The Effects of Sorghum Volatiles and Sugarcane Aphids on *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) Attraction to Plants

Authored by Apuleyo Yanez

Texas A&M University

Edited by Hannah Welch

Abstract*: Coccinella septempunctata* and *Harmonia axyridis* are incredibly effective controls for insect pests in crops. We tested their ability to use olfactory cues to choose the plant with aphids on it. We used two strains of *Sorghum bicolor* one being the normal susceptible strain while the second was resistant to aphids. The tests conducted were ultimately to determine which strain of sorghum the ladybeetles would favor, and whether the ladybeetle would prefer the plants with aphids on them. When aphids were present, no significant difference between susceptible and resistant was found, but when there were no aphids the susceptible plant attracted more predators. This could suggest that there is chemical or volatile that the susceptible plant has that the susceptible plant does not. Further study of the volatiles that the two strains produce would be necessary as well as more replicants of the two strains with no aphids.

Keywords: Sorghum, volatiles, Sugarcane Aphid, *Melanaphis sacchari, Harmonia axyridis*

The sugarcane aphid *Melanaphis sacchari* (Hemiptera: Aphididae) (Zehntner) has been a major crop pest in all part of the world but recently it was discovered in North and Central America (Villanueva 2014). In 2013 it was first detected in sorghum in South and East Texas, southern Oklahoma, eastern Mississippi, northeastern Mexico and parts of Louisiana, where it caused a significant loss in crop yield due to the aphids large amounts of honeydew, a waste product produced by the sugarcane aphid composed of mainly sugar and water, which is then followed by the growth of a black sooty mold that can inhibit photosynthesis (Villanueva, 2014). The aphids use of a stylet to pierce the sorghum can lead to vectoring plant viruses (Smith and Boyko, 2006). In 2014 the number of states with aphids in sorghum crops went up to eleven states and 311 counties, then in 2015 it went up to 17 states and over 400 counties (Bowling, 2015). This rapid distribution through North America is accredited by many to the aphid’s ability to reproduce at such incredible rates and wind dispersal (Brewer, 2015).

An effective form for controlling aphid populations is the use of predaceous coccinellids, which have been used for over a century (Gordon, 1985) (Hodek, 1970) (Hodek, 1996) (Obrycki and Kring, 1998). *Harmonia axyridis* (Pallas) (Coleoptera:

Coccinellidae) also known as the 'Asian ladybeetle' has spread so several continents and has become a major biological control because of its voracious behavior (Gordon, 1985). *Harmonia axyridis* has been successful in controlling pest aphid species on several crops including soybean, maize, alfalfa, and winter wheat (Koch, 2001).

Coccinella septempunctata (Coleoptera: Coccinellidae), also known as the 'seven-spot ladybird' is a common species with voracious adults and larvae that can feed on several insect pests (Karpacheva, 1991). Previous studies have focused on both *C. septempunctata* and *H. axyridis* in order to find out whether or not they detect or are influenced in any way to the volatiles of aphids and aphid infested plants. An experiment conducted by Ninkovic and Pettersson tested whether or not *C. septempunctata* uses volatiles from barley plants or pheromones from the aphid to determine where its prey was, the conclusion is that the lady beetles were significantly attracted to volatiles emitted from barley plants infested with aphids, having four times as many ladybeetles going to the plant with aphids, but not from healthy plants or from aphids alone (Ninkovic et al. 2001). In 1986 Obata performed a similar experiment with *H. axyridis* using fleabane, he found that *H. axyridis* was more attracted to infested leaves than healthy leaves (Obata, 1986).

Past studies have tested the ladybeetle's ability to use olfactory cues to successfully choose a plant with aphids, but they have not tested the susceptible strain and the resistant strains together. The resistant strain of sorghum is resistant to aphids as found by Dr. Salzman in 2004 but it was not tested to see if it was also more attractive to aphid predators. It is possible that the resistant strain of sorghum may not be as attractive to the ladybeetles as the susceptible strain. When attacked by aphids, the sorghum will release volatiles and chemicals that help defend it from the aphids. Some volatiles also attract predators which could be the case for the susceptible strain of sorghum, but this may not be the case for the resistant strain. For this study we will first test how the predator responds to the plants with and without aphids, and finally test the two strains of sorghum against each other in order to demonstrate if the resistant plant is not only more resistant to aphids but also more attractive to predators.

Material and Methods

Plants and Insects

Two strains of *Sorghum bicolor* were used, one being susceptible to aphids and the other being resistant. Both strains were planted in 4.5x4.5x4.5" pots in a greenhouse in 24x24x24" bug dorms (Bioquip. Rancho Dominguez, CA) and were watered 2-3 times a week. The plants used in the experiment were all 3 weeks old, 25 ± 5cm with 4-5 true leaves. *Melanaphis sacchari*, and *Harmonia axyridis* were collected at a sorghum field off of FM 60 in College Station, Tx (30.550350, -96.439606). *Coccinella septempunctata* were collected at research park in Texas A&M university (30.598554, -96.361275). Both *C. septempunctata* and *H. axyridis* were then placed in plastic one-gallon cylindrical containers 20x17x17cm (manufactured in China, distributed by Michaels Store inc.)

and stored in Percival units, set at 25 ± 2° C and 50 ± 8% r.h., for rearing.

Y-tube Olfactometer Setup

A two-chamber air delivery system was used with two 4L glass chambers used to store the sorghum plants (ARS Gainesville, FL, OLFM-ADS-2AFM1C). Two ¼" Teflon hoses were used to connect each chamber to the Y-tube where the ladybirds were placed. The olfactometer was set to have an outlet pressure of 15 psi.

Experiment parameters

The experiment was run in blocks, each block consisted of 24 separate runs using 12 different ladybirds, three blocks were run over the course of the experiment. The first block contained six separate combinations between resistant plants with aphids, resistant plants without aphids, susceptible plants with aphids and susceptible plants without aphids. After the first block of data, the combinations were changed to non-resistant with aphids vs. non-resistant without aphids, and resistant with aphids vs. resistant without aphids. The sorghum plants that had required aphids for the experiment were separated from the others 24 hours before the experiment and were infested using clippings from aphid-infested sorghum in the greenhouse. Before the experiment began the aphids were counted and scrubbed off until each plant contained 45 ± 5 aphids. The ladybirds would be run two per plant combination. The Y-tube would be cleaned out with 70% ethanol and distilled water and dried with a hair drier after every run. The chambers would be cleaned with 70% ethanol and distilled water before every change in plant.

Statistical Analysis

RStudio (version 1.0.143) was the statistical program used to calculate the Chi-squared test for independence of the data collected.

Left	Right	Predator 1	Predator 2
S w/a	S w/o	S w/o	S w/o
S w/o	R w/o	N/C	R w/o
S w/a	R w/a	S w/a	R w/a
S w/a	R w/o	S w/a	N/C
R w/a	S w/o	S w/o	N/C
R w/a	R w/o	R w/a	R w/a

Table 1. Experimental combinations and their respective outcomes.

Results

The first block tested the six possible combinations between Resistant with aphids (Rw/a), Resistant without aphids (Rw/o), susceptible with aphids (Sw/a), and susceptible without aphids (Sw/o).

Six different combinations were used, as well as which plant the predator preferred (Table 1). Two predators were used for each combination. The first combination tested was Sw/a vs. Sw/o, from this both predators chose the susceptible plant without aphids. From Sw/o vs. Rw/o, one chose Rw/o and the other did not choose (N/C). Sw/a vs. Rw/a resulted in one predator going for susceptible

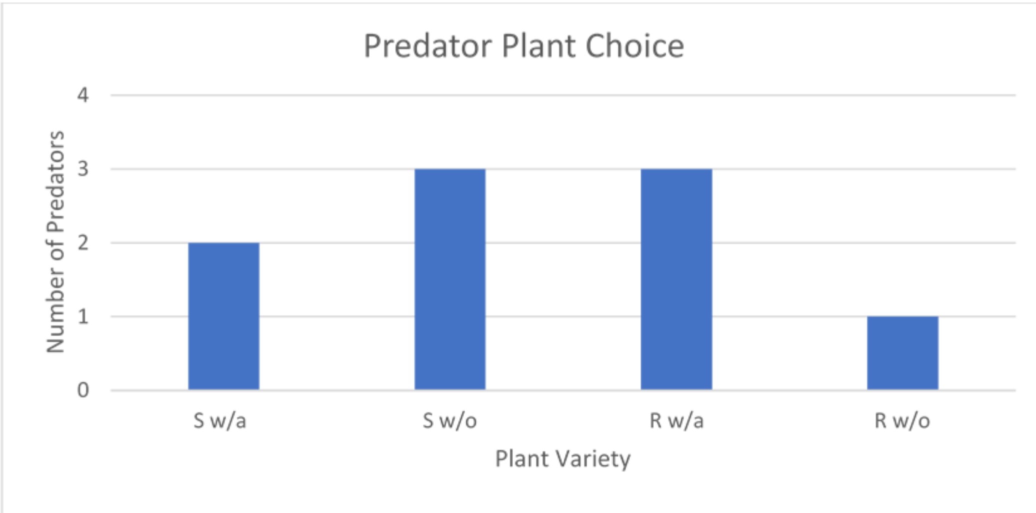

Figure 1. Predator plant preference for trial one.

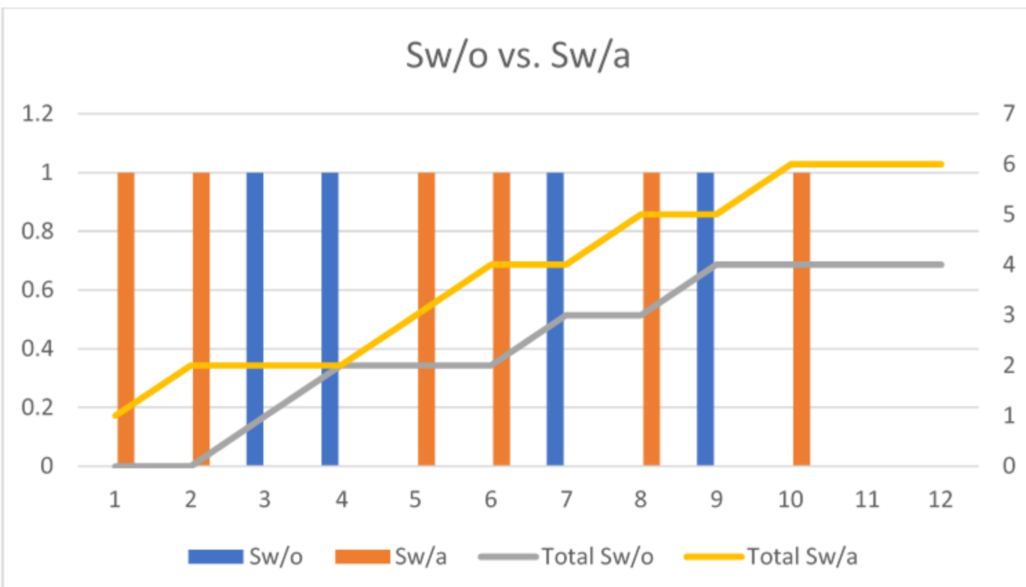

Figure 2. Second trial using only susceptible sorghum. Vertical bars represent individual predators, trend line represents predator increase over time. X^2 = 8.8824, df = 2, p = 0.01178

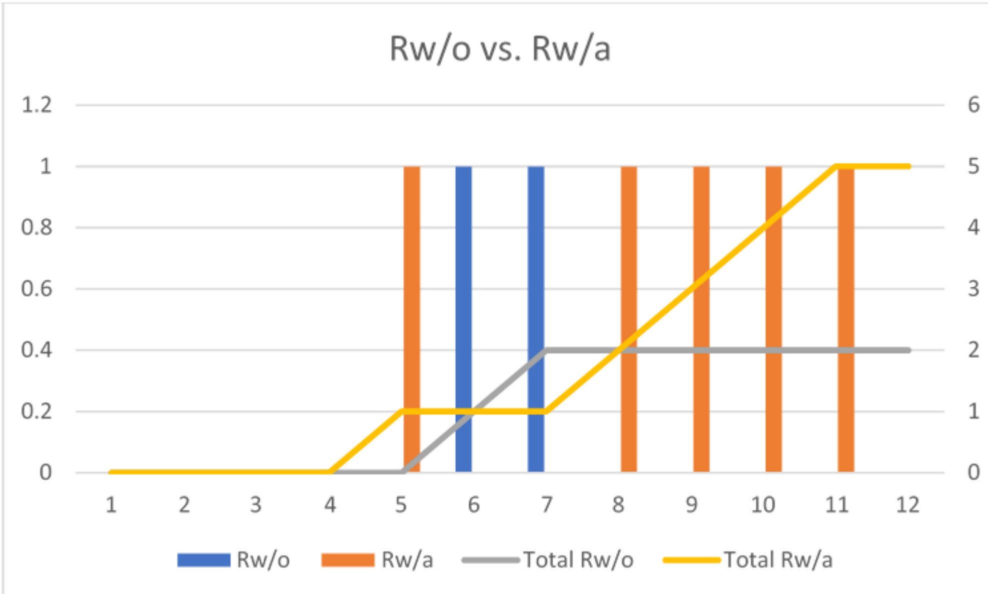

Figure 3. Results from second experiment concerning resistant strain of sorghum. The bars represent individual predators per run, while the lines represent the increase in predators that choose that plant over the whole course of the experiment.

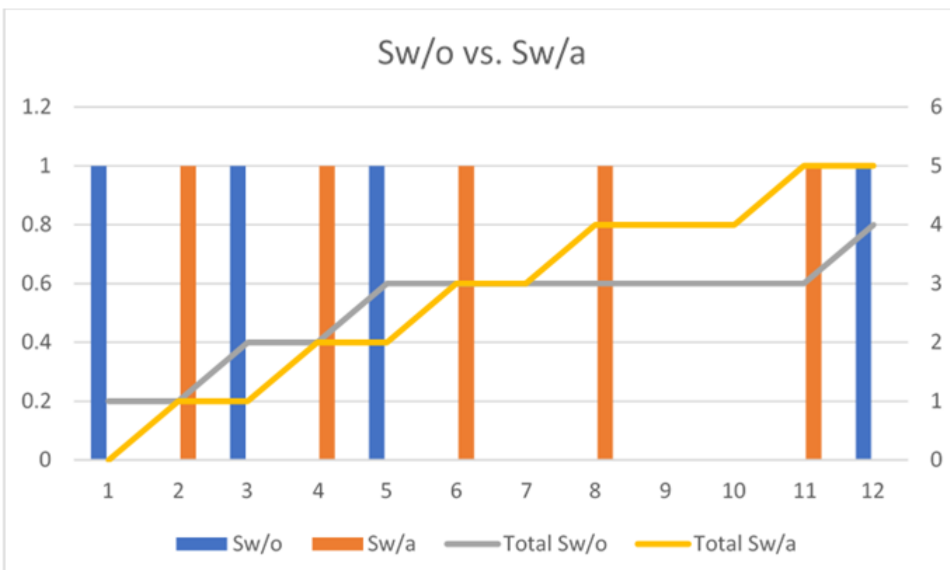

Figure 4. Results from third experiment dealing with only the susceptible strain of sorghum. The bars represent the individual predator and the lines represent the total number of predators over the course of the experiment.

with aphids and one for resistant with aphids. Sw/a vs. Rw/o had one predator go towards susceptible with aphids and the other predator did not choose. Rw/a vs Sw/o had one for susceptible without aphids and the other did not choose. Rw/a vs. Rw/o resulted in both predators having chosen resistant with aphids.

The second block run was strictly Rw/o vs. Rw/a, and Sw/o vs. Sw/a. The combinations were interchanged randomly, and each setup had two predators run through them. In total, both combinations had a total of 12 predators run through them each.

either one (Figure 2) (X-squared = 8.8824, df = 2, p-value = 0.01178). For the resistant plants, five chose the plants with aphids, two

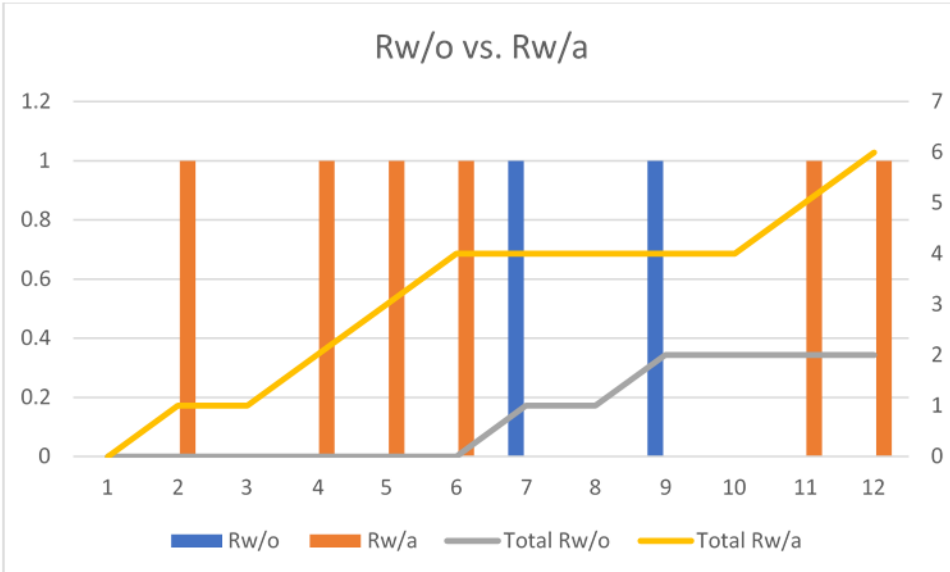

Figure 5. The results of the third experiment dealing with only the resistant strain of sorghum. Each bar represents a single predator while each line represents the progression of predators that chose that certain plant.

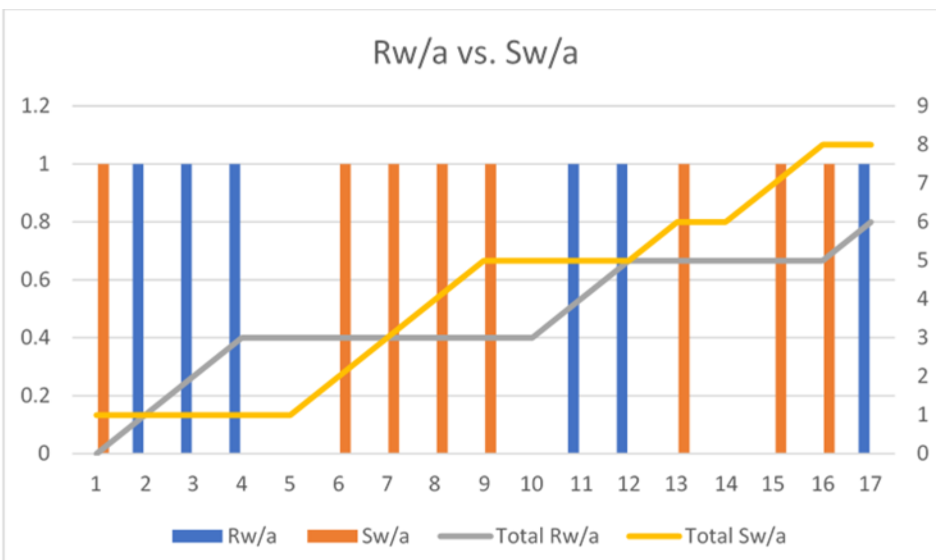

Figure 6. The results of the final experiment using both strains of sorghum (Susceptible and resistant), both with aphids. The bars represent a single predator while the lines represent the total number of predators that chose that certain plant.

For the susceptible plants six of them chose the plants with aphids, four of them chose the plants with no aphids, and two did not choose

chose the ones with no aphids and five did not choose either of the plants (Figure 3) (X-squared = 10.938, df = 2, p-value = 0.004216).

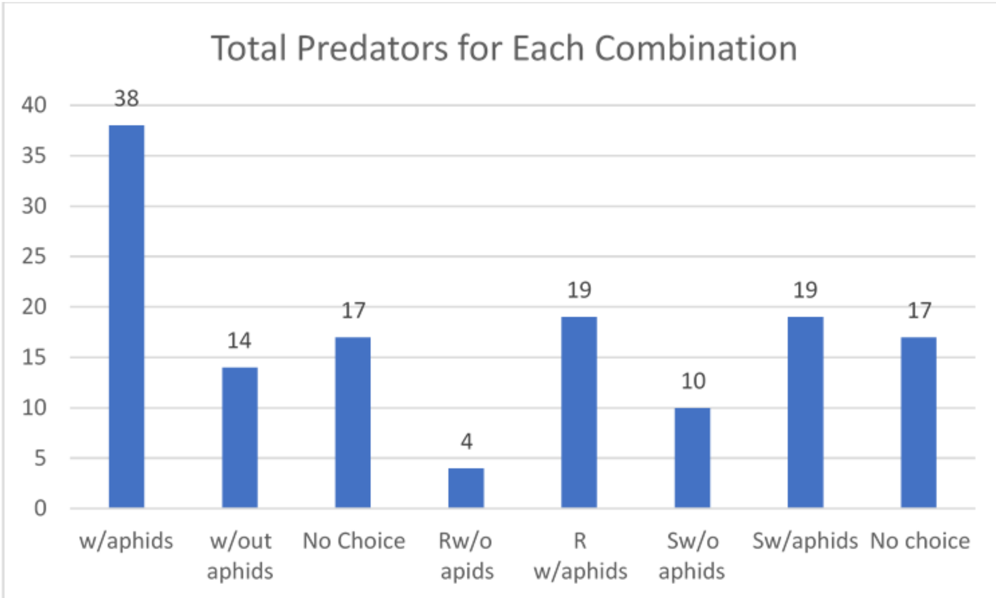

Figure 7. Final results of all the blocks put together. The graph depicts the total number of predators that chose each plant as well as how many chose plants with aphids over plants without aphids.

The third block of data was over the same two combinations of resistant with resistant and susceptible with susceptible. For these runs

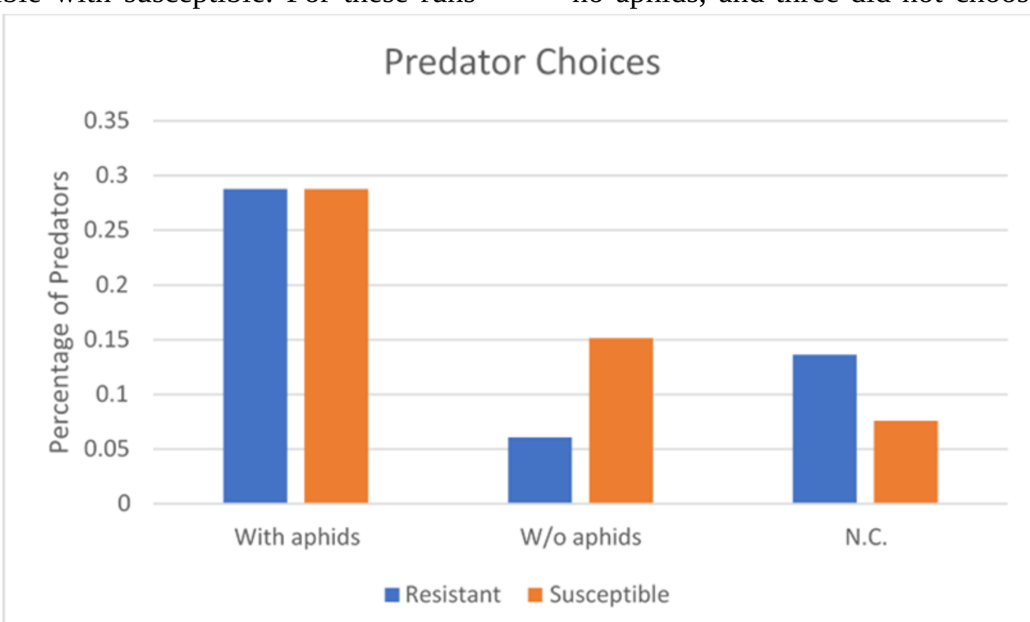

Figure 8. Percentage of predators that chose each of the choices as well as the number that did not choose anything.

the *H. axyridis* was used. Two predators were used for each setup and each setup was repeated three times randomly.

For the susceptible plants five chose the plants with aphids, four chose the ones with no aphids, and three did not choose (Figure 4) (X-squared = 8.8824, df = 2, p-value =

0.01178). As for the resistant plants, six chose the plant with aphids, two chose the plant with not aphids, and four predators did not choose (Figure 5) (X-squared = 10.938, df = 2, p-value = 0.004216).

were rotated randomly as well as rotating which plant went in which jar.

Eight of the predators chose susceptible with aphids while six chose resistant with aphids, and three did not choose anything (Figure 6)

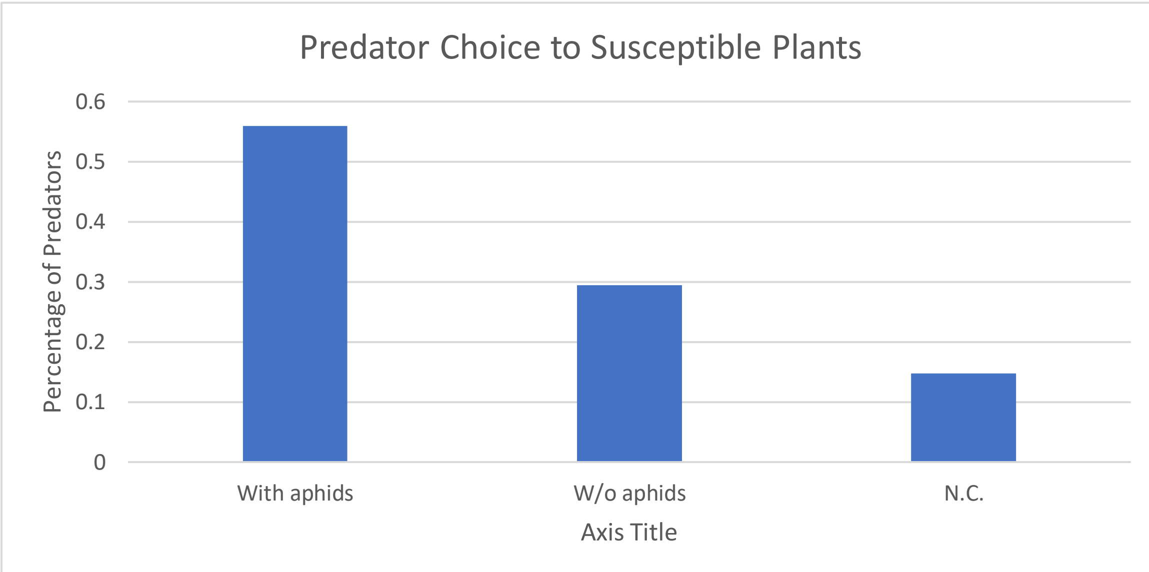

Figure 9. Percentage of predators that chose each option in the tests involving susceptible plants.

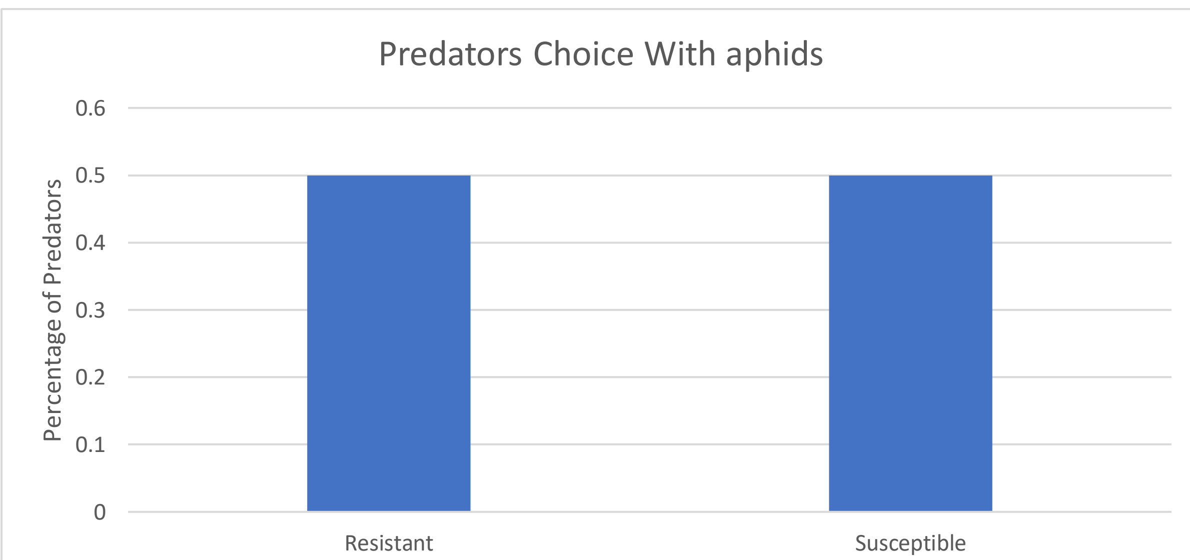

Figure 10. Percentage of Predators that chose susceptible with aphids or resistant with aphids.

The fourth and final block consisted of only resistant plant with aphids vs. susceptible plants with aphids. This was run a total of 17 times using *Harmonia axyridis.* The plants

(X-squared = 3.657, df = 2, p-value = 0.1607).

The percentage of each choice was also found: 28.7% of the predators chose

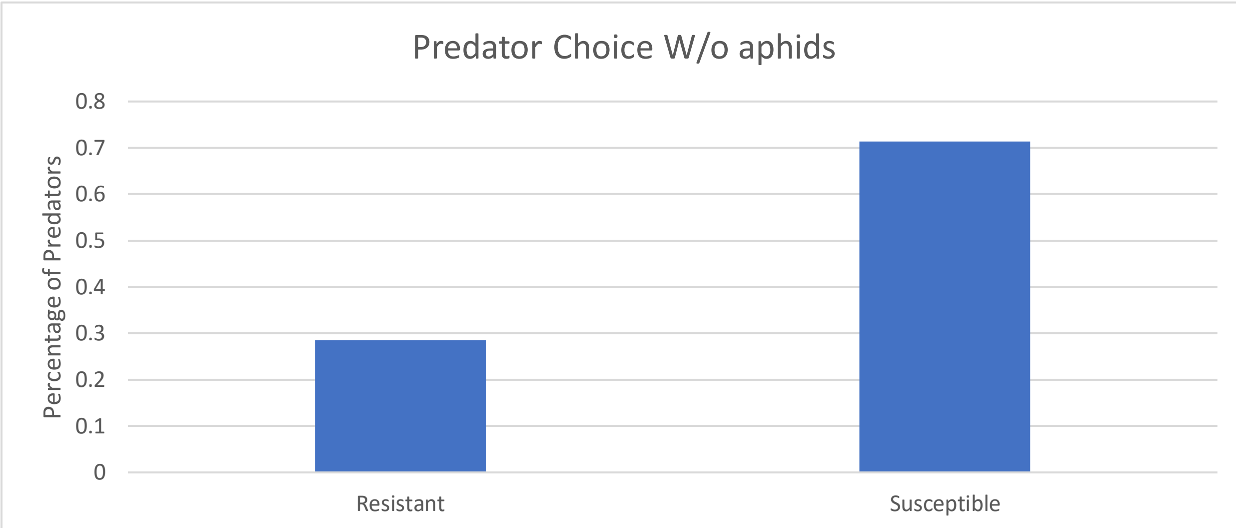

Figure 11. Percentage of predators which chose resistant or susceptible without aphids.

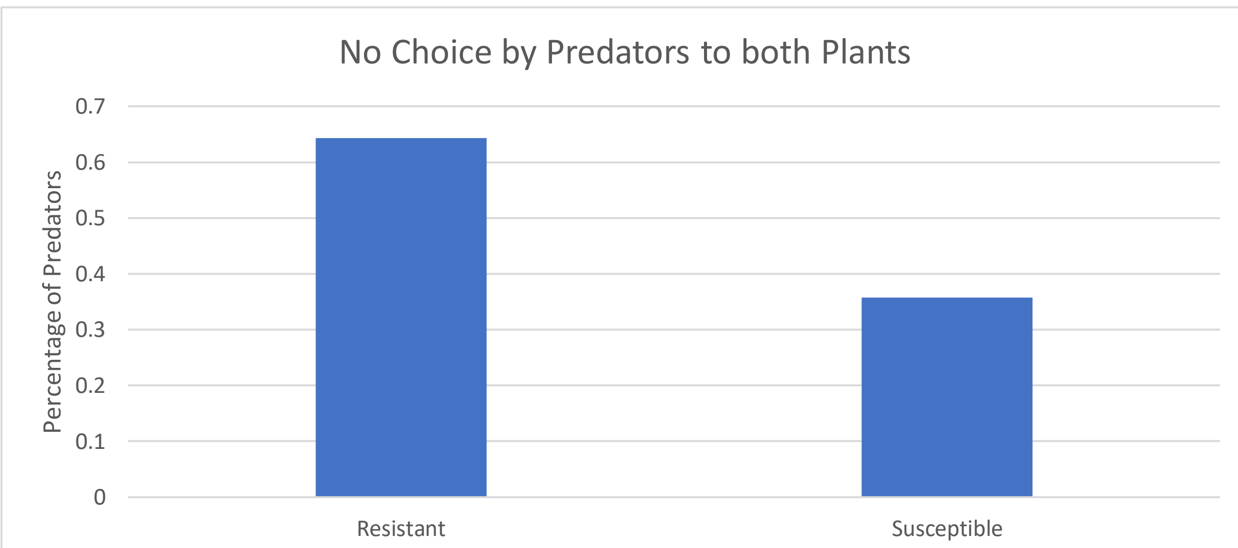

Figure 12. Percentage of predators that did not choose either plant, for the test only using resistant plants and the tests only using susceptible plants.

susceptible with aphids and 28.7% chose resistant with aphids, 6.0% chose resistant without aphids, 15.1% chose susceptible without aphids, the resistant tests had 13.6% of the predators not choose, while susceptible had 7.5% no choice (Figure 8) (With aphids vs. Without aphids: X-squared = 14.87, df = 2, p-value = 0.005904).

Discussion

Ladybeetles did not seem to prefer either of the two strains over the other. The chi-square test showed that there was no significant difference between the two values and when looking at the percentage of predators that preferred susceptible with aphids over resistant with aphids, the percentages were 50-50, then when seeing the percentage that chose those two options over all other options they both came out to the exact same 28.78% each. The ladybeetles do not seem to have a preference when aphids are present, since both choices had the same number of predators, meaning aphid presence is more significant than whether or not the plant is resistant or susceptible. When looking at the chi-square test again, the significance that susceptible with aphids over susceptible without aphids has compared to the significance that resistant with had to resistant without is less. This could support that something from the resistant plant attracts more predators when attacked by aphids. In the susceptible plant tests the number of predators that preferred "with aphids" over "without aphids" was only one more which gives reason to believe that the lady beetles do not necessarily depend on the smell of the aphids to determine which of the susceptible plants they go on. These results seem to correlate with what Ninkovic found in his experiment with *C. Septempunctata* which stated that the adults may not be receptive to volatiles emitted from the aphids (Ninkovic et al. 2001). In similar studies done by Nakamuta, he found that β-farnesene (an aphid alarm pheromone component) did not have any effect on *C. septempunctata* (Nakamuta. 1991). In an earlier study Nakamuta also tested *C. septemppuntata's* visual sense and its ability to use visual cues to successfully capture an aphid, he would place an aphid on a filter paper and have a ladybeetle walk by the aphid at several distances to scope the distance required for a response by the ladybeetle; the ladybeetles eventually reacted to the aphids at less than 7 mm away in a lit room, at 8 mm away they would not interact or notice the aphid at all, when in the dark the ladybeetles needed to be less than 2mm away, they needed to come in contact with the aphids to know they were there (Nakamuta 1984). This suggests the ladybeetles lack of olfactory senses to find the aphids. In the resistant tests, the predators that chose "with aphids" over "without aphids" was more than three times higher, suggesting that there is something the plant may be doing or releasing to attract predators that is more significant to predators. The percentage of predators that chose susceptible without aphids over the number that chose resistant without aphids was 71.4% to 28.6% and in the total number of predators 15.1% chose the susceptible without aphids while only 6.0% chose resistant without aphids. This could mean that the susceptible strain's volatiles are more attractive to the predator, but when aphids start invading the difference in strain becomes less significant, which could be a problem for farmers that want to decrease the degree of damage that their sorghum receives; the sooner the predator lands on the plant the sooner it can start feeding on aphids, so if it is there even before the aphids invade it will be able to start feeding on them before they grow to huge numbers and cause more damage. The resistant plants may be lacking some of the volatiles that the susceptible plant has, for example when maize is attacked by beet armyworm larvae it will release a combination of 11 compounds that attracts the parasitoid *Cotesia marginiventris* (Cresson) (Turlings.1991). Future studies could be to discern whether the ladybeetles react to mechanical injuries on the resistant sorghum to see if the volatiles that attract the ladybeetle are from the plant itself. We would compare how and if the ladybeetle reacts to cutting/scratching the plant, to puncturing it with a small needle to replicate an injury caused by an aphid, in order to test if the plant releases different volatiles when injected by a proboscis and if those volatiles are what attracts the ladybeetle. In a study by Ninkovic and Pettersson, they studied the effect that barley plants had on the ladybeetles without any aphids and they found that there was a positive correlation between the barley and *C. septempunctata's* olfactory response hinting that the ladybeetles may use the scent of the plant itself rather than the scent of the aphids in the wild (Ninkovic et al. 2003). Many studies have focused on whether or not plants release volatiles or chemicals when attacked by aphids and how those chemicals affect how the plant responds to the aphid attack, for example in one study involving *Sorghum bicolor* it was found that the plant would in fact respond to aphid attacks by releasing chemicals, proteins, and several other compounds to try to stop the aphids, one of these defense mechanisms was to produce more cyanogenic glucoside dhurrin (Salzman, 2004), which when degraded, is a potent toxin to herbivore insects, (Tattersall et al., 2001). The ladybeetles reacted the same way to both susceptible plants with aphids and resistant with aphids, but when there were no aphids, the predators preferred the susceptible plant. By having more replicants for this scenario, we could be able to more accurately predict how the ladybeetles react to each plant in the while and have a better understanding of how these ladybeetles find prey in the wild. We did not have many predators at the time and the number of aphids we had was also limited, but we also did not test susceptible w/o aphids to resistant w/o aphids against each other, the possible direction to take for this study could be to focus on just the plants with no aphids and observe how the predators react to each plant. A study using mechanical injuries to mimic herbivorous eating behavior would be a great way of testing how the ladybeetle responds to the plant's volatiles without the presence of aphids. Past studies have found a connection between certain volatiles and an increase in predators, for example the release of methyl salicylate is a way in which *Glycine max* attracts predators such as *C. septempunctata* to it when infested by *Aphis glycines* (Zhu et al., 2005).

Acknowledgements

The author would like to express his gratitude to Prof. M. Eubanks as well as Jeremy Hewlett of Texas A&M University for allowing me to work in their lab and allowing me to use their equipment to collect the data for my study, as well as using their ladybeetles and sorghum plants. I would also like to thank them for reading and revising my paper.

References

Bowling, R. D., et al. 2016. Sugarcane Aphid (Hemiptera: Aphididae): A New Pest on Sorghum in North America. *Journal of Integrated Pest Management,7*(1), 12. doi:10.1093/jipm/pmw011

Bowling, R., M. Brewer, S. Biles, and J. Gordy. 2015. 2015 Occurrence of sugarcane aphid in the U.S. and Mexico with reference to occurrence in 2013 and 2014. Texas Plant Protection Conference, Bryan, TX (http://ccag.tamu.edu/sorghum-insect-pests/).

Gordon R.D. 1985. The Coccinellidae (Coleoptera) of America North of Mexico. *J NY. Entomol*. 102, 209-216

Hodek I. 1970. Cechtoccinellids and the modern pest management. *BioScience.* 20, 534-5552

Hodek I., Honek A. 1996. *Ecology of Coccinellidae.* Dordrecht:Kluwer. 464

Koch R.L. 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. *J Insect Sci*, 3, 1-16

Nakamuta, K. 1984. Visual orientation of a ladybird *Coccinella septempunctata* L. (Col.:Coccinellidae), towards its prey. *J. Exp. Biol.* 199, 141-146.

Nakamuta, K. 1991. Aphid alarm pheromone component, (E)-β-farnesene, and local search by a predatory lady beetle *Coccinella septempunctata bruckii* Mulsant (Coleoptera:Coccinellidae). *Appl. Entomol. Zool.* 26, 1-7.

Ninkovic, V., Abassi, A.S., and Pettersson, J. 2001. The influence of aphid-induced plant volatiles on ladybird beetle searching behavior. *Biological Control.* 21, 191-195

Ninkovic, V., Pettersson, J. 2003. Searching behavior of the sevenspoted ladybird, *Coccinella septempunctata* effects of plant-plant odour interaction. *OIKOS*. 100, 65-70.

Obata, S. 1986. Mechanism of prey finding in the aphidophagous ladybird beetle *Harmonia axyridis*(Coleoptera:Coccinellidae). *Entomophaga*, 31, 303-311

Obrycki, J.J., and Kring, T.J. 1998. Predaceous coccinellidae in biological control. *Annu. Rev. Entomol.* 43, 295-321

Salzman K.Z., Salzman R.A., Ahn J.E., Kiowa H. 2004. Transcriptional regulatioin of sorghumdefense determinants against a phloem-feeding aphid. *American Society of PlantBiologists*

Smith, M.C., Boyko, E.V. 2006. The molecular bases of plant resistance and defense responses to aphid feeding; current status. *Entomologia Experimentalis et Applicata*. 122, 1-16

Tattersall D.B., Bak S., Jones P.R., et al. 2001. Resistance to an herbivore through engineered cyanogenic glucoside synthesis. *Science.* 293, 1826-1828

Turlings, T.C.J., Tumlinson, J.H., et al. 1991. Isolation and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventrus*(Cresson), to the microhabitat of one of its hosts. *J. Chem. Ecol.* 17 2235-2251.

Villanueva, R.T., et al. 2014. Sugarcane aphid: a new pest of sorghum, 4 pp. Texas A&M AgriLife Extension, College Station, TX. (2014 Villanueva)

Zehntner, L. 1897. Die plantenluizen van het suikenet. Arch. Suikerind. Ned. Ind. 5:551.

Zhu, J., Park K.C. 2004. Methyl Salicylate, a soybean aphid-induced plant volatile attractive to the predator *Cocinella septempunctata. MSTRS Technologies Inc.*