.

Seed Germination: Figure 4 shows the maximal height of a seedling after 7 weeks in each treatment which closely mirrors the trends found in the seedling experiment. While the statistical analysis yielded no significant differences, this was probably due to small sample size from lack of enough germination. It also may be slightly different if we were able to do a repeated measures ANOVA instead of a univariate, but that would require more data points than we had.

Figure 5 depicts the average dry weight of seedlings after 7 weeks of germination. The trends are slightly different from the other measurements, and similarly to the other statistical tests, there were not any statistical significance of charcoal's affects on garlic mustard. This again could be attributed to the small sample size and the way the data was statistically analyzed. The data was analyzed using a univariate ANOVA instead of a repeated-measures ANOVA like the field trial, but without more data points this could not be done. Another possible reason why the data showed a loss of mass-growth on seedlings could be do to competition in each pot. Since we used field soil from each site, when we allowed the seeds to germinate there were a lot of weeks growing in the pots as well. The weeds could not be pulled until they were large enough to be sure they were weeds and not columbine. The differing amount of weeds in each pot could have added competition against the columbine seedlings causing some pots of seedlings to grow less.

These results raise a few significant conclusions that should be discussed. First, given the data and the small sample size, our results do seem to show that activated carbon could be used as a control method to help stop the damage done by the allelopathy of Garlic Mustard. This is especially valuable to farmers who could help increase crop yield by reducing the amount of phytotoxins in the ground. Land managers could help keep their properties healthy by reducing alleopathic compounds in the soil through the use of activated carbon. Even restoration ecologists could use activated carbon to increase the resiliency of their restored habitats. Another conclusion is that land managers and restoration ecologists could use burning as a possible control method for Garlic Mustard since the charcoal left by the burn would help adsorb and lower phytotoxin levels in the soil.

Conclusions

Pierce Cedar Creek is being invaded by Garlic Mustard. In the six years since the last plant survey, Garlic Mustard has entered Pierce Cedar Creek property along multiple vectors. If action is not taken immediately the Garlic Mustard is sure to travel across the majority of the property and driving out native understory vegetation along the way.

While Garlic Mustard did not have a significant effect on seedling growth, there is good reason to believe that if another experiment had a larger sample size over a larger period of time there could be a statistical significance. As for the effect of activated carbon as a control method, there was a statistical significance for Columbine to grow taller when in the presence of Garlic Mustard and treated with activated carbon. If this method can be further tested and the consequences examined more deeply, this could be a possible boon to farmers who have crop damage from Garlic Mustard.

Literature Cited

Prati , Daniel. And Oliver Bossdorf. "Reduced competitive ability in an invasive plant." Ecology Letters 7(2004): 346-53.

Hille, Marco, and Jan den Ouden. "Charcoal and activated carbon as adsorbate of phytotoxic compounds - a comparative study." OIKOS 108:1(2005): 202-07.

Jonstone, I. "Plant invasion windows: a time-based classification of invasion potential." Biol. Rev. 61(1986): 369-394.

Kelly, T, and RC Anderson. "Examination of the allelopathic properties of garlic mustard (*Alliaria petiolata*)." Trans Ill State Acad Sci 83(1990): 31-32.

McCarthy, BC. "Response of a forest understory community to experimental removal of an invasive nonindigenous plant (*Alliaria petiolata*, Brassicaceae)." Assessment and Management of Plant Invasions (1997): 117-30.

McCarthy, BC, and SL Hanson. "An assessment of the allelopathic potential of the invasive weed *Alliaria petiolata* (Brassicaceae)." Castanea 63(1998): 68-73.

Nuzzo, V. "Current and historic distributions of garlic mustard (*Alliaria petiolata*) in Illinois." The Mich. Bot, 32(1993): 23-33.

Rice, E. L.. Allelopathy. Orlando, Florida: Academic Press, 1984.

VanEtten, C. H., and H. L. Tookey. "Glucosinolates." Naturally occurring food toxicants. 1983: 15-20.

Zackrisson, O., and M. C. Nilsson. "Allelopathic effcts by *Empetrum hermaphroditum* on seed germination of two boreal tree species." Can. J. For. Res. 22(1992): 1210-1319.

Acknowledgements

I would like to first thank the Pierce Family Foundation for the chance to experience an undergraduate research grant. I would also like to think the staff for their continued support and their help to finding all of my answers. Lastly, I would like to thank Rob Aicken for his continued support and finding all the tools and fencing I could ever need.

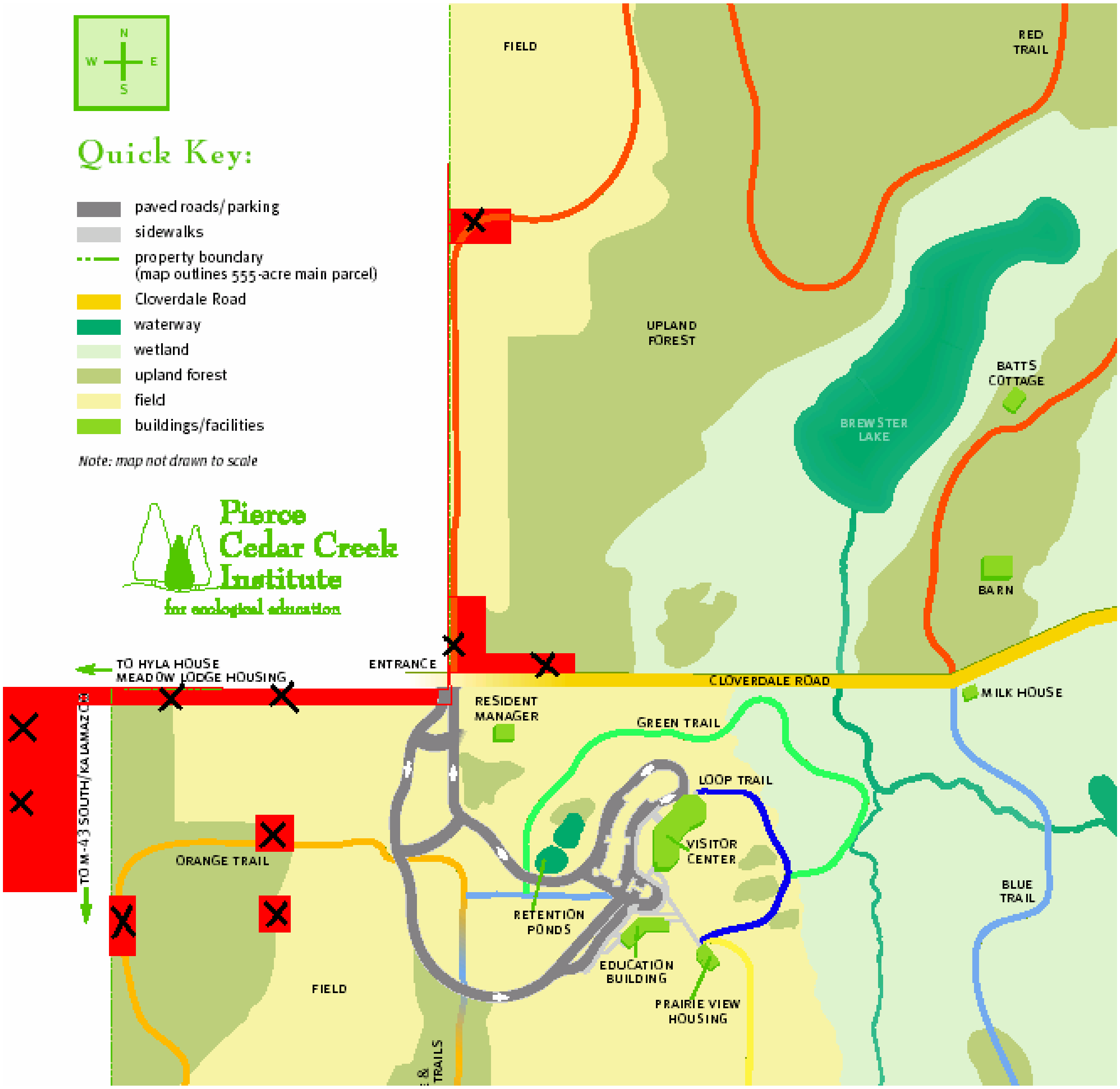

Figure 1 Current map of Pierce Cedar Creek (Hastings, Michigan) red areas denote invasive stands, X's mark the 10 chosen sites.

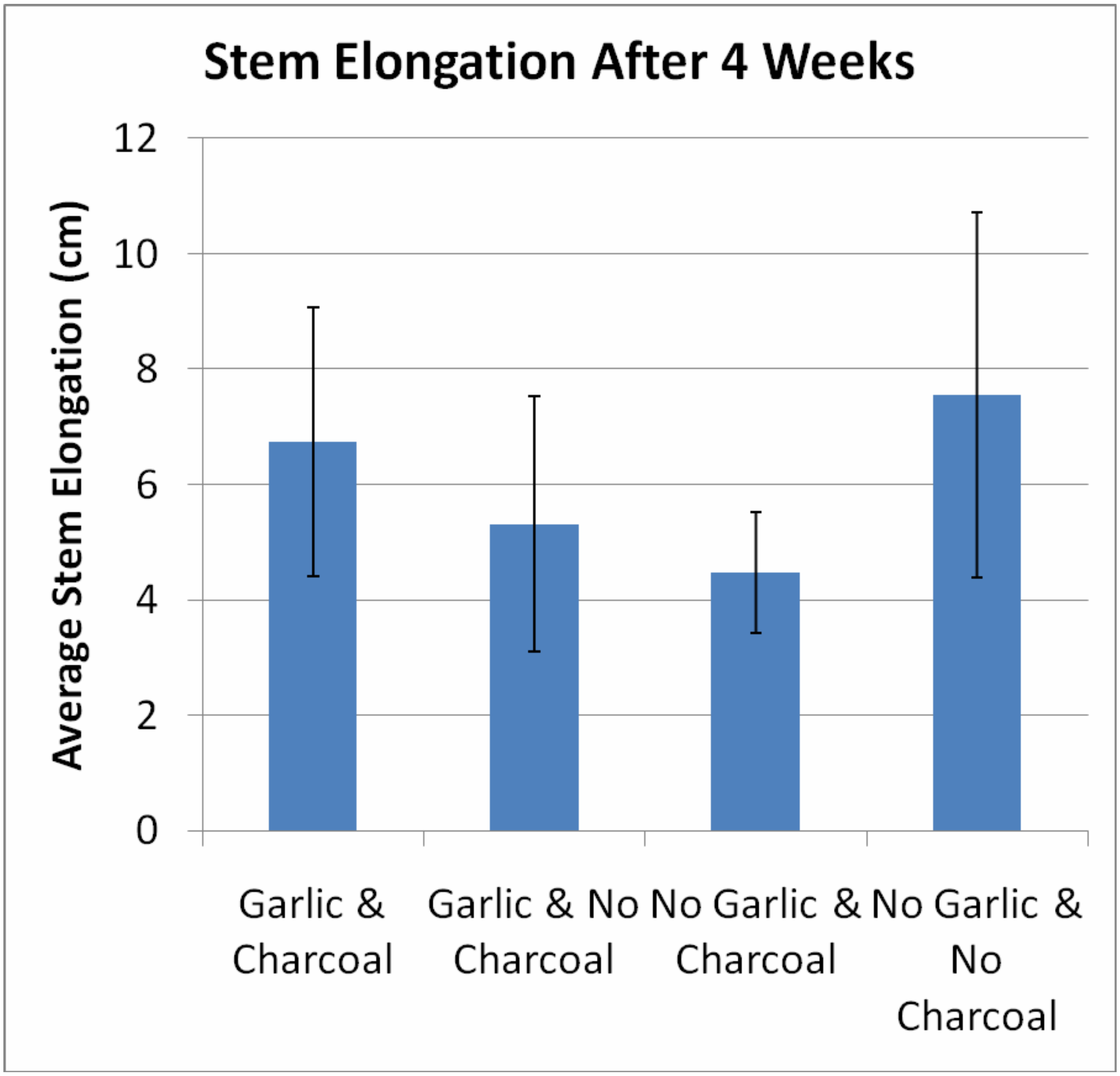

Figure 2 Average stem elongation per treatment during the seedling experiment (over 4 weeks). ANOVA Garlic Mustard X Charcoal Interaction, F (1,6) = 11.23, p = .015

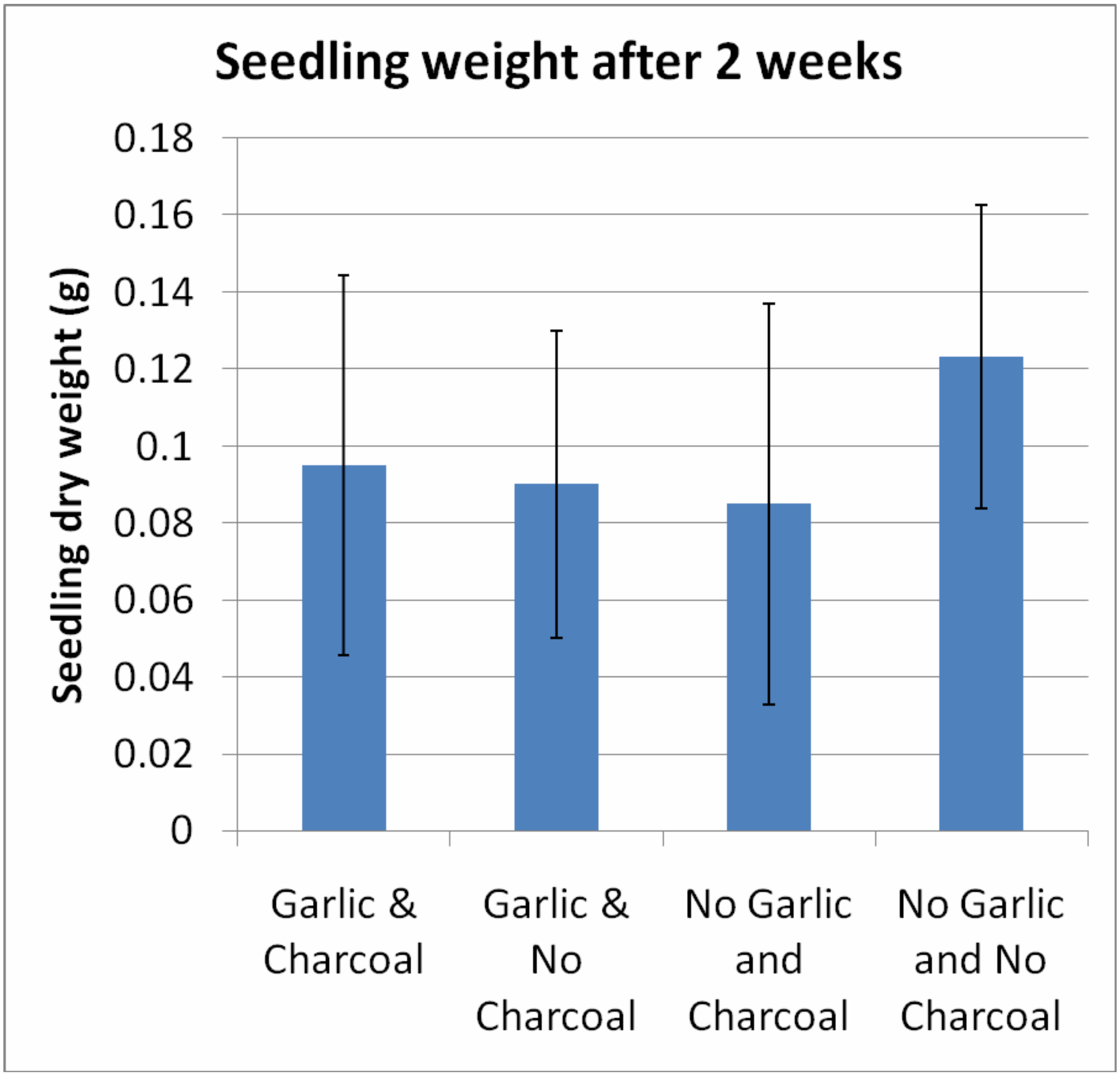

Figure 3 Average seedling dry mass per treatment during the seedling experiment (after 2 weeks). ANOVA F(1, 5)= 1.568 P=0.266

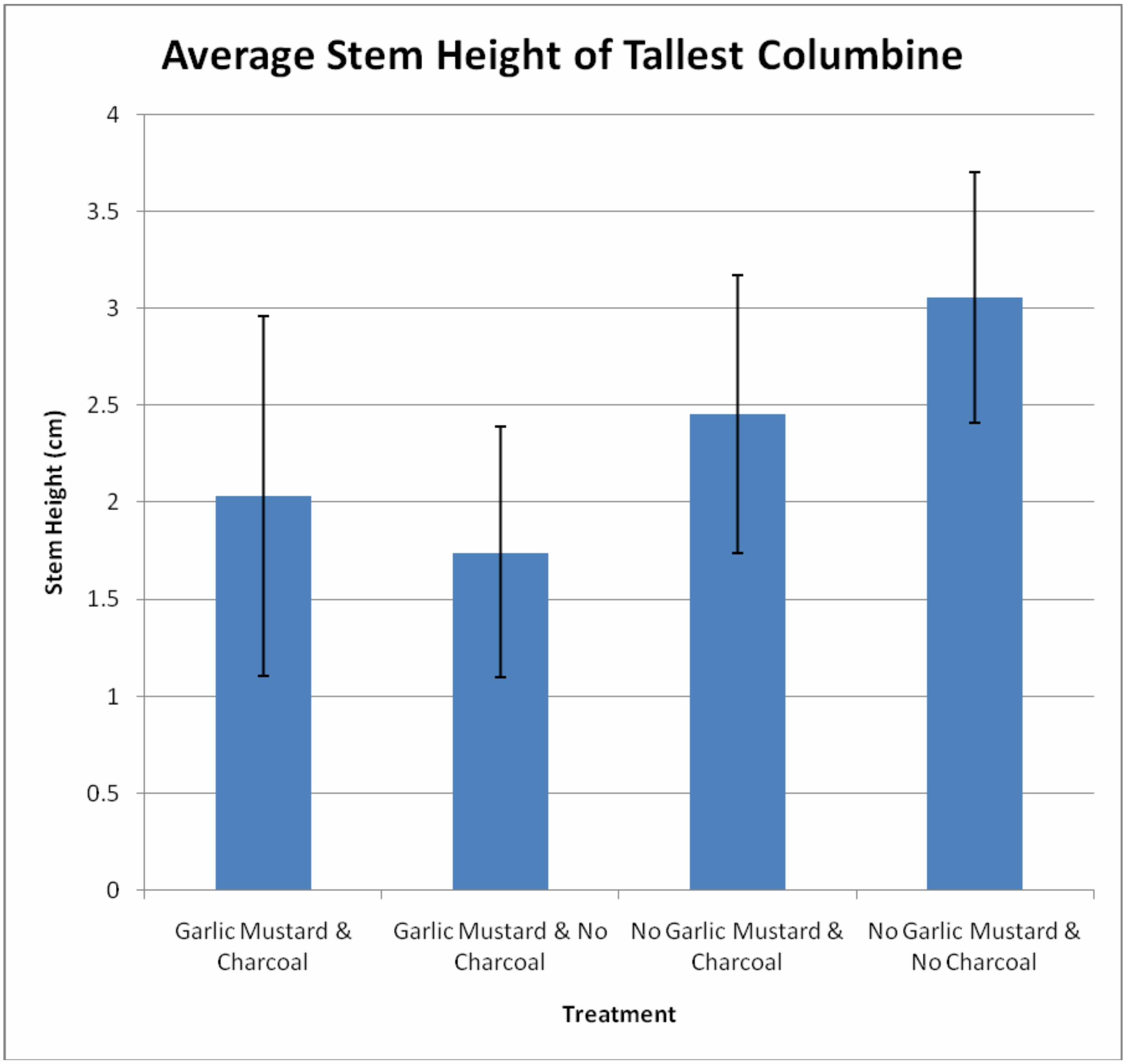

Figure 4 Average Stem height of tallest Columbine seedling per treatment in seed germination experiment (after seven weeks). Univariate ANOVA F(1, 3)= 1.255 P=.138

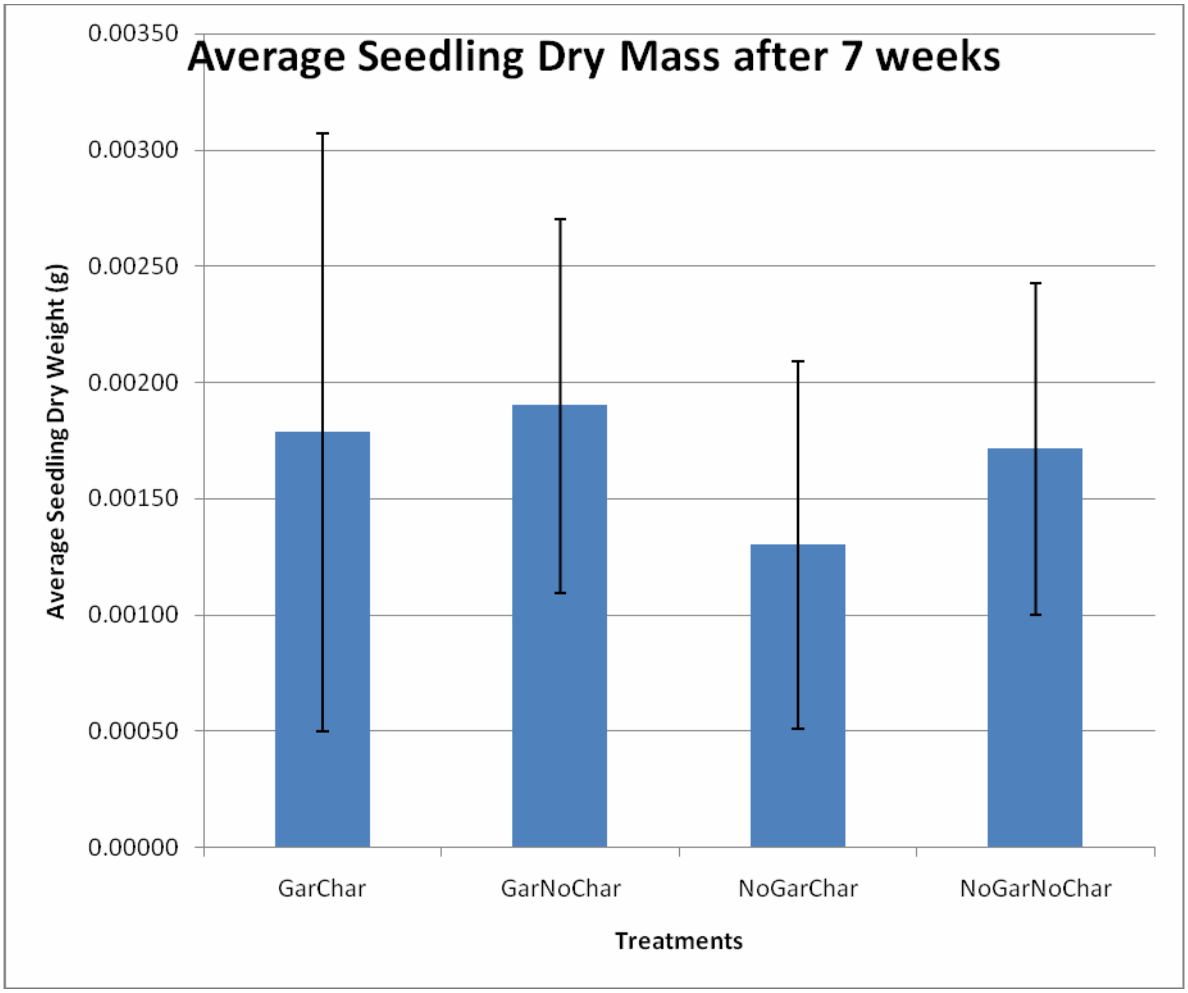

Figure 5 shows the average dry weight of a seedling per treatment during the seed germination experiment. Univariate ANOVA F(1, 6)= 0.244; P=0.639