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Effects of used coffee grounds on *M. cribraria* preferences, soil characteristics, and soybean growth

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Abstract

Megacopta cribraria, also known as the kudzu bug, was first discovered in the southeastern United States in 2009. *M. cribraria* feed on soybeans (*Glycine max*), which causes a significant decrease in overall soybean yields. Currently, there is no information regarding environmentally safe control methods of this particular invasive species. Used coffee grounds have been implemented in many agricultural settings as a fertilizer and as a preventative of invasive pests. The purpose of these two greenhouse experiments is to determine whether the application of used coffee grounds has the potential to control or deter *M. cribraria* herbivory on soybeans. We hypothesize that UCG application will cause a decrease in *M. cribraria* herbivory on soybeans. The first experiment evaluated used coffee grounds (UCG) with six treatments of soil mixture measured by volume with 0% UCG (control), 2.5% UCG, 5% UCG, 10% UCG, 15% UCG, and 20% UCG. The second experiment evaluated used coffee grounds as a mulch applied to the soil surface with UCG application and without UCG (control). Our results demonstrated that used coffee grounds, when applied as a 20% UCG mixture, are able to decrease *M. cribraria* preference for soybeans. The control soybeans were preferred despite a lower plant quality that could have been a result from nutrient deficiency, toxic heavy metal presence, or decreased rhizobia interactions. However, UCG do not deter *M. cribraria* when applied as a mulch to the soil surface.

Introduction

Megacopta cribraria is an insect that is native to Asia and it was accidentally introduced to the southeastern region of the United States (Eger et al. 2010, Zhang et al. 2012). The first documented case of this invasive species was in 2009, where it was discovered feeding on kudzu, *Pueraria montana*, located in nine northeastern counties of Georgia (Suiter et al. 2010). *M. cribraria*, or the kudzu bug, has now spread across the southeast in very high densities feeding on leguminous plants. The primary host plants include kudzu and soybean, *Glycine max* (Zhang et al. 2012). The adults and nymphs of *M. cribraria* are able to oviposit and successfully develop with a high survival rate of 90% for first-generation individuals on soybean crops (Del Pozo-Valdivia et al. 2013). These pests suck plant sap from the stems, petioles, and leaves that reduce overall soybean yields by an average of 18% (Greene et al. 2012).

Quite frequently alternative methods to insecticides are used to remove or prevent certain pest species, such as the application of used coffee grounds. Used coffee grounds (UCG) are able to contribute a large amount of organic matter to the soil, which can increase soil water holding capacity as well as nutrient retention (Kasongo et al. 2011). Nutrient holding is key to prevent leaching of elements like potassium and sodium, and studies have shown that used coffee grounds reduce the loss of potassium from 45% to 13% and sodium from 58% to 43% (Kasongo et al. 2011). UCG can also influence other characteristics such as soil pH, which can ultimately affect the amount of iron available for plant absorption (Morikawa and Saigusa, 2008).

Used coffee ground application has been used by organic growers as a fertilizer to benefit soils, yet UCG do not always benefit plants (Yamane et al. 2014). According to a study examining lettuce, *Lactuca sativa*, as the treatment amount of used coffee grounds increases, lower amounts of organic nitrogen become available for plant uptake. This nitrogen deficiency can cause negative effects like pigment loss, protein degradation, and lower plant biomass (Cruz et al. 2012). However, legumes like soybeans are known to tolerate this stress due to biological nitrogen fixation (Yamane et al. 2014). Legumes have a symbiotic relationship with rhizobia bacteria that are able to fix nitrogen to ammonia that the legume can uptake and, in return, provide sugars to the bacteria by forming root nodules (Zahran 1999). Therefore, the benefits of used coffee grounds to deter agricultural pests can be exploited without negatively effecting soybean growth.

UCG may also act as an insecticide. For instance, the larval development of *Aedes aegypti* with the most efficient concentration of UCG produced 100% mortality (Guirado et al. 2007 and Laranja et al. 2003). Another study on the mosquito, *Ochlerotatus notoscriptus*, in anthropic environments sought to determine the toxicity of larval survivorship for public health concerns. The results after one month indicated that the UCG treatments of 0.01 g/ml and 0.05 g/ml (41.6% and 59.6%, respectively) had a significantly lower survivorship percentage compared to the control of 78.7%. (Derraik et al. 2005). Caffeine is believed to be the toxic ingredient and in many cases, plants such as coffee and tea are able to use caffeine as a chemical defense mechanism against herbivory (Cruz et al. 2012).

Other methods of removal have been explored to control *M. cribraria* in agricultural and urban settings, such as insecticides and biological control (Seiter et al. 2013). However, these management strategies have been proven in past events to cause more ecological or economical harm. These costly insecticides can cause water contamination, target beneficial species, and diminish effectiveness over time as species develop genetic resistance. Biological control, another potential way to control an invasive pest species, requires thorough research to determine whether the new potential species could become invasive. For example, the parasitoid fly, *Strongygaster triangulifera*, has been suggested to be a potential biological control agent of *M. cribraria*. Although the parasitoid fly has shown to successfully use *M. cribraria* as a parasitoid host, *S. triangulifera* has a large range of other host species (Golec et al. 2013). Therefore, more research is needed on the overall community effects as other important insects may be targeted and negatively affected.

There is no information available regarding the effects of used coffee grounds on *M. cribraria* herbivory. This greenhouse experiment seeks to determine the overall effects used coffee grounds have on soil characteristics, soybean growth, and ultimately *M. cribraria* preference and herbivory on soybeans. We predict that used coffee grounds will reduce kudzu bug herbivory on soybeans by improving plant quality.

Methods

Adult *M. cribraria* individuals were collected from kudzu in Pensacola, Florida in March 2015 and maintained with ample soybeans in a controlled environment. A total of 125 soybeans, *Glycine max*, were planted on February 17, 2015. Each soybean seed was inoculated with rhizobia to allow root nodulation and nitrogen fixation to occur. The soybeans (butterbean variety) were planted to the corresponding UCG treatment in 8.9 cm wide by 8.9 cm height pots in Sunshine professional growing mix soil made up of 75-85% Canadian Spagnum peat moss, horticultural grade perlite, and dolomitic limestone. The soybeans were randomly assorted and kept in the Georgia College, Department of Biological and Environmental Sciences, greenhouse from mid-February to early-April. The used coffee grounds were collected in a 5 gallon bucket from a local coffee shop in Baldwin County, GA. There were two experiments that examined the effects of UCG on soybeans and *M. cribraria*.

Experiment 1: Used Coffee Ground mixture effect on M. cribraria preferences

The first experiment compared used coffee ground soil mixtures measured by volume with 0% UCG (control), 2.5% UCG, 5% UCG, 10% UCG, 15% UCG, and 20% UCG. All six of the soil mixtures successfully yielded at least 11 of 25 soybeans for each treatment with the highest germination rate occurring in the 5% UCG, 15% UCG, and 20% UCG treatments.

When plants were 41 days old the kudzu bug feeding trial began. Six visually equivalent soybeans, one from each UCG treatment, were randomly placed into a bugcage. This was replicated ten times. The bugcages were 60x60x60 cm made up of a clear plastic and polyester netting to allow observation and ventilation. For each bugcage, 15 *M. cribraria* individuals were introduced to the bugcage and given 48 hours to acclimate. The number of kudzu bugs on each soybean was recorded at 2:00 PM of the second day.

Soil analysis

Soil water holding capacity was determined by collecting 3 samples from each UCG treatment and measuring the field capacity and hygroscopic amounts. An advanced electrochemistry meter probe was used to determine soil water pH from 3 samples of each treatment in experiment 1. Trace heavy metals were measured using an X-ray fluorescence spectrometer from 6 measures per soil sample for each treatment.

Plant characteristics

After the *M. cribraria* preference tests, all soybeans were harvested. Plants were oven dried and weighed. Aboveground biomass, belowground biomass, and root nodulation were recorded.

Experiment 2: Used Coffee Ground mulch effect on M. cribraria preferences
The second experiment compared used coffee ground application to the surface of the soil as a mulch rather than a homogenized mixture. Two treatments were tested, soybeans with mulch application and soybeans without mulch (control). There were ten replicates, each with two visually equivalent soybeans randomly placed within a bugcage. The treated soybeans were not exposed to UCG until the preference test began. Once in the bugcase, the UCG mulch was applied to the top layer surrounding a single treated soybean. For each replicate, 15 *M. cribraria* individuals were introduced to the bugcage and given 48 hours to acclimate. The number of kudzu bugs on each soybean was recorded at 2:00 PM of the second day.

Statistical analysis
M. cribraria preferences and plant characteristics recorded in experiment 1 were statistically analyzed using a one-way analysis of variance (ANOVA) with a significance level of $P < 0.05$ and further analysis using Tukey HSD. Soil characteristics recorded in experiment 1 were statistically analyzed using linear regression analysis. *M. cribraria* preferences recorded in experiment 2 were statistically analyzed using a two-sample t-test assuming unequal variances.

Results

Experiment 1: Used Coffee Ground mixture effect on M. cribraria preferences
The application of the 20% UCG treatment significantly decreased *M. cribraria* preferences as compared to the control 0% UCG (Fig. 1: $F_{5,54}$=2.834, P=0.024). *M. cribraria* herbivory was reduced from an average of 3.2 individuals per plant in the control to 0.8 individuals in the 20% UCG treatment. All other treatments were not significantly different from one another.

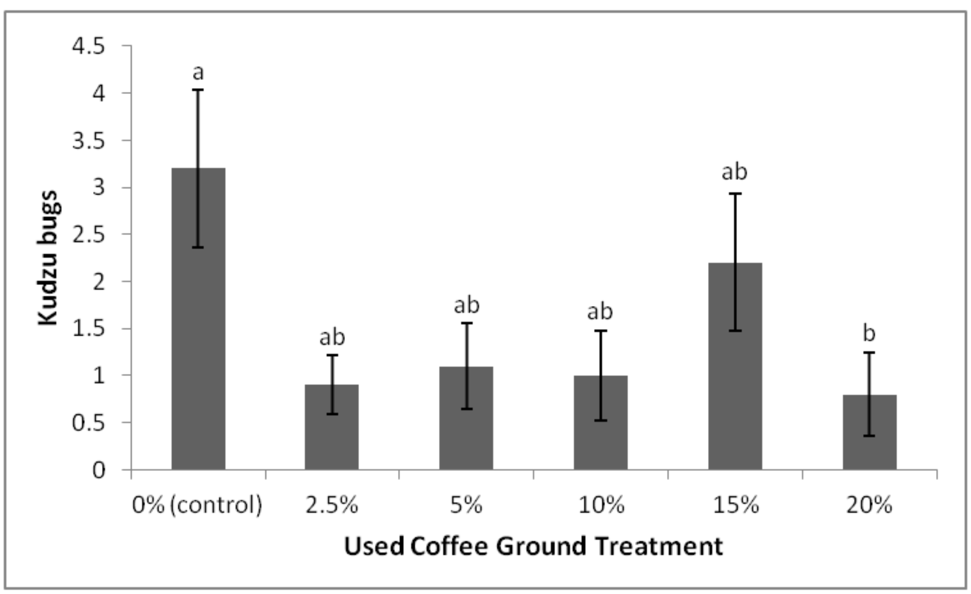

Fig. 1. Preference of *M. cribraria* exposed to six treatments. Different letters indicate a significant difference between groups.

Used Coffee Ground mixture effect on soil characteristics

The XRF spectrometer results showed that as the amount of UCG in the soil increased, there was a trend towards increasing concentrations of K and Si (Fig. 2: R^2=0.38, P=0.19; Fig. 3: R^2=0.40, P=0.18). As the treatment level increases, the amount of zinc (Zn) decreased (Table 1: R^2=0.27, P=0.23). Copper was detected in the control and absent in all of the UCG treatments, therefore a statistical analysis could not be conducted. The soil pH, porosity, and plant available water measured in experiment 1 were not significantly different among treatments (data not shown: soil pH $F_{5,12}$=2.26, P=0.102; porosity $F_{5,12}$=0.563, P=0.727; plant available water $F_{5,12}$=0.019, P=1.000).

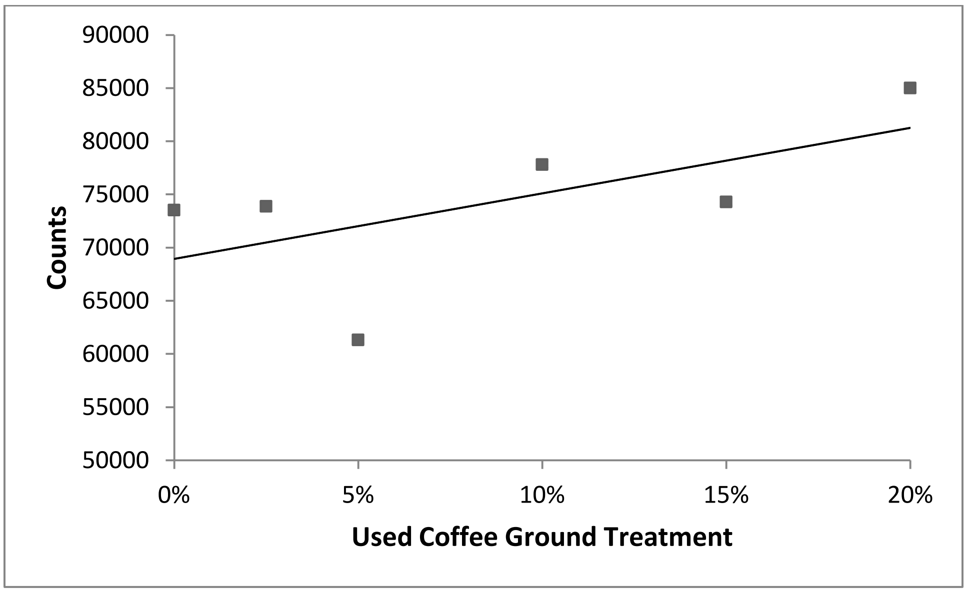

Fig. 2. Mean counts of potassium (K) found in each treatment.

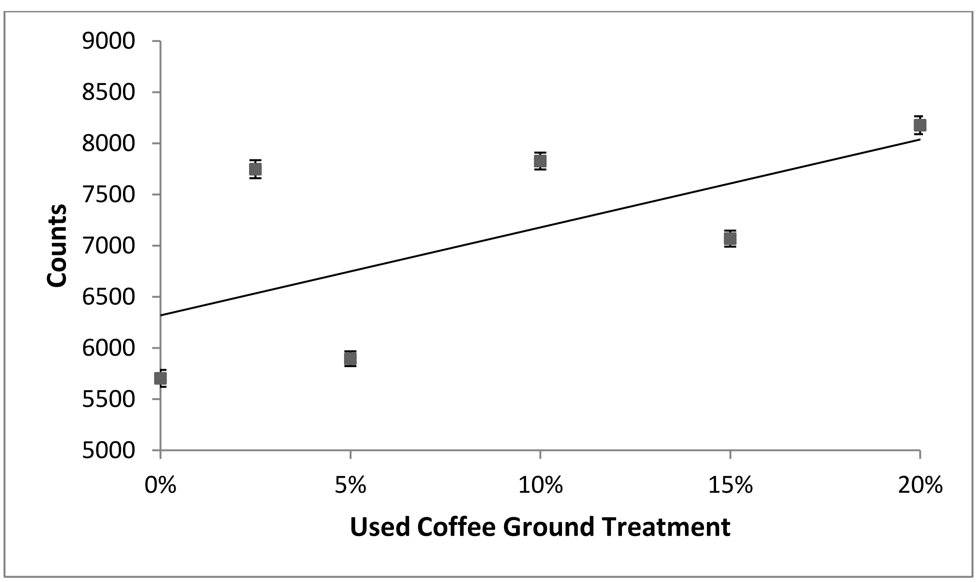

Fig. 3. Mean counts of silica (Si) found in each treatment.

Table 1. Mean counts of zinc (Zn) and copper (Cu) in each treatment.

UCG Treatment	Zinc	Copper
0% (control)	106.50	40.20
2.5%	29.27	0.0
5%	20.79	0.0
10%	29.74	0.0
15%	26.84	0.0
20%	29.40	0.0

Used Coffee Ground mixture effect on soybeans

Observations from experiment 1 indicate an increase in soybean germination as the treatment level increases. The control, 2.5% UCG, 5% UCG, 10% UCG, 15% UCG, and 20% UCG successfully germinated 11, 12, 16, 12, 17, and 16 soybeans, respectively and out of 25 soybeans for each treatment. Aboveground plant biomass showed a difference where treatments of 2.5% UCG, 10% UCG, and 20% UCG were significantly lower in weight than the control (Fig. 4: $F_{5,54}$=3.877, P=0.004). Root nodulation showed a difference where treatments of 2.5% UCG, 5% UCG, 10% UCG, and 15% UCG were significantly higher in number of root nodules than the control (Fig. 5: $F_{5,54}$=5.775, P < 0.001). Belowground plant biomass did not show a significant difference between treatments (data not shown: $F_{5,54}$=1.467, P=0.216).

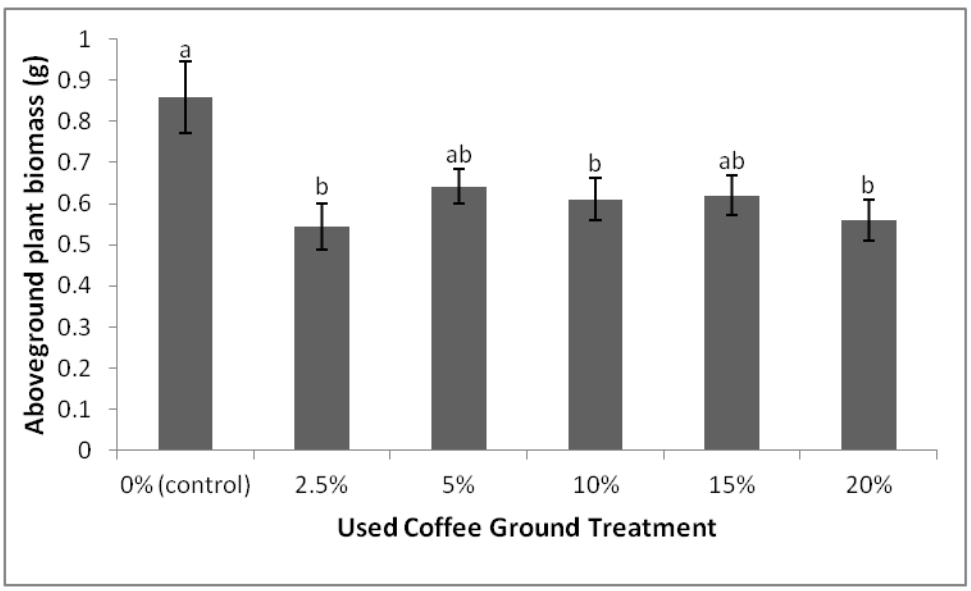

Fig. 4. Aboveground plant biomass of soybeans exposed to six treatments. Different letters indicate a significant difference between groups.

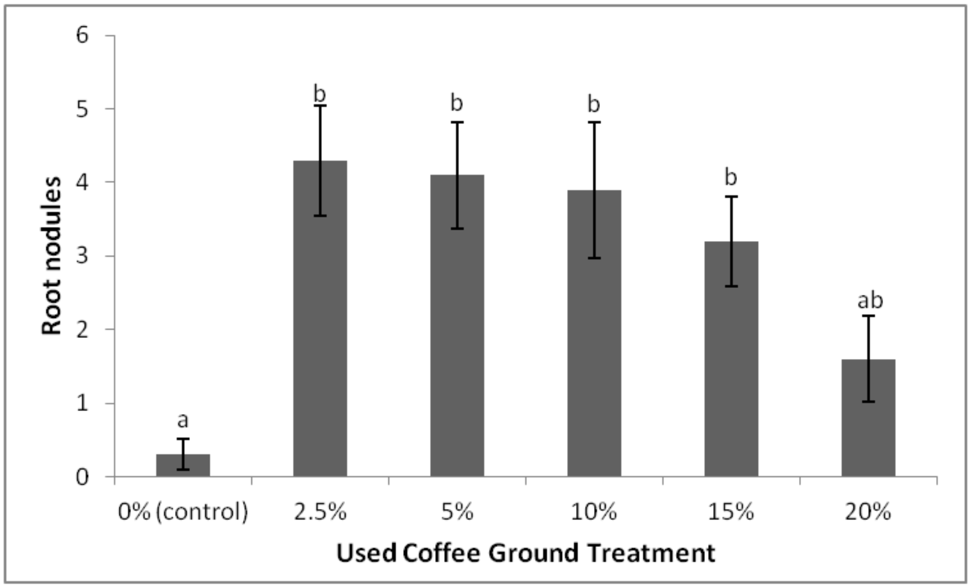

Fig. 5. Root nodulation of soybeans exposed to six treatments. Different letters indicate a significant difference between groups.

Experiment 2: Used Coffee Ground mulch effect on M. cribraria preferences

M. cribraria herbivory increased from an average of 4.3 individuals on the control to 6.7 individuals in the UCG mulch treatment (Fig. 6). However, there was not a significant difference between the two treatments (t_{18}= 1.756 p= 0.096).

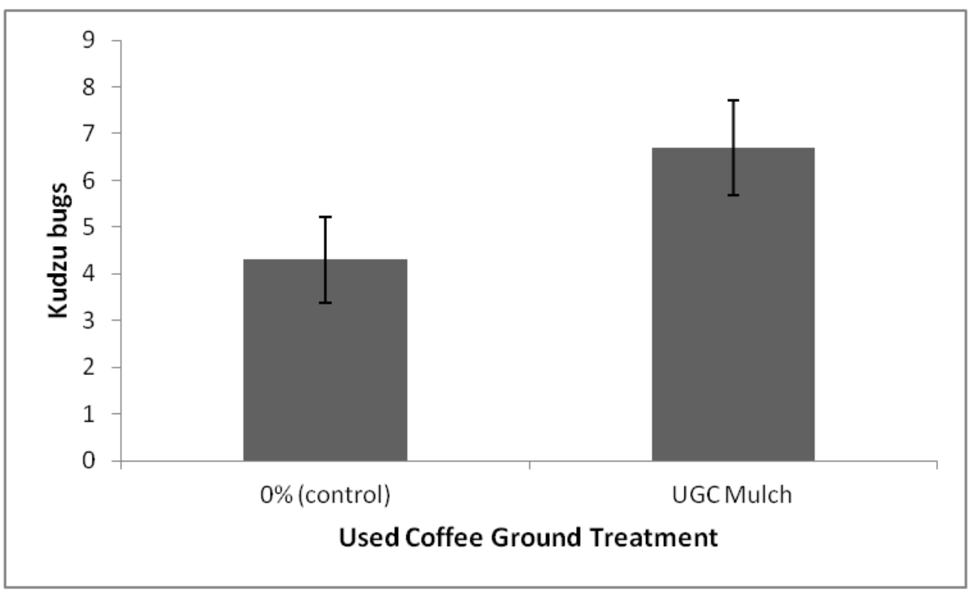

Fig. 6. Preference of *M. cribraria* exposed to soybeans treated with UCG mulch and untreated soybeans (control). Each bar represents that average of 10 replicates.

Discussion

The results from experiment 1 show that *M. cribraria* preferred the control soybeans over the 20% UCG treatment. This finding supports the hypothesis that used coffee grounds reduce *M. cribraria* herbivory when applied to the soil as a mixture. The application of UCG as a mixture illustrates the potential to increase plant quality. Plant quality is considered to be the most important factor in influencing the preference of herbivores. This concept is noted in other studies where plants are able to use direct and indirect modes of self-protection in larger quantities given a certain nutritional diet. For instance, thriving soybeans are able to produce more metabolites and organic compounds to actively deter herbivory (Timbo 2014). Therefore, intensity of these defense mechanisms depends on the health and interactions of the plant.

Soybeans treated with used coffee grounds had a greater number of root nodules as compared to the control soybeans. Root nodules are found on soybean roots, which give fixed nitrogen to the plant in return for carbon produced by the soybean (Lee and Hirsch, 2006). This symbiotic relationship between rhizobia and legumes has been shown to promote plant defenses. For instance, higher amounts of jasmonic acid were found in soybeans containing more root nodules when exposed to the insect herbivore, *Heliocoverpa zea* (Dean 2014). The rhizobia-legume interaction is also inversely related to the level of available nitrogen in the soil (Allos 1959). Therefore, it is evident that the used coffee grounds applied in experiment 1 may reduce the amount of available nitrogen, like nitrate and ammonium in the soil, thus increasing the dependence for nitrogen fixation to convert atmospheric nitrogen to available nitrogen. Whereas, the control soybean plants contain a larger amount of available nitrogen and can uptake nitrogen without forming root nodules. Future studies should examine how UCG effects soil nitrogen, and ammonia should also be measured to determine the amount of available nitrogen in each treatment.

The aboveground plant biomass results show that the control soybeans weighed more than the UCG treated soybeans. This is similar to a study on soybeans where a larger dependence of rhizobia yielded a lower aboveground plant biomass (Dean 2014). It was also observed in experiment 1 that the control soybeans contained more seedpods, which could have contributed a larger biomass to the plant. Both observations of more seedpods and yellow, shriveled leaves in the control could suggest that the plants were exposed to stress causing the weaker plant to undergo rapid pod production.

Potassium and silica are plant nutrients that effect photosynthesis and enzyme activation, and in a deficient amount can reduce the quality of the plant. These deficiencies can be caused by an abundance of nitrogen fertilizer, which may have caused the results seen in experiment 1. Potassium and silica deficiency can cause a number of negative effects, but deficiencies ultimately restrict the amount of nitrate or available nitrogen absorbed by soybean roots needed for development (Pettigrew 2007). Copper and zinc were also detected in the control soils of experiment 1, and these heavy metals are known to inactivate enzymes and important macromolecules at high levels (Ivanova 2010). It is still unknown why the used coffee ground treatments contained lower values of copper and zinc, and higher values of potassium and silica than the control treatment.

The results from experiment 2 show that *M. cribraria* did not show a preference between the control soybeans and the UCG mulch treatment. Although it is evident that the application of UCG as a mulch is not effective, it further supports the results provided by experiment 1. *M. cribraria* is not deterred by UCG as an odor irritant, but rather that plants may gain more plant defense mechanisms through the interaction with rhizobia and UCG mixed into the soil.

Overall, the results demonstrated that used coffee grounds, when applied as a 20% UCG mixture, are able to decrease *M. cribraria* preference for soybeans. The control soybeans were preferred despite a lower plant quality that could have been a result from nutrient deficiency, toxic heavy metal presence, or decreased rhizobia interactions. Further research in an agricultural field would be needed to confirm the effects of the present study to show UCG to be a reliable insecticide. The results from experiment 1, however, are able to support the hypothesis that an increase in plant quality by used coffee grounds can deter *M. cribraria.* This is most likely due to the used coffee grounds decreasing available nitrogen in the soil, thus increasing the rhizobia symbiotic relationship with soybeans to form root nodules, which couples to increase plant defense mechanisms against the agricultural pest, *Megacopta cribraria*.

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