Assessing Effects of Ant-Aphid Mutualisms on Grain Sorghum Health

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Abstract

Invasive insect pests are known to cause yield loss and economic damage to grain sorghum crops (*Sorghum bicolor*). This damage can be exacerbated by invasive insect mutualisms, or beneficial interactions. For example, tawny crazy ants (*Nylanderia fulva*) (Hymenoptera:Formicidae) (TCA) tend sugarcane aphids (*Melanaphis sacchari*) (Hemiptera:Aphididae) (SCA) for the honeydew or sugary excretion that they produce from feeding on the plant. This tending can reduce plant nutrients, which decreases plant health. Currently, it has not been studied whether the mutualism between TCA and SCA has any effects on grain sorghum health. To access potential plant health effects of this mutualism, potted grain sorghum plants of similar development were evaluated in a greenhouse environment. Plants were evaluated in pairs, with each individual plant having 25 sexually mature SCA and the treatment plants having an addition of 300 TCA workers. Plants were enclosed in individual fine mesh cages and placed inside a Fluon lined bin to prevent the organisms from escaping. Damage was assessed by measuring the dry root weight, change in leaf chlorosis, and change in plant stem diameter after the 14-day experiment. There were no statistically significant differences found in the treatments for dry root weight ($P = 0.61$), leaf chlorosis ($P = 0.37$) or stem growth ($P = 0.25$). Future studies should increase the duration of the experiment to determine potential effects of long-term interactions of TCA and SCA on grain sorghum health. Monitoring and controlling ant-aphid mutualisms may be important for the Integrated Pest Management for this agricultural crop.

Keywords: Tawny crazy ant, sugarcane aphid, mutualism, grain sorghum, plant damage

Introduction

In the world of agriculture, there are many obstacles that need to be solved in order to produce a successful harvest. The presence insect pests in agriculture can significantly reduce overall yield and economic profit (Zehnder et al. 2007). These insect pests can reach damaging economic levels, therefore, making it problematic for farmers and businesses. A recent pest, the sugarcane aphid (*Melanaphis sacchari*) (Hemiptera:Aphididae) (SCA) began to attack grain sorghum in Texas and Louisiana during 2013 (Bowling et al. 2016).

When SCA invade grain sorghum, they damage plants by reducing the amount of nutrients available to the plant causing a decrease in overall health. When aphids feed on the plant, it suffers from nutrient, water, and sugar loss as well as a decrease of photosynthesis efficiency due to sooty mold buildup from the aphid's honeydew excretion (Bowling et al. 2016). The introduction of SCA on grain sorghum can cause necrosis, discoloration of plant tissue, and result in a reduction of grains yield per harvest (Burton 2018).

According to Bowling et al. (2016), a monetary loss of $25 and $175 per acre in grain farms is due to large infestations of SCA. This monetary loss can be reduced by controlling the SCA population. Pest management of SCA includes biological control (i.e. the introduction of natural predators) or the use of insecticide (White 2001). An effective biological control of SCA includes the use of organisms such as parasitoid hymenopteran, lacewings, ladybirds, and hoverflies, cause the greatest mortality to aphid populations (Singh et al. 2004). On the other hand, chemical controls include the use of such as disulfoton, disyston, phorate and others as being effective at controlling aphid populations (Singh et al. 2004). Although these two methods of control may alleviate the aphid pest problem, there is another interaction to consider.

Recently, a mutualistic relationship between the tawny crazy ant (*Nylanderia fulva*) (Hymenoptera:Formicidae) (TCA) and SCA has been found (Personal communication: Jocelyn R. Holt). This relationship happens because SCA exude honeydew as a waste product. This honeydew is a sugary substance that serves as a liquid food source for ants. TCA are reported to both tend and protect aphids and other hemipterans for this honeydew food source (Sharma et al. 2013).

The damaging effects of ant-aphid interactions to grain sorghum, along with the plant damage by SCA in the presence and absence of TCA will be assessed. These assessments will include measurements of dry root weight, leaf chlorosis, and plant growth.

Interactions between SCA and TCA could contribute to influencing the health of commercial grain sorghum. This experiment will bring awareness to the synergism that invasive species mutualisms can have and to the potential damage they can cause. Finding out how this mutualism affects grain sorghum health can help can lead to better pest management strategies.

Materials and Methods

Colony Maintenance. Both SCA and TCA were collected in the field and raised in the lab under controlled conditions. The use of lab colonies allowed for the uniformity of aphids used in this experiment while keeping TCA colonies in the lab allowed for easy access to specimens for use in experiments.

The SCA were fed grain sorghum that was grown in a greenhouse. The aphid colony was kept in a pop-up cage (Bioquip, Compton, CA) covered with a fine mesh bag to prevent aphid escape. The aphid colony was provided with 2-4 pots each having an individual grain sorghum plant. When plants became chlorotic in at least 60% of total leaves, new plants were added to the colony. Any dead sorghum plants were removed during colony health checks.

Several TCA colonies were collected from the population in Bryan, Texas and kept in buckets lined with talcum powder (Johnson & Johnson, New Brunswick, NJ). The ants were fed vials of water and a sugar water solution of dextrose, fructose, and sucrose. The resources of water and sugar water were regularly checked and maintained to keep the colonies healthy and alive.

Plant Rearing. Grain sorghum was grown in the greenhouse with average temperatures between 18.89 °C and 29.44 °C. The plants were grown for a duration of 14 days before the experiment. At the end of the 14-day period, the plants were chosen for the experiment based off of developmental uniformity (Burd 1992). There were pairs of similar sized plants selected for the experiment.

Sorghum Greenhouse Experiment. The selected plants were placed in plastic two-gallon buckets that were lined with talcum powder to prevent the ants and aphids from escaping. For the cages, rectangular PVC frames (length = 43.4 cm, width = 26.7 cm, height = 76.6 cm) were constructed to fit the size of the clear plastic arenas (length = 62.7 cm, width = 44.0 cm). The plastic arenas were lined with Fluon (Bioquip, Compton, CA) to prevent the ants from escaping. The PVC frames were used to support a fine mesh cage that was open from one side only. In order to provide easy access to each experimental unit, the side of the mesh cage that opens is oriented face up and is closed off during the experiment with a string and a simple knot that can be fastened and unfastened.

Twenty-five mature apterous or alate aphids were placed on each of the individual plants. Depending on the availability of the most abundant aphid type, either one was used for the experiment, as ants were observed tending both types of aphids. Half of the total number of plants for the experiment were introduced with sub-colonies of ants. The ant sub-colonies were aspirated from lab managed colonies and consisted of 300 workers. The experiment ran for a total of 14 days allowing the aphids to freely reproduce and feed. After 14 days, plant health was assessed using dry root weight, leaf chlorosis, and plant growth.

Aphids were estimated for each plant in the experiment using field scouting techniques (Robert Bowling 2016). The aphids and plant without roots were collected and placed in a Ziploc bag (S. C. Johnson & Son, Racine, Wisconsin) and left in a freezer until time of processing. Ants were removed from the plants via aspiration and vouchered in a vial of 95% ethanol.

Plant Health Assessment.

Dry Root Weight. Aphids and ants were removed from each sorghum plant. The pot containing a sorghum plant, was submerged underwater in a 5-gallon bucket and soaked for 25 minutes. This allowed the contents in the pot to be easily removed. Each root was gently pulled out of the pot and re-submerged into the bucket of water. Then, the pot was set aside. The roots were gently massaged with fingers to remove the remaining soil. When the water became too cloudy with debris, the plant was carefully removed and set aside. The contents that were in the bucket were disposed and the bucket was refilled with clean water to submerge the plant material again. This process was repeated approximately 5-8 more times until the fine pieces of soil contents could not be removed from the roots. Fine particles attached to the roots were removed by hosing them with a gentle stream of water in the sink using a back and forth and side to side motion. If any of the thicker and main roots become detached during any part of the process, the sample pieces were cleaned, set aside and left with the correct sample throughout the process. Fine root pieces were ignored for this experiment if they broke off.

Once the roots were free of soil, the roots were gently pressed with paper towels to remove excess water. The roots were trimmed with scissors as close to the crown as possible. The roots were dried in a drying oven set at 100°C for 24 hours. After 24 hours, the root mass was weighted on a scale (mg) and the data was recorded (Girma 2018).

Leaf Chlorosis. Chlorosis was measured using a scale of One to Nine that was modified based upon that used by Burd et al. (2018). In the modified scale, a One represents a healthy plant leaf, the leaf is dark green; Two, the leaf is a light green in color; Three, some spotting is starting to be noticeable; Four, streaky appearance and some yellowing of the leaf, from 1% to 4%; Five, chlorosis 5% to 25% of total leaf area is yellow, well-defined streaks; Six, chlorosis 26% to 50% of total leaf area is yellow; Seven, chlorosis 51% to 75% of total leaf area is yellow; Eight, chlorosis 76% to 100% of total leaf area is yellow; Nine, leaf death, or beyond recovery (Burd et al. 2018).

Each leaf was observed on the top side only. The leaves were assessed bottom-up from the crown. The first leaf appearing from the soil that may or may not be present, was not represented in the data. The rating for each of the plant's total chlorosis was taken as average of the chlorosis of each leaf. The average leaf chlorosis data was collected and calculated in a spreadsheet and the total chlorosis values were stored in a separate tab of the same spreadsheet.

Plant Growth. For the plant growth analysis, a digital caliber was used to take the stem diameter at the beginning of the experiment and the measurement was recorded on a spreadsheet. The stem diameter was measured at about 30-40 mm from the crown. At the end of the experiment, the stem was measured at approximately the same place and the data was put in the spreadsheet as the end measurement. In order to obtain the total plant growth, represented by the stem diameter, the starting stem diameter measurement was subtracted from the ending measurement.

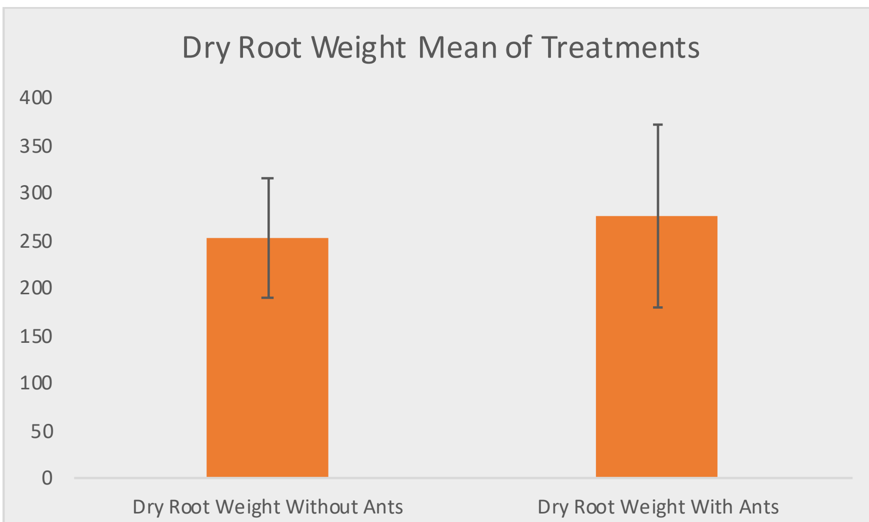
Data Analysis. The free online GraphPad Software (GraphPad, San Diego, CA) was used to analyze the data. All variables were analyzed using a paired two-tail t-test. The mean, SD, SEM, and N for each group were analyzed and recorded into an Excel spreadsheet. In addition, the degrees of freedom, t-value, and p-value were recorded

Results

Dry Root Weight. In the experiment, no difference was observed between the two treatments. There were no statistically significant differences in the dry root weight between plants with only aphids and plants with both aphids and ants (df = 5, t = 0.55, P = 0.61). The dry root weight of grain sorghum between treatments with aphids only and treatments with aphids and ants indicated that there were no trends (figure 1).

Leaf Chlorosis. After 14 days, the plants were observed for the average amount of leaf chlorosis. In the experiment, there were no statistically significant differences were found (df = 10, t = 0.95, P = 0.37). The average change in leaf chlorosis indicated that there were no trends for neither a positive or negative affect on grain sorghum health between treatments with only aphids and treatments with aphids and ants (figure 2).

Stem Growth. The stem diameter of the plants was measured at the start and end of the experiment. There were no statistically significant differences found in stem growth between the plants with only aphids and the plants with both aphids and ants (df = 10, t = 1.25, P = 0.25). The



stem diameter growth in grain sorghum indicated that there were no trends for the plants treated with either only aphids or with aphids and ants (figure 3).

Fig. 1. The mean of the dry root weight treatments. SEM of plants without ants is 63.37. SEM of plants with ants is 96.42.

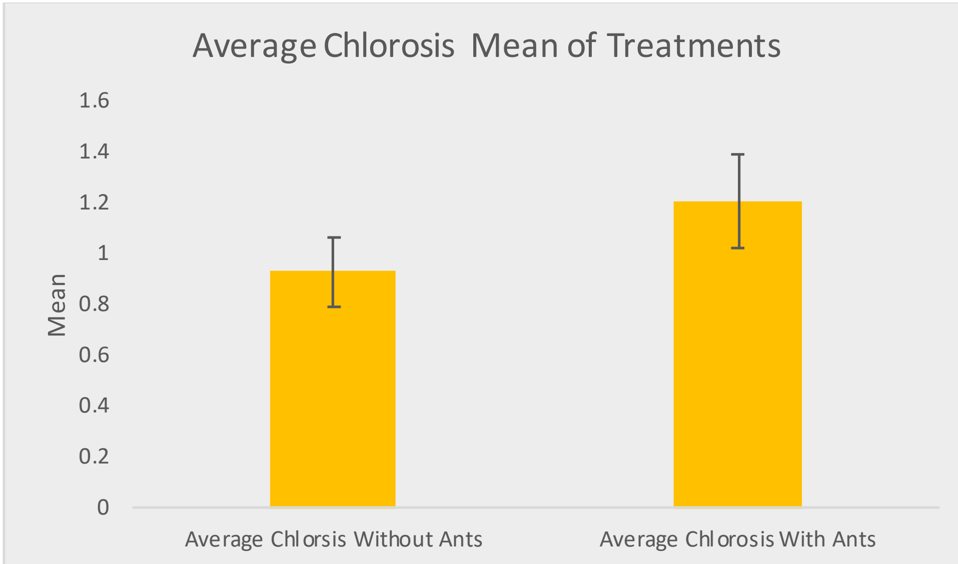


Fig. 2. The average change in leaf chlorosis mean between treatments with aphids only and treatments with both aphids and ants. The SEM of average leaf chlorosis without ants is 0.1362. The SEM of average leaf chlorosis with ants is 0.1853.

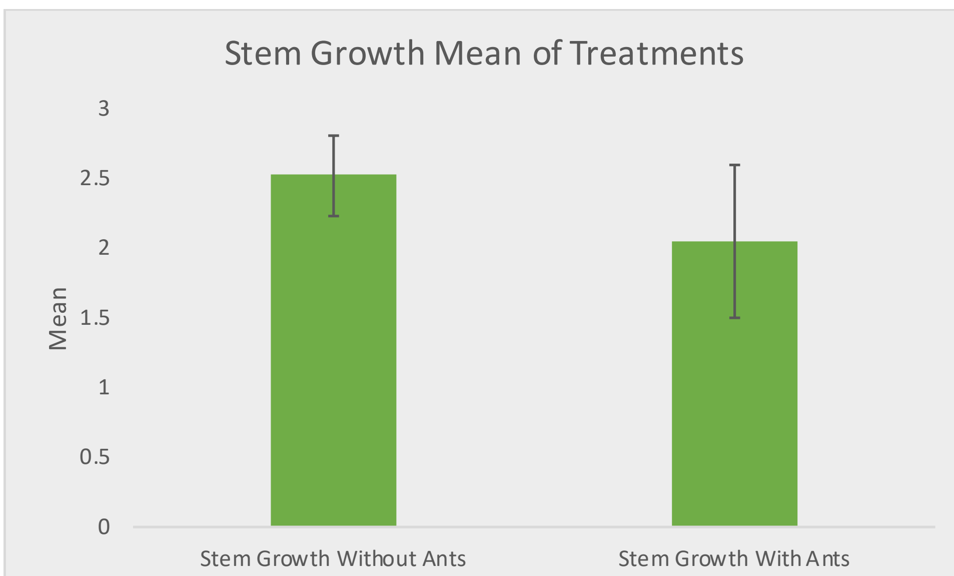


Fig. 3. The stem growth of grain sorghum means with either only aphids or with both aphids and ants. The SEM of stem growth without ants is 0.2889. The SEM of stem growth with ants is 0.5470.

Discussion

Currently, no studies have examined the effect of SCA and TCA interactions on grain sorghum health. Understanding this is important because both of these insects are invasive species in the continental USA. In addition, these insects have been reported interacting in sorghum fields, which could cause increased damage to this crop. The aim of this experiment was to develop an understanding of the potential damage mutualisms between TCA and SCA can cause to the grain sorghum plants.

Although the experiment did not reveal statistically significant differences, there are still trends that show potential negative effects for grain sorghum in the presence of SCA and TCA mutualisms. Some differences in dry root weight, leaf chlorosis, and stem diameter were noticeable between the plants with aphids and the plants with ants and aphids.

A potential reason that there were no statistically significant differences is because perhaps the sample size was too small. The number of samples examined (N=11) did not provide enough data points to compare in case there were any flaws during the experiment. Furthermore, the experiment was tested using a 14-day period which seems to not be enough time to allow the aphids to produce significant damage to the plants. By increasing the duration of the experiment, future studies can have better results. Also, the number of TCA present during each treatment could have some influence as to why there were no statistically significant results. There were 300 TCA present for each treatment and increasing the number of TCA per treatment in future studies could increase the likelihood of TCA and SCA interacting. Thus, the increase of this interaction could mean that TCA and SCA mutualisms can potentially increase the amount of damage to grain sorghum.

In summary, TCA and SCA mutualisms needs further investigation to determine whether they cause more damage to grain sorghum crops. In this experiment, trends were examined that TCA and SCA mutualisms cause an increase to leaf chlorosis, reduced stem diameter, and a decrease in dry root weight. Future studies need to increase the duration of the experiment, increase the sample size, and increase the number of TCA used per treatment in order to have results that better represent the mutualism effects on grain sorghum. The investigation of this mutualism will determine whether farmers will have control of both ants and aphids or just focus on aphids. If this mutualism does increase the overall damage to grain sorghum, new IPM strategies will have to be implemented for pest control of both TCA and SCA. Understanding the impact of this ant-aphid interaction can help farmers manage their crops better. This means that growers can focus on the statistically important causes in order to maximize efficiency against the problem. The experiment also brings awareness about potential damages that invasive species interactions can cause.

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