Examining the use of biochar on spotted knapweed, *Centaurea stoebe* L., and its impact on the success of two introduced biocontrol agents, *Larinus minutus* Gyllenhal and *Cyphocleonus achates* Farhaeus (Coleoptera: Curculionidae).

A Thesis

Presented in Partial Fulfillment of the Requirements for the

Degree of Masters in Science

with a

Major in Entomology

in the

College of Graduate Studies

University of Idaho

by

Toriani Kent

Approved by:

Major Professor: Stephen Cook, Ph.D.

Committee Members: Andrew Nelson, Ph.D.; Deborah S. Page-Dumroese, Ph.D

Department Administrator: Edwin Lewis, Ph.D.

May 2022

Abstract:

Spotted knapweed (*Centaurea stoebe* L.) is potentially the most devastating invasive weeds in rangelands in the western United States, causing a reduction in rangeland productivity, foraging habitat for wildlife, and increasing erosion and stream sedimentation. Two of the most widely used biological control agents against spotted knapweed are *Larinus minutus* Gyllenhal (seed-head weevil) and *Cyphocleonus achates* Farhaeus (Knapweed root weevil) (Coleoptera: Curculionidae). However, their presence alone has not been enough to meet management needs. Biochar has been investigated as a potential control of invasive plants because it can be an alternative to removal or biological controls that have not been able to meet management's needs. This work examined the effects of biochar as a soil amendment on the susceptibility of spotted knapweed to *L. minutus* and *C. achates*, two biological control agents feeding on different parts of the plant. In a nursery, two biochar amendments (10% and 25% by volume) were used in standard nursery media. Biochar amendments increased the attack and presence of offspring in spotted knapweed attacked by *C. achates.* The attack by *L. minutus* was not as straightforward. Biochar appeared to make spotted knapweed seed heads less acceptable to attack while also decreasing the total number of seed heads produced by the plants. However, despite the success in the nursery, the field experiment did not provide supporting data. I used three biochar treatments (0, 10% and 25%) on field plots located near Bovill, ID and found no clear trend. Additional long-term monitoring of plants may be necessary as biochar can take time to move into the mineral soil profile after surface soil application. The majority of the results suggest that using biochar as a soil amendment in areas with spotted knapweed present would be most effective in

conjunction with *C. achates* as the biological control agent, and the lower concentration is this study (10% by volume) of biochar added to the soil provided better results compared to the higher concentration tested.

Acknowledgments

First and foremost, I would like to thank the members of my committee. Dr. Stephen Cook has been much more than my major professor the last two years. His guidance and support are what got me through my masters. I thank Dr. Andrew Nelson for his time and expertise in areas I was unfamiliar with. I thank Dr. Debbie Dumerose for her passion that helped push my work as well as support through my project. My thesis is stronger with their guidance and support. I would also like to thank the faculty and staff of the Department Entomology, Plant Pathology and Nematology at the University of Idaho for strengthening my knowledge and giving me an environment, I would thrive in.

This study was supported by funds from the United States Forest Service and Idaho Agricultural Experiment Station. I would also like to thank Conner Peterson, Abigail Crawford, Curtis Martin, Paul Brusven, Don Regan, Carol Randall, Idaho Department of Land, Latah County Weed Commission for their invaluable assistance through this process.

Table of Contents

List of Tables

List of Figures

Introduction

Spotted knapweed (*Centaurea stoebe* L.) is native to Eurasia that was introduced in the Pacific Northwest US in the late 1800s (Sheley et al. 1998) It has become a devastating invasive species across the western United States and Canada. It was first introduced to North America by accident in clover and alfalfa seeds from Asia (Smith and Story, 2002). Spotted knapweed is found in a wide range of disturbed sites that have varying ecological conditions, soil types, and elevations (Minnesota Department of Agriculture). Spotted knapweed is a perennial plant with a high seed production rate that allows it to dominate the western rangelands where it was introduced (Corn et al., 2006). Areas that are invaded by spotted knapweed have a reduction in rangeland productivity, less foraging habitat for wildlife, and often have increased erosion and stream sedimentation which leads to the need for an improved management strategy for the control of spotted knapweed in North America.

Since the accidental importation of spotted knapweed, twelve species of insect biological control organisms have been introduced and become established in attempts to manage this weed in North America (Smith and Story, 2002). Even with the introduction of twelve natural enemies, the control of spotted knapweed has only shown tolerable levels of suppression in some populations. (Smith and Story, 2002). Two of the most widely used biological control agents against spotted knapweed are *Larinus minutus* Gyllenhal and *Cyphocleonus achates* Farhaeus (Coleoptera: Curculionidae). *Larinus minutus* also known as the seed-head weevil was named for feeding, mating, and laying their eggs in knapweed flower heads (Van Hezewijk and Bourchier, 2012). This weevil is native to Europe and first released in the United States in 1991 as part of a biocontrol program against spotted and diffuse knapweed (Lang, 2021) The seed-head weevil overwinters in surface organic horizons as adults and emerges to start feeding on the vegetative portion of knapweed. Once flowers have developed on the knapweed, *L. minutus* will finish feeding in the flowers which is required for the development of the beetle's ovaries (Minteer et al., 2011). *Larinus m*inutus has also been used in the control of other plant species and was found to control populations of other invasive plants, including thistle (*Carduus nutans* L.) and several knapweed species (*Centaurea stoebe* L. and *Centaurea diffusa* Lam.) found in Canada (Crawley, 1989).

Cyphocleonus achates, the knapweed root weevil, feeds on the roots of knapweed resulting in decreased plant density and stature. This insect is native to southern Europe and the Mediterranean region and was introduced to the United States in 1987 to control both spotted and diffuse knapweed (Lang, 2021). *Cyphocleonus achates* is one of the larger weevils (5-17mm long) and the relatively large larvae results in substantial damage to spotted knapweed roots leading to the girdling of the vascular tissue (Van Hezewijk and Bourchier, 2012). Studies in Europe demonstrated the effectiveness of this root weevil on spotted and diffuse knapweed roots and shoot biomass (Corn et al., 2006).

Studies in other countries have been able to demonstrate the use of *L. minutus* and *C. achates* to be effective in controlling spotted knapweed, but in North America that is not the case. The combined attack by *L. minutus* and *C. achates* on spotted knapweed should increase the damage caused by the two control agents; With two different feeding sites on the plant, it can be weakened, and its dispersal interrupted. In North America the level of control provided by the biological control agents has not sufficiently met management needs for knapweed control (Van Hezewijk and Bourchier, 2012). Adding another tool to combine with insect control is needed. A new control tactic, biochar, can alter soil properties to enhance knapweed control by biocontrol organisms.

Biochar is the product of burning organic material via pyrolysis (Biederman and Harpole, 2013). The end-product is a high carbon substance that contains about 40-90% of the original carbon, depending on pyrolysis method (Cooperman, 2016). Biochar is an amendment for restoring degraded agricultural, mine, urban, and forest soil. Biochar can be a potential control method to limit growth or reproduction of invasive plant species and used either alone or in combination with removal or biological control agents. Biochar generally increases soil organic matter and carbon to increase desirable plant species numbers and growth, while also immobilizing soil nitrogen. Nitrogen immobilization stresses invasive plant species that thrive in a nitrogen rich environment (Blumenthal et al., 2003). This added stress can be used to reduce knapweed or other invasive species biomass and potentially enhance attack by biological control organisms. An earlier study found that the addition of biochar reduced the weeds *Cirsium arvense* L. and *Setaria faberi* Herrm. biomass at a prairie site in Minnesota by 54% (Blumenthal et al., 2003). Biochar did not affect some invasive *Lespedeza cuneata* Dum.Cours. height or biomass but did increase the height and biomass of the native plant *Andropogon gerardii* Vitman. (Adams et al., 2013). A meta-analysis found that biochar, in variable site conditions, has neutral to positive effects and has shown an increase in plant productivity (Biederman and Harpole, 2013). This potential increase in plant productivity could limit the usefulness of biochar if the positive effects on the spotted knapweed outweigh the negative effects.

The objectives of my project were to determine in the nursery and field: (1) the survival of adult *L. minutus* and *C. achates* when exposed to biochar and to ensure biochar will not kill the biological control agents, (2) response of spotted knapweed effect to biochar and (3) a change in spotted knapweeds susceptibility to attack by *L. minutus* and *C. achates* while comparing the impact of feeding by seed versus root as a biocontrol agent.

Methods and Materials

1. Experimental organism:

Two species of curculionids, *L. minutus* and *C. achates*, were collected from field sites in western Montana (2020) and Nez Perce County, Idaho (2021) and transported to Moscow, ID in paper tube containers with ice packs. The curculionids were maintained in a cold incubator for several hours before being placed into randomly assigned treatments for each experiment. The curculionids collected in 2020 were used in the adult survival study and those collected in 2021 were used in the nursery and field study. Sixty Spotted Knapweed plants of similar size were collected in Moscow, ID (46.728549, -117.005057) and repotted the same day during the first week of June 2021.

2. Biochar:

The biochar used in the experiment was furnished by the U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station which was produced by pyrolysis of sawmill residues in a gasification system manufactured by Tucker Engineering Associates (TEA), located in Locust, North Carolina. The residues used to produce the biochar were from mixed conifer tree species, predominately Douglas-fir (*Pseudotsuga menziesii* var.) and lodgepole pine (*Pinus contorta* var.) The characteristics of the biochar after the gasification process consisted of a pH = 10.2, %H2O = 2.94, bulk density (dry) = 0.165 Mg m-3, %C = 91.5, %N = 0.89, C:N = 102.8, BET surface area = 15.0 m2g-1, energy = 33.98 MJ kg-1 and a particle size distribution of <44 μm to 6.350 mm, centered around 0.84 mm (Anderson et al. 2013).

3. Adult survival:

This study was conducted for a 7-day period at the end of July 2020. Four soil and biochar treatments were used to determine if exposure to biochar had an impact on *L. minutus* and *C. achates* survival. Treatments were: (1) 0.5g of biochar alone, (2)10g of soil alone, (3) 0.5g of biochar scattered across the surface of 10g of soil, and (4) 0.5g biochar mixed with 10g of soil. Once established, each treatment was misted with distilled water to maintain natural moisture conditions. Three curculionids were randomly assigned to 500 ml containers with air holes added to the lids containing one of the treatments and placed in a 20°C incubator. Each treatment contained a total of five replicates, (total of 120 curculionids= 4 treatments x 2 insect species x 5 replicates x 3 specimens). Weevils were examined daily for seven consecutive days to monitor survival.

4. Nursery experiment:

The nursery experiment was conducted at the University of Idaho's Franklin H. Pitkin Forest Nursery in Moscow, ID starting at the beginning of June 2021 and running through September 2021. Sixty spotted knapweed plants were assigned to three growing media treatments (4 curculionid treatments x 3 soil treatments x 5 plants/treatment =60 total pots): (1) standard nursery media mix (control) with peat moss + perlite mixture (50:1), low (90%

standard nursery media mix and 10% biochar) and high (by volume, 75% standard nursery media mix and 25% biochar). The standard nursery soil mixture was made by adding 1 L of perlite to 50L of peat moss. The plants were potted into 10 cm x 10 cm x 23 cm pots with mesh lining the bottom of the pots to prevent other insects from contaminating the study. Once potted, the plants were brought to an outdoor area at the nursery, where they would remain for the duration of the experiment.

Nine weeks into the experiment Ion-Exchange Resin Capsules (IERC, UNIBEST International ©) were added to five plants in each treatment (15 total). It was not known if the curculionidae would interact with the IERC's, so they were placed in separate treatments to avoid issues that could arise. The IERC's release hydrogen and hydroxide into the soil similarly to how plant roots release these ions in exchange for available nutrients in soil. Once the nutrients were absorbed into the IERC's they would provide a predictive measurement of the nutrients released by the soil that is available to plants.

This experiment was conducted from June 2021 through September 2021. During June and July, the plants were watered for 15 minutes every third day. In August spotted knapweed watering was reduced to twice weekly and this was continued for the duration of the experiment. Biweekly observations were made on the plants and height measurements were taken weekly on all the plants that were not placed in mesh nets (see below).

5. Field experiment:

The field experiment was conducted on a site controlled by the Idaho Department of Lands, located approximately 8 km from Bovill, ID (46.76179, -116.248727). This site was chosen for the high abundance of spotted knapweed over a large, contiguous area. The soil classification at this site was Andosols 41% Cambisols 31% Luvisols 11% Albeluvisols 6% Podzols 3% ("Soilgrids Web Portal."). Ten plots that were 50 cm x 50 cm were established, and each large plot had three soil treatments (1) control, (2) low and (3) high biochar treatment. Treatments within a plot were marked using whiskers of different colors. The control had no biochar added, the low biochar treatment plots received 56g of biochar, and the high biochar treatment plots received 112g of biochar. This can also be thought of as the low treatment having 22400 kg/ha and the high treatment having 44800 kg/ha. The biochar was dispersed evenly within the plot.

This experiment was conducted from June 2021 through September 2021. Data was collected weekly to determine spotted knapweed height, browning (as an indicator of environmental damage from drought and/or high temperatures), presence of the released curculionids, and presence of other insects.

6. Release technique for the biological control agents:

Of the two curculionid species used during these experiments, *L. minutus* emerges earlier in the summer than *C. achates*. Therefore, releases of the curculionids occurred at different times. In mid-June, *L. minutus* were released in both the nursery and field sites. For the nursery site ten plants in each treatment (30 total) each had ten *L. minutus* placed with them. Half the plants had only the *L. minutus* released onto them while the other half also received a *C. achates* treatment later in the summer as this curculionid became available. Once the curculionids were added to the pots, nylon netting was placed around the pots in a tent-like fashion using a bamboo pole and rope ties for support that kept the insects within the pot area for the duration of the study.

For the field release experiment, the curculionids were released on a 25 cm x 38 cm wood board placed in the middle of each plot to allow the curculionids to make an unbiased choice on direction of movement. Fifteen *L. minutus* were released per plot and it required 30 minutes for the curculionids to disperse from the boards. In early August, *C. achates* were released. This was done in the same manner as the *L. minutus* release, except the number of individuals released differed between the field and nursery experiments. In the nursery, three individual weevils were added to the pots of each plant, while four individuals per plot were released in the field experiment.

7. Resin capsules and plant data collection nursery and field

In the nursery, the IERC's were removed after eight weeks and sent to UNIBEST International © for analysis. Each IERC was removed using nitrile gloves to prevent contamination and rinsed with deionized water before being placed into whirl-paks and shipped. Once the IERCs were extracted, the plants were also removed from the pots and placed in zip-type bags. The remaining plants at the nursery had received the curculionid treatments (*L. minutus, C. achates* or both). Each harvested knapweed was divided into roots and seed heads and observations were made on: (1) stems and leaves were dry, (2) how much foliage was at the base of the stems, and (2) seed head development and/or flowers. The seed heads were then counted from each plant and recorded before being dissected to identify the presence of attack and offspring from the *L. minutus*. The roots were also dissected to identify the presence of attack and offspring from the *C. achates*.

At the field site one plant was removed from each treatment for all the plots (30 plants in total). The plants were placed in zip-type bags and placed into a cooler to limit water loss from the heat. Seed heads were also collected, with 10 seed heads collected from a random plant in each treatment for all plots. These were placed in plastic zip-type bags and transported to the lab where they were dissected to identify the presence of attack and number of larval *L. minutus* present

The 15 nursery plants and 30 field plants were separated into root and shoot sections for further analysis. weights of the roots and shoots were recorded before being dried in brown paper bags. Once dried, the roots and shoots were reweighed. The same roots and shoots that were dried and weighed were ground using a laboratory scale. Three grams of material from each section (root and shoot) and each treatment and replicate were placed into paper envelopes and sent to JR Peters Lab Inc (location) for total nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, copper, boron, zinc, molybdenum, aluminum, and sodium analyses.

8. Data Analysis:

All data, except the plant tissue analyses, were analyzed using ANOVA with a GLM procedure in the Statistical Analysis System package. Because the plant tissue had to be pooled for chemical analyses due to weight requirements, there was only a single measure per treatment and no statistical comparison could be made. Instead, the data were graphed to identify any trends, both within each experiment and between the plants from the nursery and field sites.

Results:

<u>Adult Survival:</u>

In the first stage of my work to determine survival when confronted with biochar, I detected no significant difference in insect mortality among the four treatments (soil alone, biochar alone, biochar mixed with soil and biochar on the soil surface) for either *L. minutus* (F = 0.53; df = 3, 28; P = 0.6630) (Figure 1a) or *C. achates* (F = 0.92; df = 3, 16; P = 0.4545) (Figure 1b). There was more variation in survival of *L. minutus*, but the overall rate of survival remained high. The *C. achates* had a 100% survival rate in three of the four treatments, but survival was less in the biochar only treatment. indicating that the biochar had virtually no impact on weevils in a confined space in which they were in immediate contact with the material.

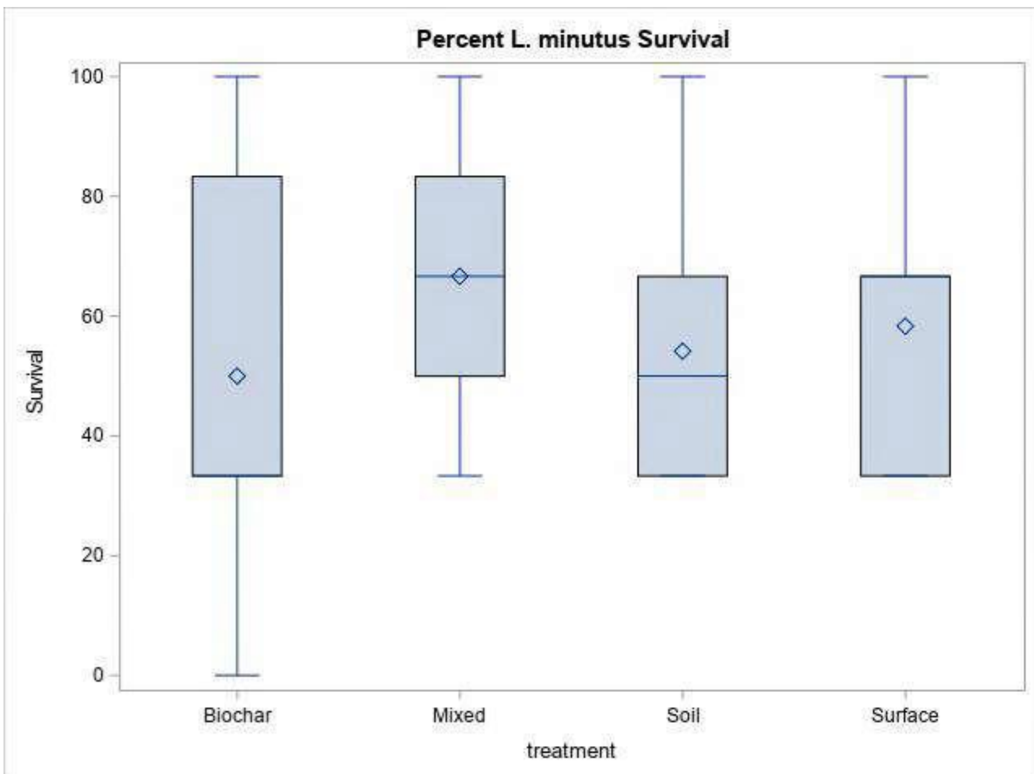

FIGURE 1a: Survival (mean + SEM) of adult *Larinus minutus* after 7 days of exposure to biochar and soil treatments.

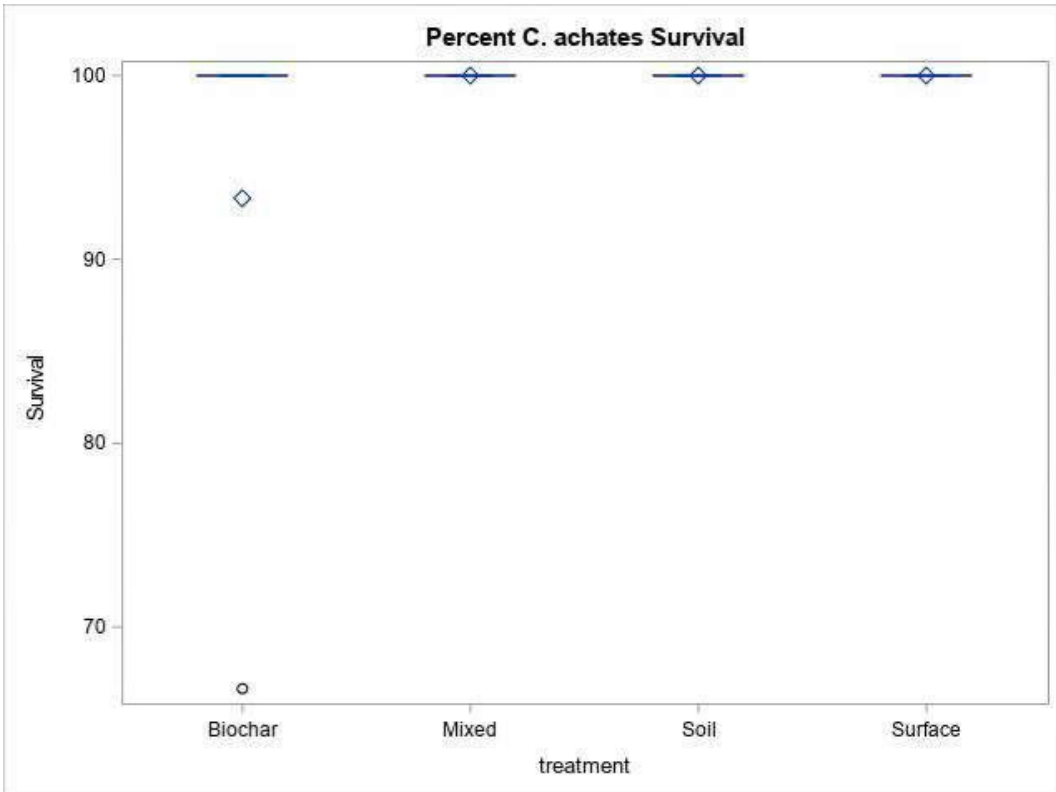

FIGURE 1b: Survival (mean + SEM) of adult *Cyphocleonus achates* after 7 days of exposure to various treatments of biochar and soil.

<u>*Larinus Minutus* Seed Head Attack and Offspring: Nursery study</u>

In the nursery study there were two weevil exposure tests, one with only *L. minutus* (figure 2a) and the other with *L. minutus* and *C. achates* (figure 2b) placed with the spotted knapweed. Similar trends were observed for seed head attack for plants that were exposed to only *L. minus* and to those exposed to a combined attack by *L. minutus* and *C. achates*. In this study, the control treatment had the greatest incidence of attack by either one or both insects. There was a decrease in the incidence of attack with an increase in the concentration of biochar. For the spotted knapweed that received only *L. minutus*, there was high variability in the control and low biochar treatments, while the high biochar treatment had no incidence of attack (F= 0.52; df = 2, 7; P = 0.6174). The spotted knapweed that was exposed to both *L. minutus* and *C. achates* had less variability in seed head attack in the control and low treatments and the high treatment was similar to the other set of plants with no incidence of attack (F= 4.97; df = 2, 8; P = 0.0396).

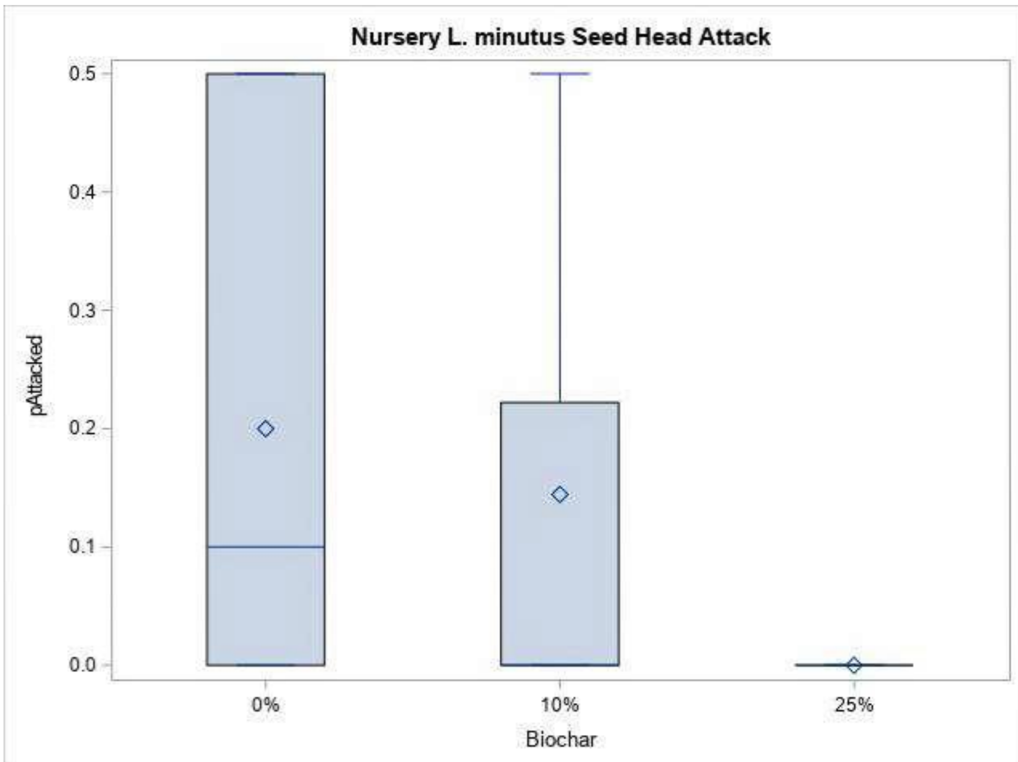

FIGURE 2a: Mean proportion (± SEM) of attacked seed heads on plants containing *L. minutus* only in the nursery study with either 0%, 10% or 25% biochar applications.

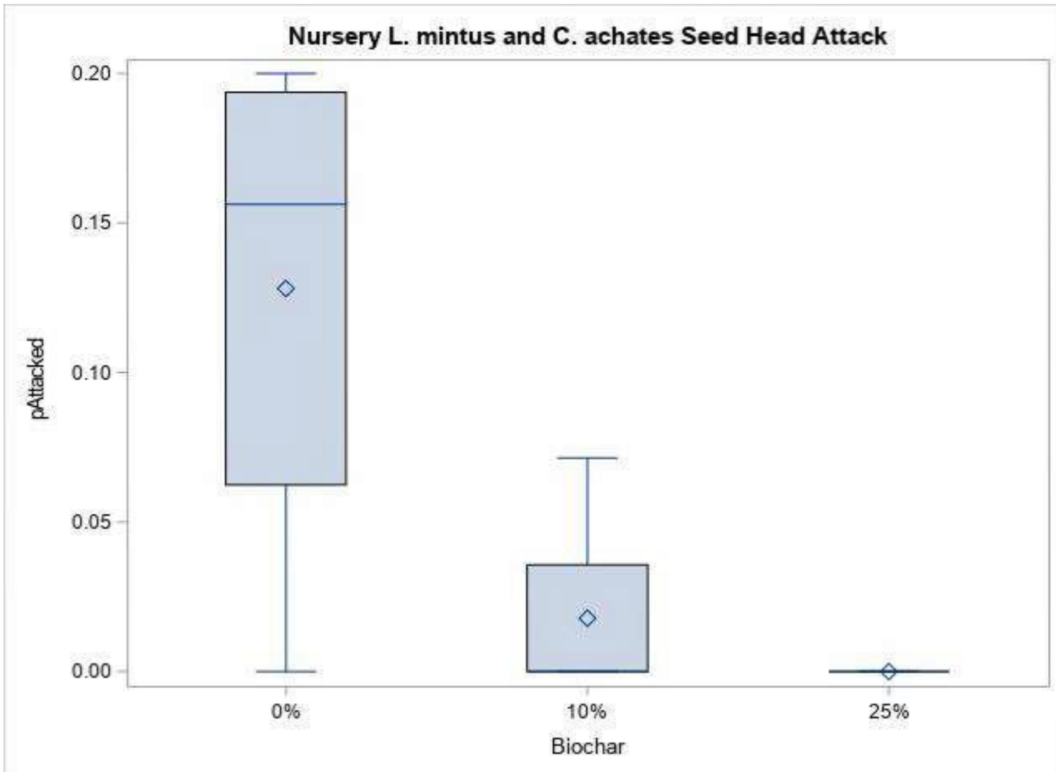

FIGURE 2b: Mean proportion (+ SEM) of attacked seed heads on plants containing *L. minutus* and *C. achates* at the nursery site with either 0%, 10% or 25% biochar applications.

When examining the attacked seed heads for presence of offspring; the plants at the nursery site had little to no offspring present. The spotted knapweed that received only *L. minutus* had offspring present in approximately a third of the seed heads attacked in the low treatment and none in the control and high biochar treatments. The spotted knapweed plants that were exposed to both *L. minutus* and *C. achates* had no offspring in any of the attacked seed heads from any of the treatments. After looking at presence of attack and offspring, an analysis was conducted to determine if the number of seed heads per plant was affected by the presence of biochar in the soil. This analysis was conducted using only the nursery plants. There was an overall decrease in the number of seed heads present per plant with the addition of biochar to the soil (F= 4.63; df = 2, 42; P = 0.0153) (figure 3).

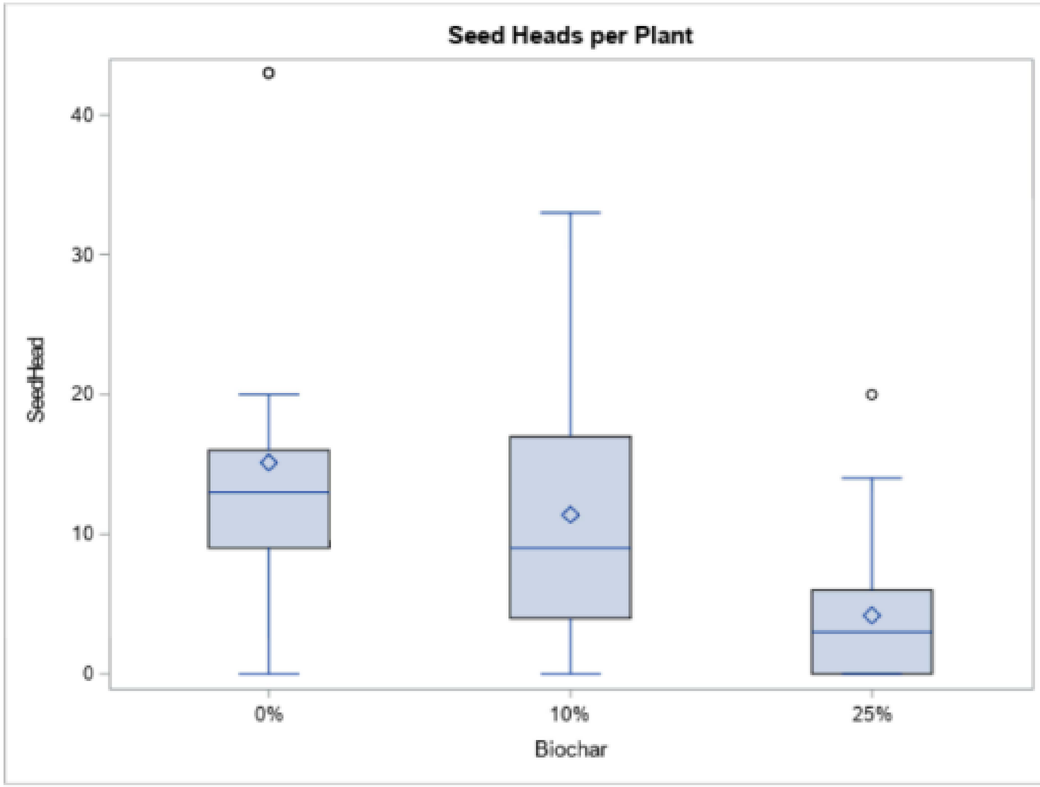

FIGURE 3: Mean number (± SEM) of seed heads found on each plant within either 0%, 10% or 25% biochar applications.

Larinus Minutus Seed Head Attack and Offspring: Field study

Seed head attacks by *L. minutus* at the field site were more prevalent in plants growing in soil treated with biochar (figure 4). Although there is substantial variability in this data, the mean for each treatment was approximately 60% of seed heads attacked by *L. minutus*. The full dataset indicated an overall high attack on seed heads in the high biochar treatment (F= 0.11; df = 2, 27; P = 0.8938).

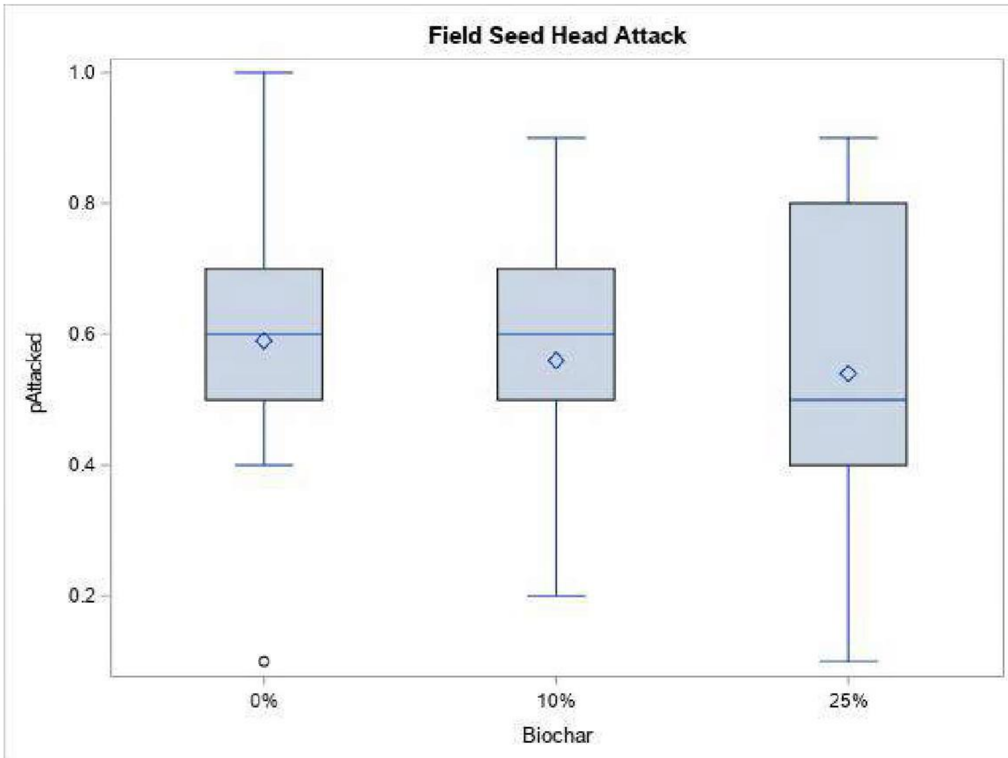

FIGURE 4: Mean proportion (± SEM) of attacked seed heads from 10 collected seed heads from each plot at the field site with either 0%, 10% or 25% biochar applications

The field site had a higher presence of offspring in the seed heads of the biochar treatments (F= 0.51; df = 2, 27; P = 0.6088) (figure 5) as compared to the control or low biochar rate treatments, but the differences were not significant. The low biochar treatment had the highest average offspring in the seed heads, as well as the highest proportion of offspring on a single plant. The lowest proportion of offspring found of the seed heads of plants in the control treatment that received no biochar.

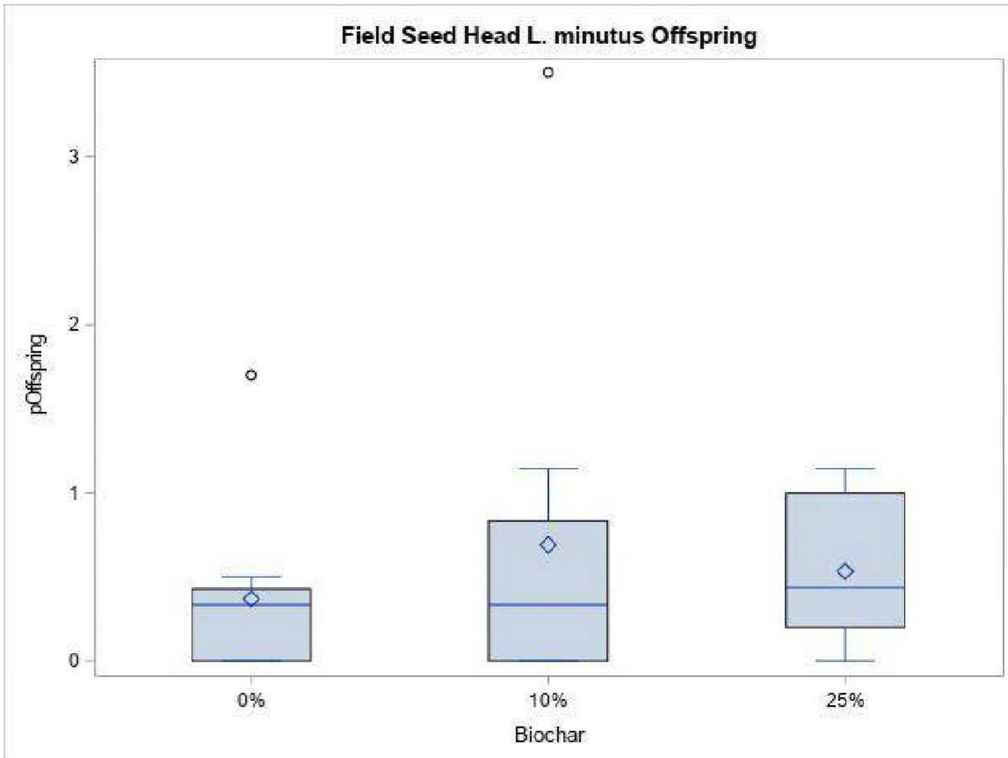

FIGURE 5: Proportion (mean ± SEM) of *L. minutus* offspring found in seed heads collected from the field site.

Cyphocleonus achates Root Attack and Offspring: Nursery study

I did not examine roots from the field study. Spotted knapweed grown in the nursery that were exposed to either *C. achates* alone or in combination with *L. minutus* had a 100% attack rate in the high biochar treatment and an 80% attack rate for the control treatment, but the treatments were not significantly different. There was slightly more variability in attack within the low treatments for both experiments with the plants that received only *C. achates* (figure 6a) (F= 0.66; df = 2, 9; P = 0.5419) having 100% attack and the plants that received *L. minutus* and *C. achates* (figure 6b) (F= 0.39; df = 2, 11; P = 0.6842) having 80% attack.

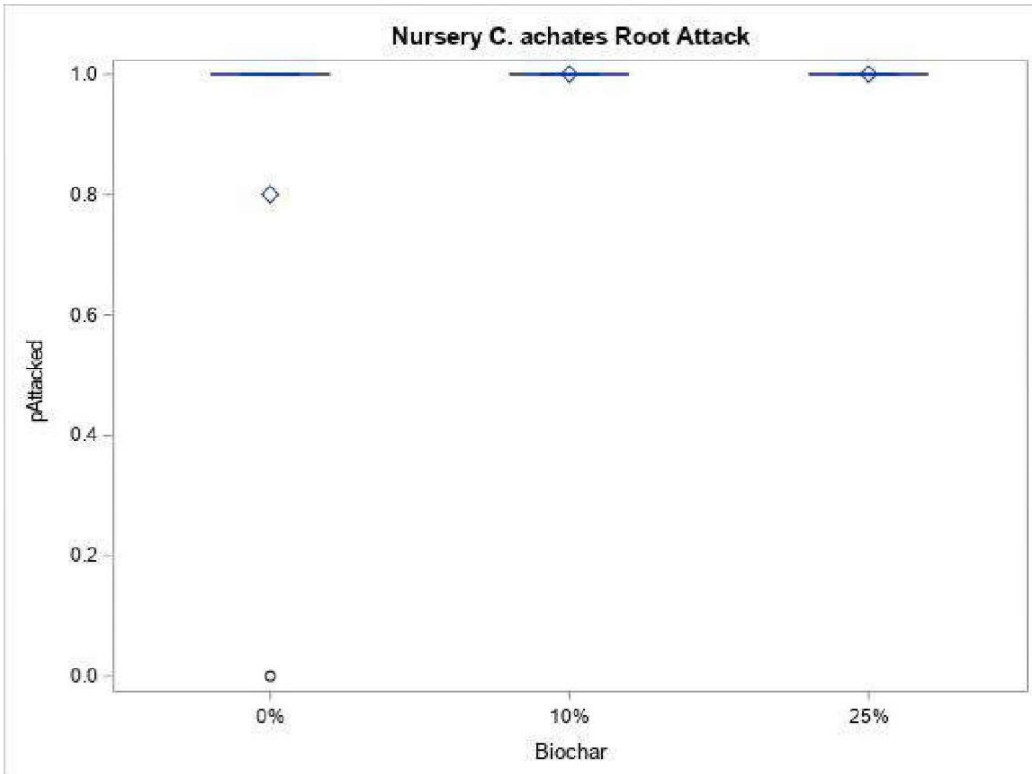

FIGURE 6a: Proportion (mean ± SEM) of *C. achates* attacked roots from the nursery site with either 0%, 10% or 25% biochar applications.

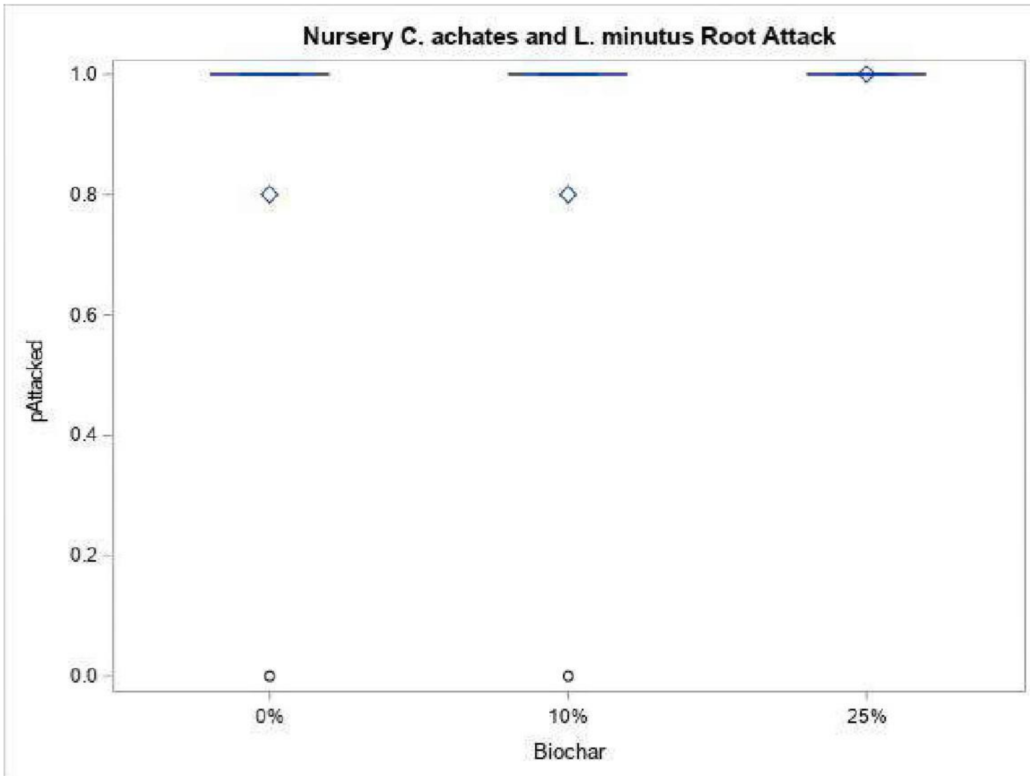

FIGURE 6b: Proportion (mean ± SEM) of *C. achates* attacked roots with *L. minutus* from the nursery site.

While the rates of root attack were similar for both sets of weevil exposures, the production of offspring differed among plants exposed to different rates of biochar. For plants that only had *C. achates* (figure 7a), there were more offspring found in the biochar treatments compared with the control plants. Plants in the low biochar treatment had the highest overall number of offspring per root and plants in the high biochar treatment had the highest number of offspring on a single root, but the treatments were not significantly different (F= 1.47; df = 2, 9; P = 0.2804). For the plants that had *L. minutus* and *C. achates* present (figure 7b), the average number of offspring increased from the control to the low treatment, while the high treatment had less overall offspring per root (F= 0.39; df = 2, 11; P = 0.6856).

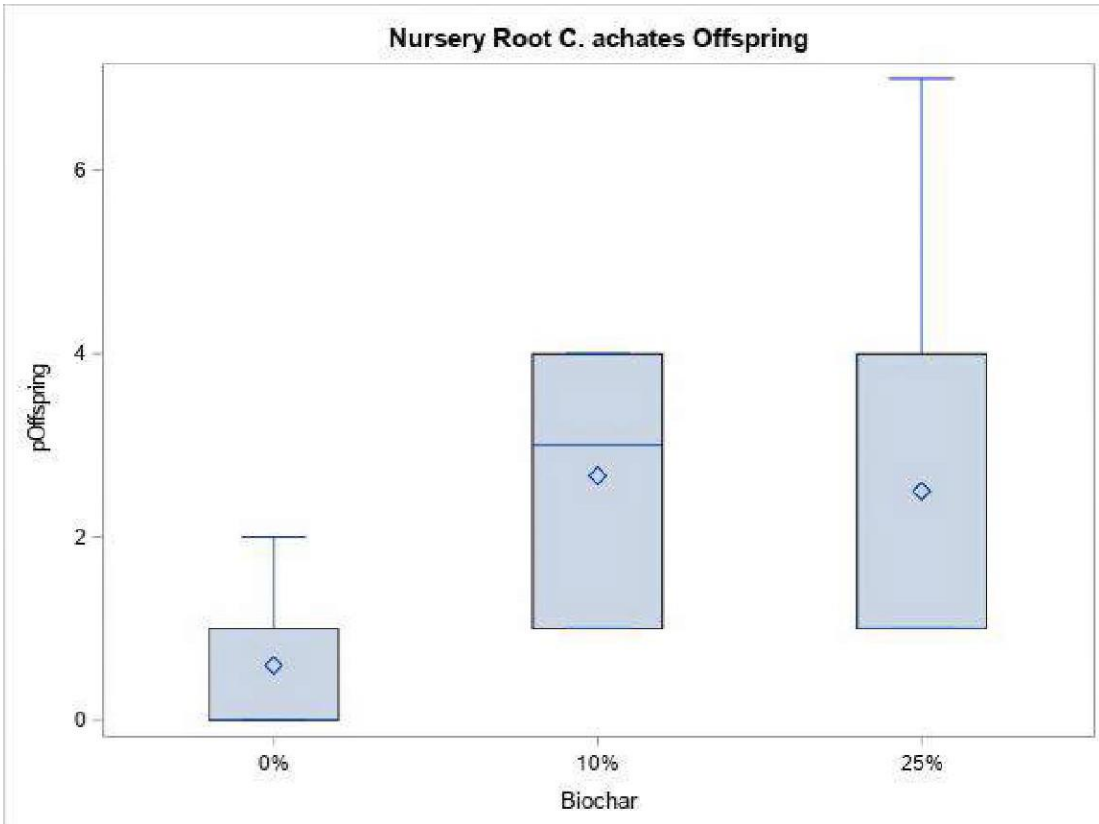

FIGURE 7a: Proportion (mean ± SEM) of *C. achates* offspring from roots attacked at the nursery site.

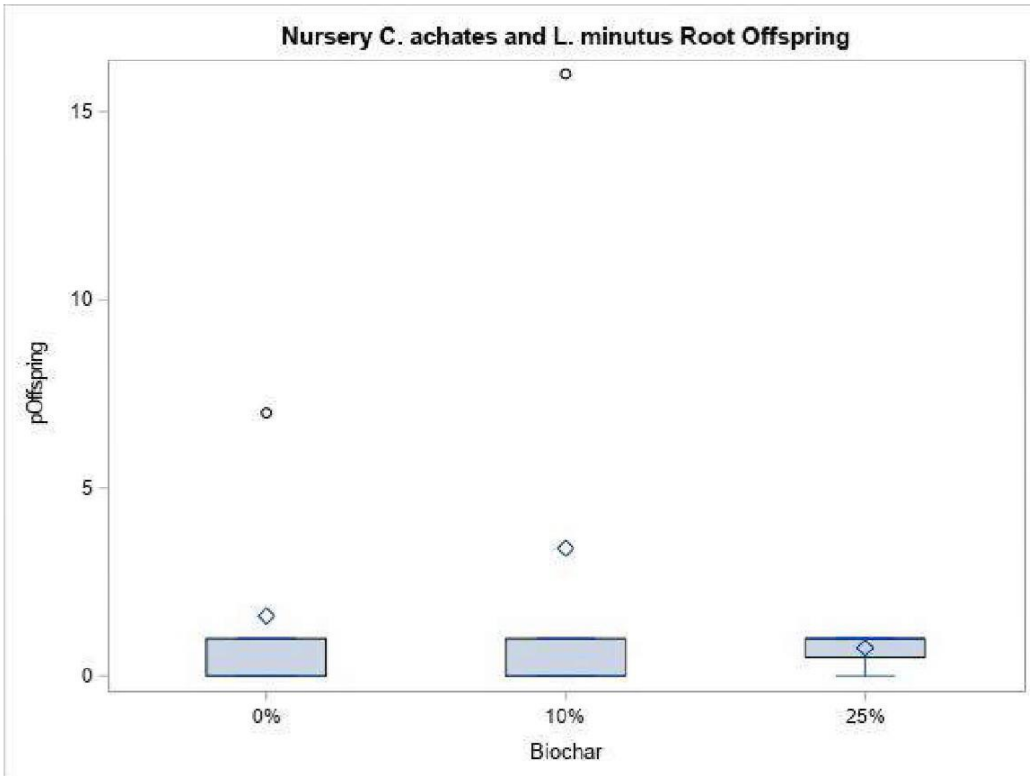

FIGURE 7b: Mean proportion (± SEM) of *C. achates* offspring from roots attacked with *L. minutus* at the nursery site.

Spotted Knapweed Chemistry:

Spotted knapweed chemistry was obtained for both the nursery and field experiments. To have enough material for the analysis, chemical composition of the plants needed to be pooled into samples from each treatment and site. Because of the necessity to pool samples to obtain an adequate sample weight, we note trends across the different treatments were only found for calcium, manganese, and aluminum (figure 8). All three of these nutrients decreased in concentration with an increase in biochar application rates. Other plant components that showed little change among the treatments were sulfur and sulfate (figure 9).

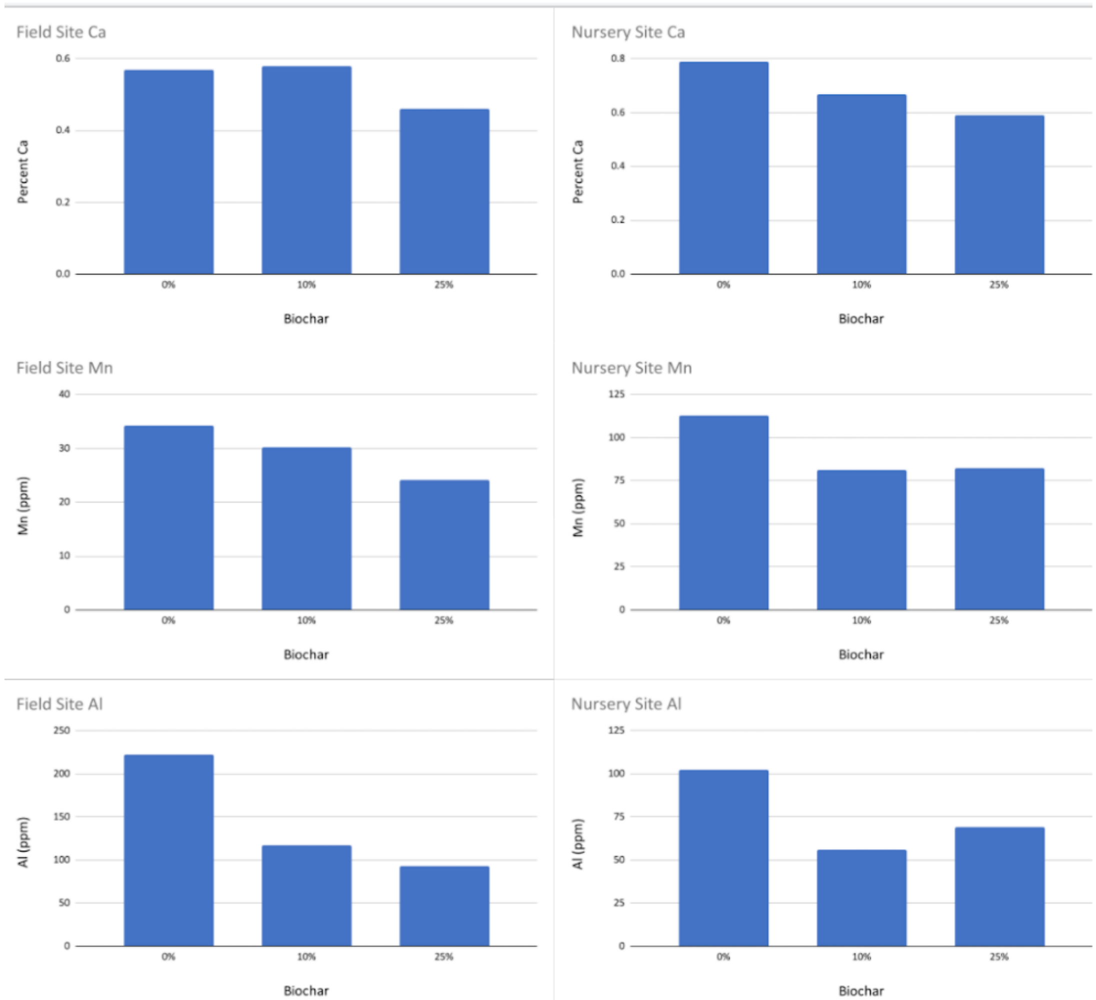

FIGURE 8: Average calcium (percent), manganese (ppm), and aluminum (ppm) as affected by increasing amounts of biochar at a field site or in the nursery. Treatments are the control (0%), low biochar (10%) and high biochar (25%).

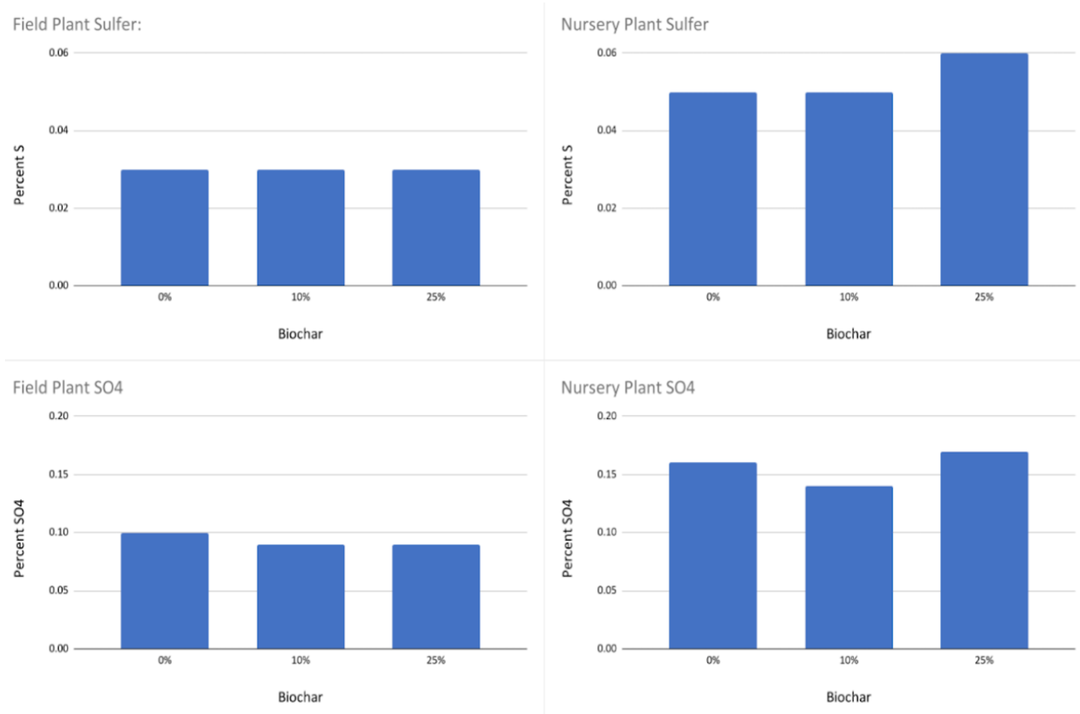

FIGURE 9: Average Sulfur (percent)and Sulfate (percent) as affected by increasing amounts of biochar at a field site in the nursery. Treatments are the control (0%), low biochar (10%) and high biochar (25%).

<u>Soil Chemistry:</u>

Soil chemistry from the pot study was evaluated using resin capsules installed in the individual pots. There were some trends and similarities found within and among the treatments. Only ammonium increased concentration as biochar amounts increased, but this was not significant (F= 0.81; df = 2, 12; P = 0.4695). Other nutrients decreased in concentration when the media and biochar increased. Aluminum (F= 0.57; df = 2, 12; P = 0.5808), zinc (F= 1.32; df = 2, 12; P = 0.3034), copper (F= 1.14; df = 2, 12; P = 0.3513), calcium (F= 0.11; df = 2, 12; P = 0.8929) and boron (F= 0.44; df = 2, 12; P = 0.6570) (figure 10) all decreased, but iron (F= 0.03; df = 2, 12; P = 0.9898) and manganese (F= 0.32; df = 2, 12; P = 0.7290) were unaffected (figure 11).

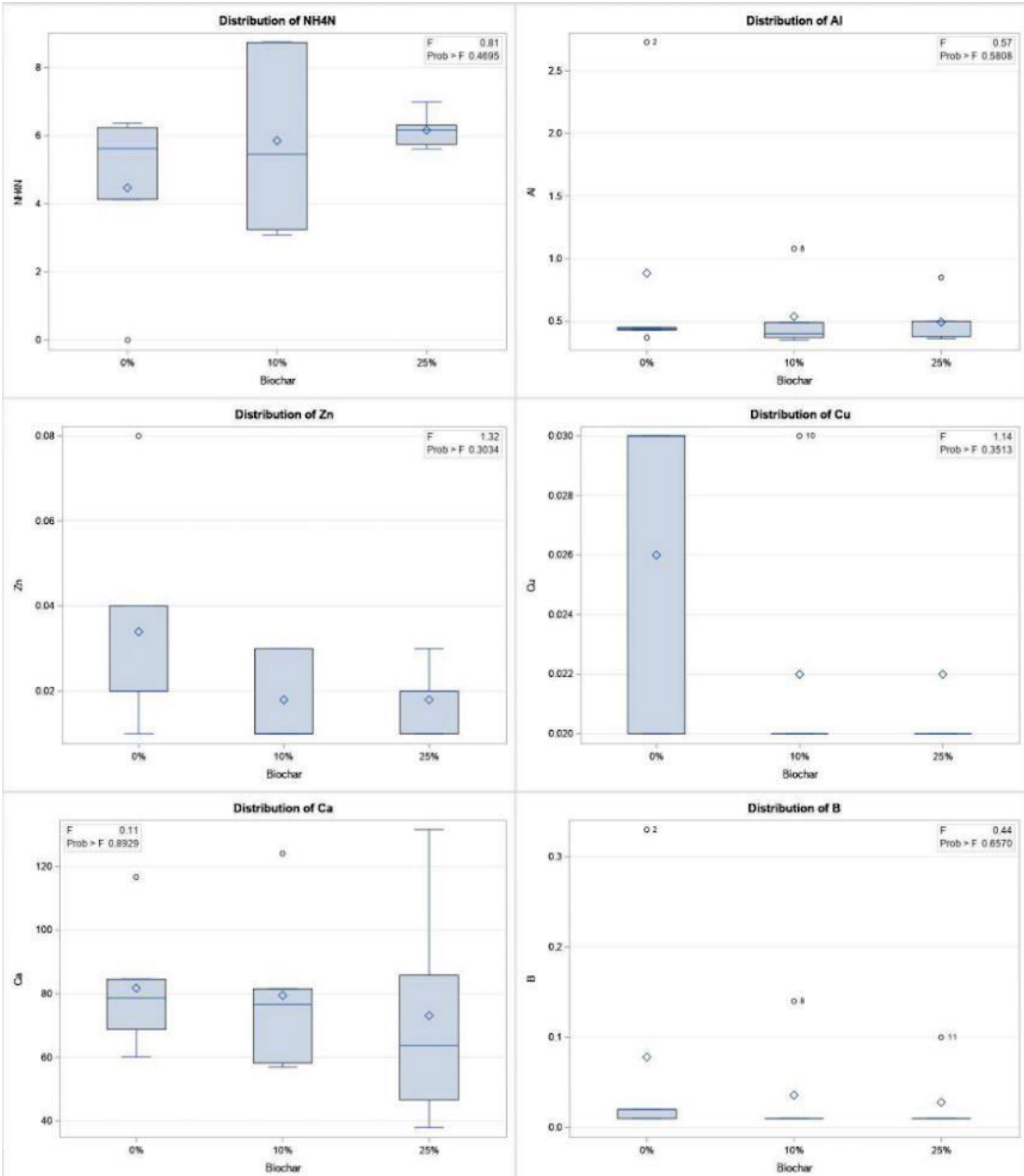

FIGURE 10: Average ammonium (ppm), aluminum (ppm), zinc (ppm), copper (ppm), calcium (ppm) and boron (ppm) as affected by increasing amounts of biochar at the nursery. Treatments are the control (0%), low biochar (10%) and high biochar (25%) mixed into standard growing media of peat moss and perlite.

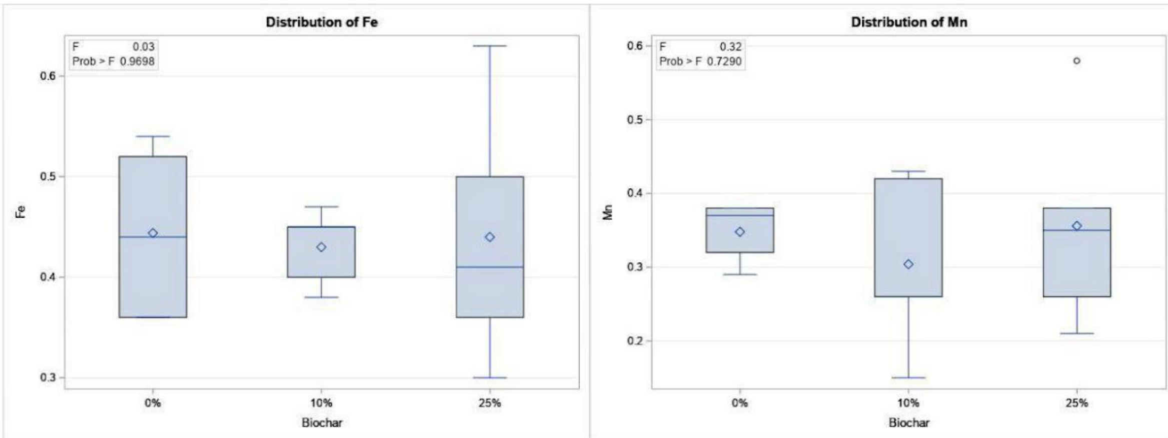

FIGURE 11: Average iron (ppm) and manganese (ppm) as affected by increasing amounts of biochar at the nursery. Treatments are the control (0%), low biochar (10%) and high biochar (25%) mixed into standard growing media of peat moss and perlite.

Discussion

Adult Survival:

Larinus minutus and *C. achates* are both known to attack spotted knapweed and cause damage leading to death. However, there is no published work that examines how the two species would react when exposed to biochar. The low variation and high survival rate of the insects of each species indicate that they would make viable biocontrol agents against spotted knapweed when combined with the use of biochar as a soil amendment.

Larinus Minutus Seed Head Attack and Offspring:

My results from the nursery and field sites for seed head attack and offspring production contradict each other. In the nursery there was a decrease while the field site had an increase in presence of attack and offspring with the addition of biochar. The difference between these two studies is likely due to the biochar application method. Treatments in the nursery experiment consisted of biochar mixed into the soil, while treatments at the field site had the biochar scattered across the top of the soil. The soil at the field site could not be mixed with biochar without disrupting the topsoil and potentially damaging the plants. Due to this difference in application methods, I did not use IERC's in the soil at the field site because surface-applied biochar likely wouldn't have altered mineral soil properties within the time frame of this study. Biochar movement into the soil can take 6 months-years, depending on soil texture and biochar particle size. Once in the soil, it can take up to 6 months to begin interacting with the plant roots with further aging increasing biochar's affinity to hold nutrients (Joseph, et al., 2021). I could not compare soil chemistry in the field site treatments, so the results appear to be related to differences in application technique. Another factor that could have affected my results was that all seed heads were examined for the nursery plants, while only a randomly selected 10 seed heads were removed and examined from each treatment plot. Therefore, if infestation is not uniform across the seed heads, some may have been infested but not counted. As well as measuring attack and offspring production, I examined the effect biochar had on the number of seed heads produced by the spotted knapweed in the nursery study. Which resulted in an overall decrease in number of seed heads per plant in the nursery, which correlated with the decrease in attack and offspring. This reduction in seed heads in the nursery study was not anticipated, but it indicates that the biochar may stress this invasive species to the point of reducing reproduction, which may also contribute to the impact of seed head feeders such as *L. minutus.* Future data collection should include the impact of biochar on seed heads that makes them undesirable or unusable to *L. minutus.*, similar to the result observed in the nursery experiment or if the biochar increases attack and offspring in seed heads as recorded for the field experiment.

Cyphocleonus achates Root Attack and Offspring:

There is no literature describing the use of biochar with insect biocontrol agents. My data on root attack showed promising results for combining the use of *C. achates* with the application of biochar. The highest proportion of attacks were measured in roots in the high biochar treatment, with 100% of the roots attacked with only *C. achates* or in combination with *L. minutus*. The low biochar treatment had the second highest rate of attack which varied between 80-100% of the roots. While the control treatment had 80% attack in plants with one or two insects. These trends indicate that the addition of biochar leads to an increase in insect attack when compared to the no biochar (control) treatment.

The data for both curculionids application types (*C. achates* alone or in combination with *L. minutus*) had similar root outcomes, but there was variability among treatments with *C. achates* alone and in conjunction with *L. minutus* in the number of offspring present. For plants with only *C. achates*, more offspring were found in the biochar treatments in the nursery study, with the low biochar treatment having the highest average number of offspring per root. The high-rate biochar treatment had the highest number of offspring on a single root. Both low and high biochar application rates resulted in more offspring in spotted knapweed root systems which may result in reducing populations of this invasive weed. Because biochar has a high cation exchange capacity (Kharel et al., 2019) it was efficient in binding Ca, Mn, and Al and likely contributed to a decrease in knapweed health. In agricultural crops, biochar can suppress soil borne pathogens (*e.g., Fusarium* sp.; Matusbara et al., 2002). In our case, biochar increased the abundance of weevil offspring, which will likely lead to better biocontrol. Biochar responses are both feedstock (Ji, et al., 2022) and soil (Joseph, et al., 2021) dependent and additional studies should be performed to find the best application rates and biochar type that will enhance biocontrol efforts on invasive species. This would indicate that the low treatment appears to have the greater number of overall offspring per root with variability in the control and high treatments. This would suggest that the biochar is increasing attack, but an increase in offspring appears more likely when a lower amount of biochar is added to the soil.

Spotted Knapweed Chemistry:

There was a decrease in three of the chemical components present in spotted knapweed (calcium, manganese, and aluminum) grown with biochar in the soil. Plants require 17 essential elements for growth (Mahler, 2004) among these 17 are calcium and manganese and a decrease in these compounds may indicate plants under stress. Calcium is required for structural strength of the cell membrane and is needed for plants to coordinate response to some development cues and changes in the environment (White and Broadley, 2003). A decrease in manganese can lead to premature aging of leaves, browning interveinal chlorosis and an overall delay in maturity (Campbell and Nable, 1988). Therefore, declines in both compounds could indicate that biochar is decreasing the overall health of the spotted knapweed and potentially increasing the plant's susceptibility to herbivores. Aluminum is considered non-essential for plants and a decrease in its concentration could be more positive for the plant because it would decrease the possibility for aluminum toxicity which can lead to limited plant growth and development (Mossor-Pietraszewska, 2001).

Two other components measured in the spotted knapweed were unchanged by the addition of biochar (sulfur and sulfate). Although there were differences in these components observed between plants in the field versus nursery experiments, these differences are likely due to the different soil types. Sulfate is the readily available form of sulfur taken up by plants, due to its mobility in soil (Stewart, 2010). Sulfur is essential in crop production and has shown to have a close association with nitrogen. Both sulfur and sulfate play a role in chlorophyll formation and sulfur also plays a role in the conversation of nitrate to amino acids (Stewart, 2010). Having these components unchanged should have no significant impact on the spotted knapweed.

Soil Chemistry:

Of the soil nutrients analyzed from the soil solution, ammonium was the only one that had an increase with the addition of biochar. This was interesting because the assumption was that biochar would reduce the amount of ammonium in soil solution (Cabeza et al. 2018). However, the temperature at which the biochar is created, and feedstock type determine how much ammonia is available in soil solution. In addition, the biochar I used had been aging in super sacks for 5 years, which could have altered ammonium levels associated with the biochar. Soil under native and invasive prairie plant species with biochar added did have a decrease in available nitrogen (Adams, et al., 2013). It is possible that the increase in ammonium could be related to the addition of perlite to the soil mixture. The Miracle-Gro Perlite bought for the nursery study releases nitrogen as moisture is added and if resin capsules were in close proximity to perlite pellets, it could lead to this measured increase in the soil.

Soil solution nutrients that decreased when biochar was added were aluminum, zinc, copper, calcium, and boron. Similar to the measurements for plant chemistry, aluminum and calcium decreased in the soil when biochar was added. Zinc plays a role in a plant's ability to uptake and transport water, reduce the impact of stressors such as heat and salinity, as well as playing a role in the production of an essential growth hormone (Tsonev and Lidon, 2012). Copper is also essential for plant health as it plays a role in plant growth and development (Yruela, 2005). Further, Copper has been found to have antimicrobial properties that can help with the increase in germination and growth (Kasana, et al., 2017). Boron is an essential nutrient in plant growth and recent studies demonstrate that it has a role in maintenance of plasma membrane functions and many metabolic processes (Camacho-Cristóbal et al., 2008). The decrease in zinc, copper, boron and calcium suggests that the effect of biochar on the soil surrounding spotted knapweed may be providing a stress to the plants that could decrease its ability to resist herbivore attack.

Conclusion Management Implications:

This research is an initial attempt to examine the effects of the addition of biochar as a soil amendment on the utilization of spotted knapweed by two biological control agents *L. minutus* and *C. achates*. There was considerable variation in experimental sites, application methods and collectible data. The majority of the data suggest that using biochar as a soil amendment in areas with spotted knapweed present would be most effective in conjunction with *C. achates* as the biological control agent. The results also suggest that an addition of 10% biochar would be more useful compared with a 25% biochar treatment. The 10%

biochar treatments resulted in more *C. achates* offspring and only slight variation in the proportion of attacked roots.

Future research should be conducted to further our understanding of how biochar can help the control of spotted knapweed with biological control agents. Based on these experiments I postulate that using below ground root feeders may provide more control due to the biochar acting directly on the roots. Also, because of the decrease in seed heads that occurred with the addition of biochar, it is possible that biochar is making the seed heads more difficult to attack. However, this should not be detrimental because biochar appears to be decreasing flowers on the spotted knapweeds and so also reducing the plants potential reproduction. The field site will continue to be monitored in future years to determine if the biochar penetrating deeper into the soil changes the results from the initial year of this study. I hypothesize that in future years there will be a similar result as in the nursery study, with the seed heads being reduced in number by the biochar and the *C. achates* continuing to stress the plants below ground. These two factors should provide additional control of spotted knapweed and help meet management needs.

References

Adams, Melinda M., et al. “The Effect of Biochar on Native and Invasive Prairie Plant Species.” Invasive Plant Science and Management, vol. 6, no. 2, 2013, pp. 197–207., doi:10.1614/IPSM-D-12-00058.1.

Biederman, Lori A., and W. Stanley Harpole. “Biochar and Its Effects on Plant Productivity and Nutrient Cycling: A Meta-Analysis.” GCB Bioenergy, vol. 5, no. 2, 2013, pp. 202–214., https://doi.org/10.1111/gcbb.12037.

Blumenthal, Dana M., et al. “SOIL CARBON ADDITION CONTROLS WEEDS AND FACILITATES PRAIRIE RESTORATION.” Ecological Applications, vol. 13, no. 3, 1 June 2003, pp. 605–615., doi:10.1890/1051-0761(2003)013[0605:scacwa]2.0.co;2.

Camacho-Cristóbal, Juan J., Jesús Rexach, and Agustín González-Fontes. "Boron in plants: deficiency and toxicity." Journal of Integrative Plant Biology 50.10 (2008): 1247-1255.

Campbell, Lindsay C., and Ross O. Nable. “Physiological Functions of Manganese in Plants.” *Manganese in Soils and Plants*, 1988, pp. 139–154., https://doi.org/10.1007/978-94-009-2817-6_11.

Cooperman, Yoni. “Biochar and Carbon Sequestration.” ANR Blogs, Regents of the University of California, 30 Sept. 2016, https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=22224.

Corn, Janelle G., et al. “Impacts of the Biological Control Agent *Cyphocleonus Achates* on Spotted Knapweed, Centaurea Maculosa, in Experimental Plots.” Biological Control, vol. 37, no. 1, Apr. 2006, pp. 75–81., doi:10.1016/j.biocontrol.2006.01.003.

Crawley, Michael J. “The Relative Importance of Vertebrate and Invertebrate Herbivores in Plant Population Dynamics.” Insect-Plant Interactions, 1989, pp. 45–71., doi:10.1201/9780429290916-2.

Ji, Mengyuan, et al. "Effects of different feedstocks-based biochar on soil remediation: A review." Environmental Pollution 294 (2022): 118655.

Joseph, Stephen, et al. "How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar." GCB Bioenergy 13.11 (2021): 1731-1764.

Kasana, Ramesh Chand, et al. "Biosynthesis and effects of copper nanoparticles on plants." Environmental Chemistry Letters 15.2 (2017): 233-240.

Kharel, Gyanendra, et al. “Biochar Surface Oxygenation by Ozonization for Super High Cation Exchange Capacity.” ACS Sustainable Chemistry & Engineering, vol. 7, no. 19, 4 Sept. 2019, pp. 16410–16418., https://doi.org/10.1021/acssuschemeng.9b03536.

Lang, Ronald. “Cyphocleonus Achates (Coleoptera: Curculionidae).” *Cyphocleonus Achates*, https://biocontrol.entomology.cornell.edu/weedfeed/Cyphocleonus.php. Accessed 20 March 2021.

Lang, R. F. “Larinus Minutus Coleoptera: Curculionidae.” *Larinus Minutus*, https://biocontrol.entomology.cornell.edu/weedfeed/Larinus.php. Accessed 20 March 2021.

Mahler, Robert L. "Nutrients plants require for growth." University of Idaho. College of Agricultural and Life Science. CIS 1124 (2004).

Minteer, C. R., et al. "*Larinus Minutus*(Coleoptera: Curculionidae), A Biological Control Agent of Spotted Knapweed (Centaurea Stoebessp.Micranthos), Established in Northern Arkansas." Florida Entomologist, vol. 94, no. 2, June 2011, pp. 350–351., doi:10.1653/024.094.0233.

Mossor-Pietraszewska, T. "Effect of aluminum on plant growth and metabolism." Acta biochimica Polonica vol. 48,3 (2001): 673-86.

Smith, L., and J.M. Story. "Plant Size Preference of Agapeta Zoegana L. (Lepidoptera: Tortricidae), a Root-Feeding Biological Control Agent of Spotted Knapweed." Biological Control, vol. 26, no. 3, Oct. 2003, pp. 270–278., doi:10.1016/s1049-9644(02)00169-x.

"Soilgrids Web Portal." SoilGrids Web Portal, https://soilgrids.org/.

"Spotted Knapweed." *Minnesota Department of Agriculture*, https://www.mda.state.mn.us/plants/pestmanagement/weedcontrol/noxiouslist/spottedknapweed.

Stewart, W. M. "Sulfur – The 4th Major NutrientW." *Nutrient Stewardship*, 2010, https://nutrientstewardship.org/implementation/sulfur-the-4th-major-nutrient/#:~:text=Sulfur%20serves%20many%20functions%20in,amino%20acids%20that%20form%20protein.

Tsonev, Tsonko, and Fernando Jose Cebola Lidon. "Zinc in plants-an overview." Emirates Journal of Food & Agriculture (EJFA) 24.4 (2012).

Van Hezewijk, Brian H., and Robert S. Bourchier. “Impact of *Cyphocleonus Achates* on Diffuse Knapweed and Its Interaction with *Larinus Minutus*.” Biological Control, vol. 62, no. 2, Aug. 2012, pp. 113–119., doi:10.1016/j.biocontrol.2012.03.008.

White, Philip J., and Marten R. Broadley. “Calcium in Plants.” *Annals of Botany*, vol. 92, no. 4, 1 Oct. 2003, pp. 487–511., https://doi.org/10.1093/aob/mcg164.

Yruela, Inmaculada. "Copper in plants." Brazilian Journal of Plant Physiology 17 (2005): 145-156.

Appendix: Additional Figures and Tables

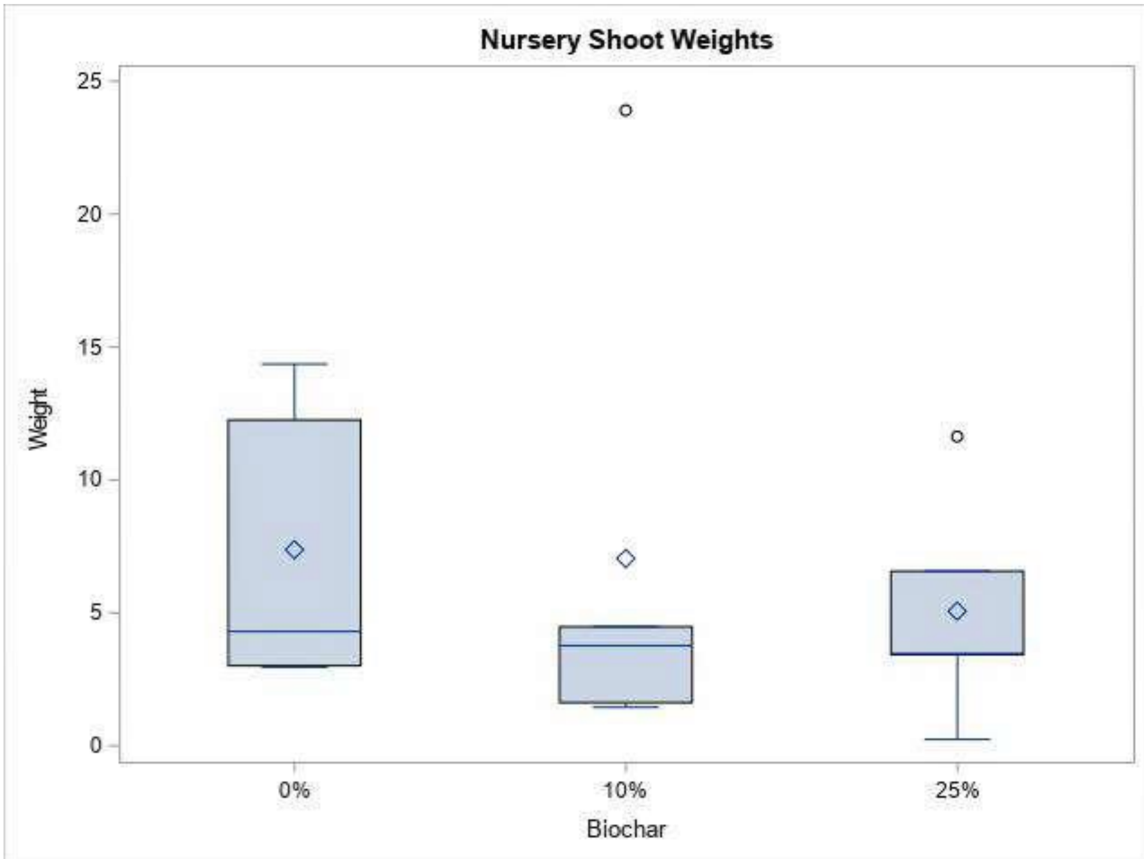

FIGURE 12a: Mean proportion (± SEM) of nursery shoot weights in grams from the three treatment groups (0%, 10% and 25% biochar)

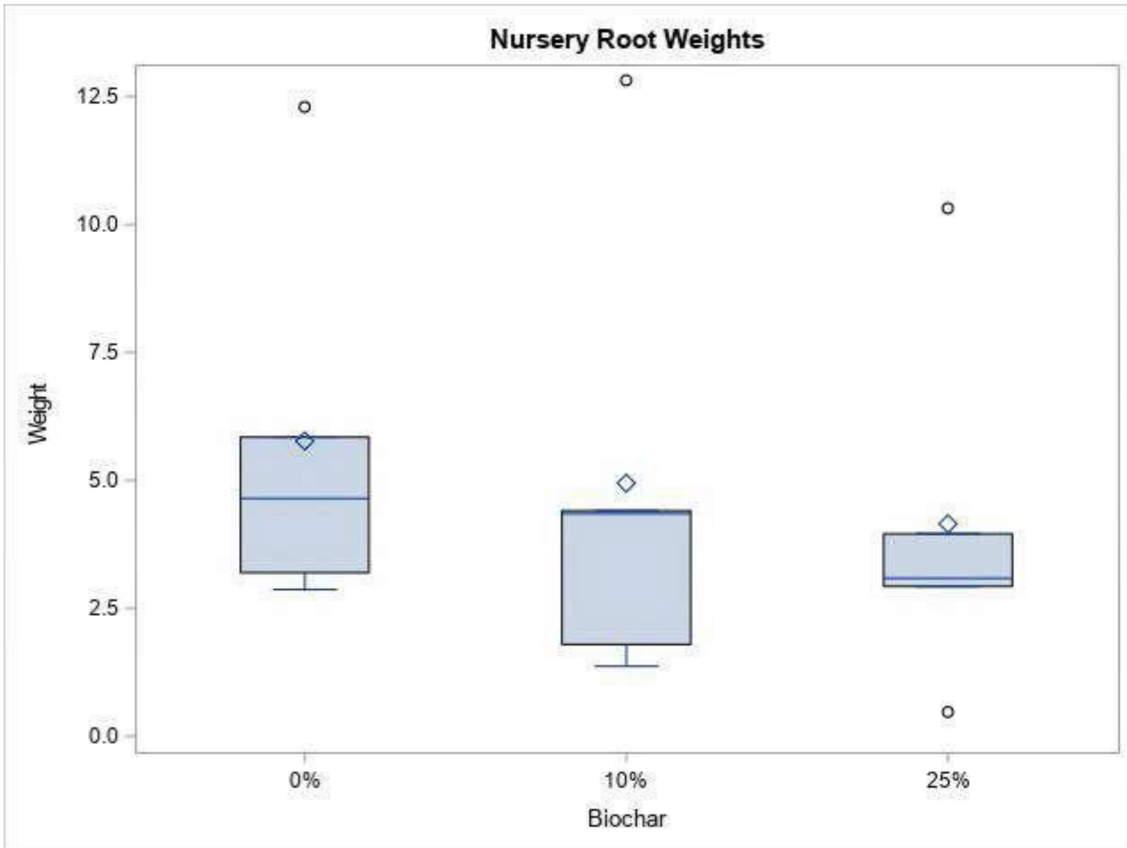

FIGURE 12b: Mean proportion (+ SEM) of nursery root weights in grams from the three treatment groups (0%, 10% and 25% biochar)

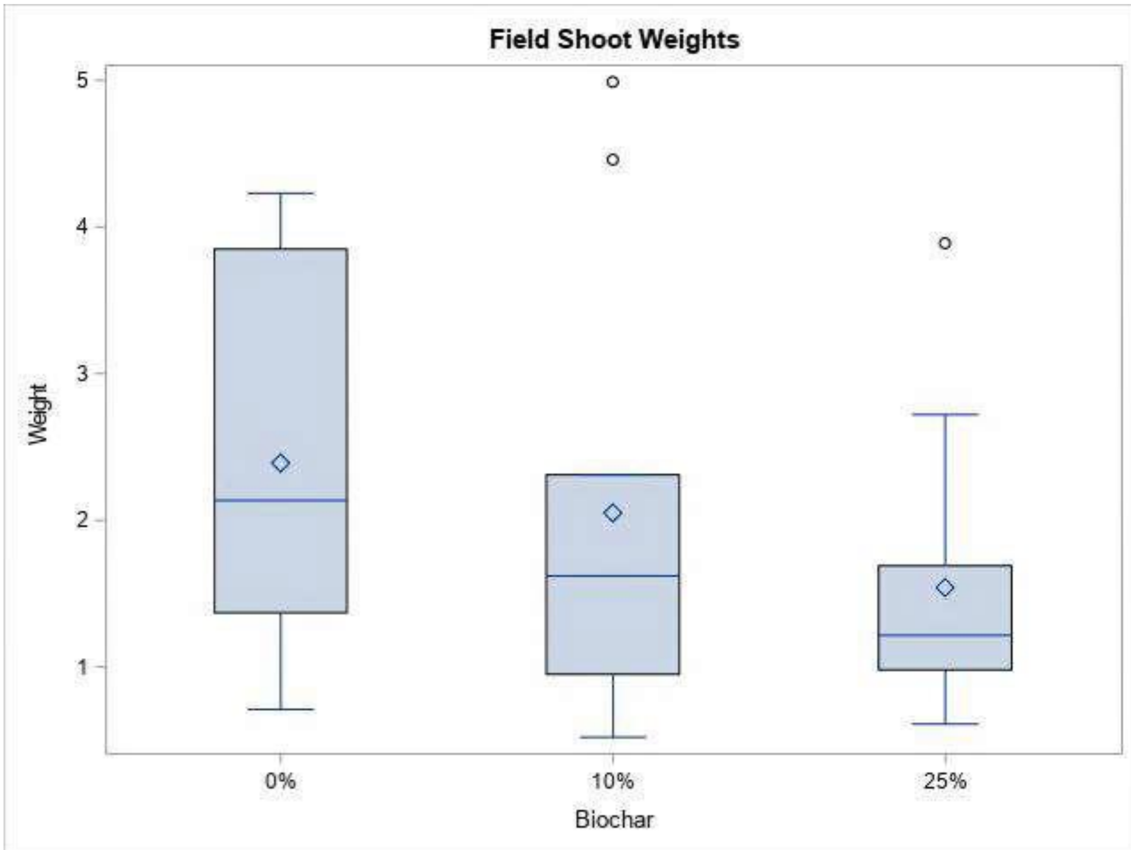

FIGURE 13a: Mean proportion (+ SEM) of Field shoot weights in grams from the three treatment groups (0%, 10% and 25% biochar)

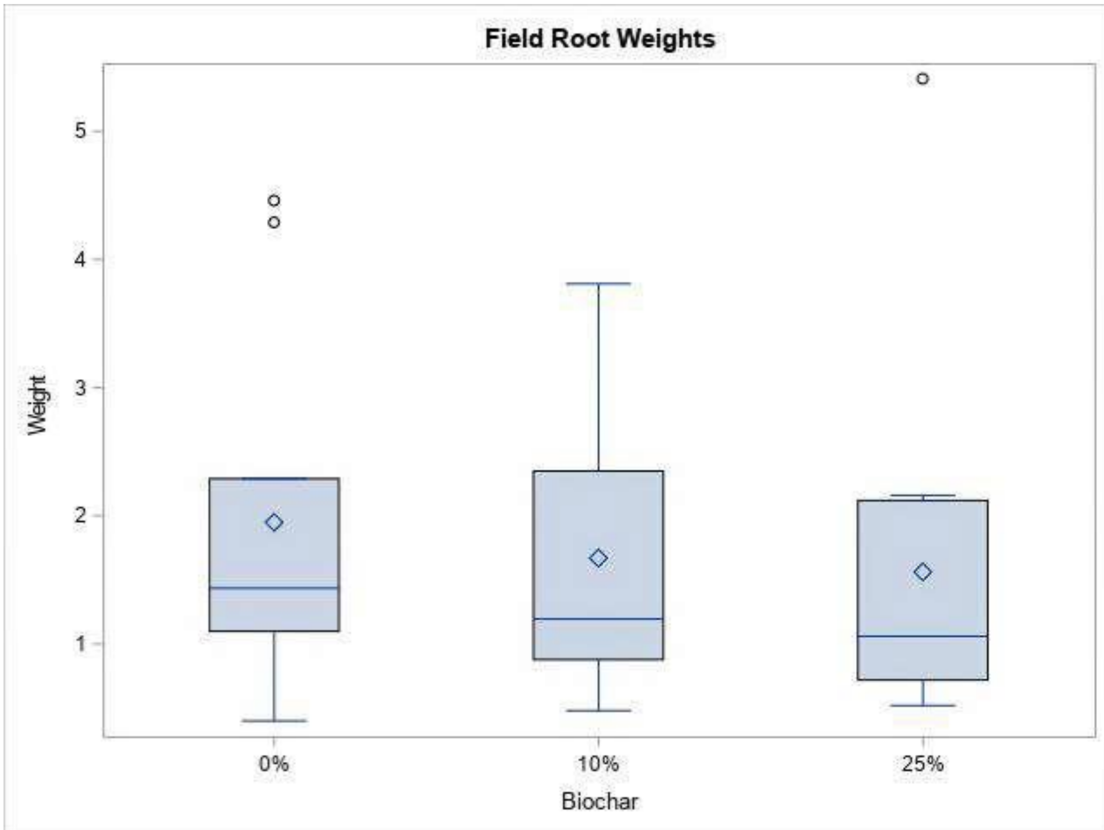

FIGURE 13b: Mean proportion (+ SEM) of Field root weights in grams from the three treatment groups (0%, 10% and 25% biochar)

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	B (ppm)	Cu (ppm)	Zn (ppm)	Mo (ppm)	Na (ppm)	Al (ppm)	P205 (%)	K2O (%)	SO4 (%)
Field Control Shoot	0	0.12	1.08	0.57	0.11	0.03	151.9	34.29	15.02	6.21	10.47	0.86	67.47	222.7	0.28	1.3	0.1
Field Control Root	0	0.16	1.31	0.55	0.09	0.06	760.8	82.59	13.46	13.87	19.14	2.6	192.8	1073	0.37	1.58	0.19
Field Low Shoot	0	0.1	1	0.58	0.11	0.03	93.84	30.26	14.99	6.87	8.79	0.48	45.84	117.7	0.23	1.2	0.09
Field Low Root	0	0.14	1.24	0.52	0.09	0.06	617.2	73.42	12.95	12.74	21.32	1.93	142.4	884.9	0.33	1.5	0.17
Field High Shoot	0	0.12	1.13	0.46	0.08	0.03	86.83	24.12	13.79	9.08	8.52	0.61	51.28	92.69	0.27	1.36	0.09
Field High Root	0	0.15	1.34	0.53	0.08	0.07	452.6	59.03	12.71	14.25	17.04	3.48	110.9	649.1	0.34	1.61	0.2
Greenhouse Control Shoot	0	0.12	0.95	0.79	0.21	0.05	1014	113.1	21.63	8.47	11.85	1.14	142.6	102.1	0.27	1.15	0.16
Greenhouse Control Root	0	0.11	0.82	0.4	0.11	0.1	829.5	81.53	11.33	11.35	33.13	1.89	1077	548.7	0.24	0.99	0.29
Greenhouse Low Shoot	0	0.16	0.99	0.67	0.19	0.05	762.1	81.3	18.78	7.98	10.27	0.55	122.6	56.27	0.36	1.2	0.14
Greenhouse Low Root	0	0.12	0.83	0.35	0.1	0.11	729.6	86.66	12.53	12.63	31.97	2.12	1398	476.8	0.27	0.99	0.33
Greenhouse High Shoot	0	0.15	1.22	0.59	0.17	0.06	903.5	82.58	25.51	3.75	11.19	0.27	182	69.35	0.34	1.47	0.17
Greenhouse High Root	0	0.1	0.65	0.35	0.09	0.11	1052	90.44	11.57	5.43	33.34	1.88	1450	652.5	0.23	0.78	0.32

TABLE 1: Average plant tissue nutrients split into roots and shoots at each experimental site. Treatments are the control (0%), low biochar (10%) and high biochar (25%).

	NO3-N	NH4-N	Al	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
Control	5.97	0	0.37	0.02	60.12	0.03	0.44	26.86	31.39	0.37	164	1.65	53.09	0.04
Control	0.27	6.24	2.73	0.33	78.59	0.02	0.36	33.01	39.65	0.29	108.65	1.57	37.69	0.02
Control	0	6.37	0.43	0.01	68.84	0.02	0.36	25.57	37.11	0.38	127.77	1.52	42.62	0.02
Control	0.18	4.13	0.44	0.02	116.67	0.03	0.54	58.75	64.87	0.38	129.5	2.75	59.25	0.08
Control	0	5.62	0.45	0.01	84.59	0.03	0.52	23.44	44.15	0.32	92.19	2.35	41.77	0.01
Low	0	8.74	0.35	0.01	56.87	0.02	0.45	59.63	31.16	0.26	92.7	2.34	47.32	0.01
Low	0	3.25	0.4	0.01	76.59	0.02	0.45	45.85	44.86	0.42	122.88	1.45	51.57	0.01
Low	0	3.09	1.08	0.14	58.3	0.02	0.47	21.26	28.94	0.15	79.74	1.2	35.66	0.03
Low	0.18	5.46	0.49	0.01	124.07	0.02	0.4	64.35	61.52	0.26	103.36	2.63	51.26	0.01
Low	0	8.75	0.37	0.01	81.51	0.03	0.38	44.99	50.67	0.43	121.6	2.25	54.12	0.03
Low	0	5.61	0.85	0.1	85.79	0.03	0.36	37.6	43.32	0.21	83.49	1.38	39.66	0.02
High	0.4	6.17	0.5	0.01	131.56	0.02	0.63	37.65	62.38	0.58	95.77	2.21	48.21	0.01
High	0	5.75	0.38	0.01	46.64	0.02	0.3	26.86	25.12	0.26	62.87	1.39	30.41	0.03
High	0.01	6.99	0.38	0.01	63.81	0.02	0.5	31.83	38.06	0.38	84.18	1.52	42.3	0.02
High	0	6.31	0.36	0.01	37.95	0.02	0.41	29.73	19.46	0.35	63.28	1.67	30.13	0.01

TABLE 2: Average soil nutrients in greenhouse plants in ppm. Treatments are the control (0%), low biochar (10%) and high biochar (25%).