LIFE HISTORY, DISTRIBUTION, AND TEMPERATURE TOLERANCE OF THE INTRODUCED HEDGEHOG GRAIN APHID *SIPHA MAYDIS* PASSERINI [HEMIPTERA: APHIDIDAE]

By

MASON TAYLOR

Bachelor of Science in Entomology

Oklahoma State University

Stillwater, OK

2020

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2023

LIFE HISTORY, DISTRIBUTION, AND

TEMPERATURE TOLERANCE OF THE INTRODUCED

HEDGEHOG GRAIN APHID *SIPHA MAYDIS*

PASSERINI [HEMIPTERA: APHIDIDAE]

Thesis Approved:

Dr. Wyatt Hoback

Thesis Adviser

Dr. Scott Armstrong

Dr. Ali Zarrabi

ACKNOWLEDGEMENTS

I would like to first and foremost thank my committee members who were the most involved in my project from the beginning to the end and giving me the opportunity to work on these projects. They have each helped and supported me in multiple ways giving me guidance and teaching me new and valuable lessons throughout this process. Dr. Armstrong helped me understand greenhouses to aphids and everything in between as well as new field sampling and research practices. Helping understand why we were doing these experiments and what real world applications these can be used for made me even more excited on what the outcome would be and what more I could do to further this knowledge. Dr. Hoback helped me greatly in getting this thesis and my defense in order as well as pushing me to do more and better things in my life as well as in this field of research. I would not being doing a master's program without him. Dr. Zarrabi helped me with creating new ideas that could be used in my aphid studies to help with experiments and help me understand more about aphid biology and how these experiments correlated. Everything I had learned from each of them were of the upmost help and were the reason I was able to keep my program moving forward and successful. I would also like to thank the people responsible for funding and facilities, because without either my research would not have been able to happen at all. None of this would have been possible without any of these great people.

I would also like to thank the faculty and students that have helped me along the way. Another big thank you goes out to Barbara Driskel, for helping me in the greenhouses and teaching me everything she knew. From the start she was there showing me how to plant and what to plant to keep everything running smoothly. Dr. Mornhinweg taught me all about planning experiments and planting barley as well as how to keep it alive while keeping the aphids happy on each plant. I would also like to thank the students Rodrigo Soares, Malea Parsons, and Haley Garonovich as fellow master students helped me so much during the program with data collections, colony care, planting, and so much more. I would also like to thank Rafael Hayashida for helping with the statistics and data analysis on most of my data collections.

Lastly, I would like to thank everyone in the department office staff, Sharon Hillock, Barbara Brown, Noah Dunagan and Lafonda Medina, for keeping me on the straight and narrow since undergraduate. I would also like to thank the professors who taught me new skills and knowledge along the way that helped me in writing and in life, Dr. Giles, Dr. Hoback, Dr. Jiang, Dr. Ochoa Corona, Dr. Ma, and Dr. Walker.

Thank you all for everything and anything in the future that may come up. I appreciate all of the help along the way and will never forget it. This has been an amazing experience that I will never forget and the great people that I have met.

Name: MASON TAYLOR

Date of Degree: MAY 2023

Title of Study: LIFE HISTORY, DISTRIBUTION, AND TEMPERATURE TOLERANCE OF THE POSSIBLY INVASIVE GRAIN APHID *SIPHA MAYDIS* PASSERINI [HMEIPTERA: APHIDIDAE]

Major Field: ENTOMOLOGY AND PLANT PATHOLOGY

Abstract: *Sipha maydis* Passerini (Hemiptera: Aphididae), the hedgehog grain aphid, is a cereal grain pest that is suspected to be a native species from Europe or Northern Africa. These aphids are also found in Asia and both Americas on crops and native grasses. *S. maydis* started to become and problem and became monitored more strictly after an outbreak on wheat in 2002 in Argentina. In 2007, the first documentation of the species in the U.S. was in California on giant wild rye with sightings spreading to the east quickly. Since this aphid is possibly invasive and in the U.S. we tested three aspects of its biology. 1) The current distribution of this aphid has increased since 2019; 2) *S. maydis* has an optimal temperature, measured by intrinsic rate of increase between 20 and 30°C; 3) Comparing the life history of *S. maydis* and *Melanaphis sorghi* will document existing host-plant cross-resistance. Testing these hypothesis we sampled in the Rocky Mountain Range and Plains states of OK, NM, CO, UT, and WY. These surveys documented that *S. maydis* has not spread or increased. However, aphids were found on a variety of grass species and a new host was identified. Optimal thermal range was tested by placing 12 aphids on the laboratory rearing host plant, Jagger wheat, in temperatures of 10, 15, 20, 25, 30 and 35°C. Aphids were observed until death and intrinsic rate of increase was calculated for each temperature. Optimal temperatures for *S. maydis* were between 20 and 25°C. Third, we compared *S. maydis* to *M. sorghi* using 5 different cereal grain varieties, TX7000 sorghum, TX2783 sorghum, KS585 sorghum, Custer wheat and Millex32 millet, observing each aphid until death to calculate the intrinsic rate of increase. Both species reproduced on sorghum with *M. sorghi* performing better on known susceptible TX7000. In contrast, *S,* maydis reproduced at approximately the same low rate on all tested plants while *M. sorghi* did not reproduce on wheat or millet. Overall, *S. maydis* does not appear to pose a threat of becoming an invasive pest, but continued monitoring is warranted.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES....................vii

LIST OF FIGURES....................viii

CHAPTER 1: REVIEW OF LITERATURE....................9
U.S. Grains....................9
Wheat....................9
Barley....................10
Sorghum....................11
Millet....................12
Aphid Pest & Damage....................13
Aphid Resistant Varieties....................14
Russian Wheat Aphid....................14
Sugarcane Aphid....................15
Greenbug/Wheat Aphid....................16
Hedgehog Grain Aphid....................17
Aphid Biology & Pathogens....................18

REFERENCES....................21

CHAPTER 2: DISTRIBUTION RANGE OF A NEWLY INVASIVE SPECIES *SIPHA MAYDIS* [HEMIPTERA: APHIDIDAE] IN THE ROCKY MOUNTAIN RANGE 25

I. ABSTRACT....................25

II. INTRODUCTION....................26

III. MATERIALS AND METHODS....................26

IV. RESULTS....................28
Sampling Trip 2021....................28
Sampling Trip 2022....................30

V. DISCUSSION....................33

VI. REFERENCES....................36

CHAPTER 3: THE EFFECT OF TEMPERATURE AND SUPERCOOLING POINTS OF *SIPHA MAYDIS* PASSERINI (HEMIPTERA: APHIDIDAE) 37

I. ABSTRACT 37

II. INTRODUCTION..37

III. MATERIALS AND METHODS...39
Aphid Culture...39
HGA Development Under Different Temperatures.................................40
Supercooling Point Experiment 41
Statistical Analysis 42

IV. RESULTS..42
HGA Development Under Different Temperatures.................................42
Supercooling Point...43

V. DISCISSION...43

VI. REFERENCES 48

CHAPTER 4: COMPARISON OF LIFE HISTORY OF *SIPHA MAYDIS* [HEMIPTERA: APHIDIDAE] AND *MELANAPHIS SORGHI* ON DIFFERENT HOST PLANTS. .57

I. INTRODUCTION..57

II. MATERIALS AND METHODS 58
Seed varieties and Planting 59
Infesting and Growth Chambers 60
Parameters...60
Data Analysis 61

III. RESULTS 61
Pre-Reproduction Days..61
Offspring..63
Longevity 66
Intrinsic Rate of Increase (rm) 68

IV. DISCUSSION...71

V. REFERENCES....74

APPENDICES....75

Chapter Page

LIST OF TABLES

Table Page

1: Positive points found from both the 2021 and 2022 distribution studies 32
2: Reproductive parameters of the hedgehog grain aphid...51

LIST OF FIGURES

Figure Page

1: Total points from both the 2021 and 2022 distribution studies 33
2: Positive sites found in 2021 distribution study...34
3: Negative and positive site found during the 2022 distribution 35
4: Supercooling point ± SE (°C) of different development stages...............................52
5: Pre-reproductive days (d) 53
6: Total average fecundity or aphids produced until death..54
7: Total average days survived or longevity 55
8: Intrinsic rate of increase (rm) shown as the average mean 56
9: Pre reproduction days (prd) for SA...62
10: Pre reproduction days for HGA 63
11: Total average offspring or fecundity of SA 64
12: the average offspring or fecundity for HGA...65
13: Longevity of the SA species 67
14: Longevity of the HGA species...68
15: Intrinsic rate of increase (rm) or output average mean for SA 69
16: Intrinsic rate of increase (rm) for HGA 71
17: Barley experiment 1...75
18: Barley experiment 2 infested 76
19: Barley experiment 3 infested 77
20: Winged adult with fungus..78
21: Winged HGA adults...79

CHAPTER I

REVIEW OF THE LITERATURE

US grains:

Cereal grains have been one of the world's most important food sources for generations due to ease of cultivation and their ability to provide a substantial amount of calories and protein (Poutanen et al., 2022). They are cultivated around the world for numerous reasons, particularly for food/beverage consumption, feed for livestock, fuel, construction, cover crops, fodder, and repurpose seeding for other crops and other systems. Between the years 2020-2021, grains totaled 2,220 metric tons worldwide ("Global grain production, 2022", 2023). The global market for grains and their production in 2021 was $295.36 billon, and the market is expected to grow by the next year ("Grain Products Market Size, Trends and Global Forecast To 2032", 2023). The most produced grains are currently wheat, sorghum, barley, and millet.

Wheat

Wheat is in the genus *Triticum,* with many different species used such as: *T. aestivum*, *T. durum*, and *T. compactum*. Wheat is primarily cultivated for human consumption. Most breaded foods, such as pasta and noodles, include wheat as a primary ingredient. Because of this, over 600 million tons is harvested annually, making it one of the top three cereal crops produced worldwide (Shewry, 2009). The leading producers are currently China, India, Russia and the US (“Wheat Production by Country 2023”, 2023). Consequently, it has the highest number of production area of any grain harvested (Awitka, 2011). In the US, Oklahoma is ranked 5th in the wheat production, following Kansas, North Dakota, Washington, and Montana (“US States That Produce the Most Wheat”, 2018).

Wheat was initially popularized due to its unrivalled ability to grow in most areas especially in the northern hemisphere and Australia. It is adaptable across a range of environments and temperature while retaining the ability produce a high yield (Shewry, 2009). For example, hard red winter wheat can withstand colder climates, while hard red spring wheat is more heat tolerant. Wheat is also used for its foraging and cover crop potential as it leaves a high and rich residue on the surface of croplands.

Barley

Barley (*Hordeum vulgare*) is a cereal grain that is part of the grass family Poaceae. It widely varies in morphology and comes in many different varieties. Though it is cultivated as a crop, barley can be found growing naturally as wild grasses all over the US and primarily in the central – north to west. Barley is drought and cold tolerant and can be planted in alkaline soils, making it one of the easier and more versatile crops in the US. It is used mostly to make malts for alcohol consumption, but can also be used for other foods such as breads and soups. Additionally, barley is used in animal feed and grown out to maturity for seed production. The US ranks 10Th globally in barley production with around 2.56 metric tons grown in the year 2021 (“Leading barley producing countries worldwide 2022/23”, 2023). Idaho, Montana, North Dakota, and

Colorado lead barley production in the US (“US farming - top states with barley production”, 2019). In Oklahoma, barley production accounts or 1% of the margin in the US, with the majority grown for variety research and seed production. Since 2003, it has been experimented with being grown as an energy source known as ethanol.

Sorghum

Sorghum (*Sorghum bicolor*) is a cereal grain in the family Poaceae and is closely related to the popular, wild, Johnson grass (*Sorghum halepense).* Initially, Sorghum was a wild grass found in Africa. Soon, it became a harvestable crop that was modified and bred to become the cereal grain we know today. Sorghum has the ability to grow in arid climates as well as temperate and tropical areas. However, tropical grown sorghums are genetically modified to survive. Most sorghums planted in arid areas are drought tolerant and are able to grow in these areas and sometimes with irrigation systems. In some of these arid areas, sorghum is grown for traditional meals, food for other livestock, and for other conventional needs which utilizes the whole plant (Onono, 2018). On the commercial scale sorghum uses include human consumption, genetic modification and plant breeding, fodder, feed for livestock, and mass seed production (Prakasham et al., 2014). Sorghum is the 5^{th} most produced cereal grain in the world with the lead producers being India, Nigeria and the USA.

In the US, only 6 states grow sorghum as a crop. As of 2021the top three producers are Kansas, Texas and Oklahoma (“Leading barley producing countries worldwide 2022/23”, 2023). Sorghum is mostly produced between the Northcentral and

Southcentral states from South Dakota to Texas. In Oklahoma, sorghum is grown in the most western part of the state, primarily the panhandle ("Sorghum Explorer", 2023).

Millet

Millet (*Pancium* spp.) is in the family Poaceae. Many species are grown in arid or semi-arid areas such as Africa and India (Baryeh, 2002) as it is drought-tolerant/resistant, heat tolerant, and able to grow in low nutrient soils (Andrews and Kumar, 1992). These attributes make it a very useful crop in areas that cannot usually other grains (Saleh et al., 2013). The two main species of millet crop are pearl millet *(Pennisetum glaucum*) and finger millet (*Eleusine coracana*). These species are primarily used to feed mass populations in areas where food resources are not as accessible. Secondary to food production, millet is used as cover crops, forage for livestock feed, fuel and construction (Andrews and Kumar, 1992). Globally, millet is the 6th most produced cereal grain with Asia and Africa accounting for 97% of the world's total production (Kishor et al., 2021). Millet is not commonly grown in the US, however, a few small farms grow millet for special use and do not generate high yield. The states which grow millet are mostly located in central US and include Colorado, Nebraska, the Dakotas, and Minnesota. Minnesota buys and imports a few different species of millet for game feed and planting small fields for forage, sport hunting, and fowl feeding. However, Proso millet (*Pancium miliaceum* L.) and foxtail millet (*Setaria italic*) are the two most produced millets in the US ("Millets" 2022).

Cereal grains are cultivated all over the world for a variety of purposes and are modified to survive a range of environments and soils. However, the most important use of these grains are for seed testing in research facilities and stations. This testing gives us multiple combinations of, varieties, genotypes, or germplasms that can be integrated into the field as crops. The purpose of creating different varieties is to modify the grain's ability to withstand heat/cold, drought, pest/pesticide/disease resistance, rate of production, and to change the types of foods that can be made for both humans and livestock consumption (Lemerle et al., 1996). Some of the most revered crop genotypes worldwide are the ones which increase resistance to pests and disease. One of the most harmful pest to crop production is the aphid. This insect is highly adaptable and can infest a crop in a matter of days with exponential growth due to high fecundity. Aphid resistant grain genotypes are constantly being tested and advanced to keep up with new and invasive species of aphid.

Aphid Pests & Damage

Since the start of utilizing different plants for crop production pests have been a hindrance for farmers and control tactics keep evolving over time. From biological control to the start of pesticide usage, technology has further advanced the control of pests on valuable crops. One of these advancements were mostly found due certain genotypes making plants more resistant than others to a certain pest. These plants were then either bred or engineered with other genotypes to make crops that have more than one valuable asset such as drought tolerance. Cereal crops around the world do have a lot of pests or diseases to keep away, but some of the most prolific are insect pests that can also transfer disease. One of these insect pests that have destroyed crops around the world is the aphid. With over 450 species of aphids found in agriculture systems these insects have become infamous to researchers and crop producers alike (Emden and Harrington, 2017). With aphids being one of the most serious pests in crop production, the worldwide annual cost of controlling and loss from this pest is variable and hard to quantify (Perring et al., 2018). These varieties have specified names for each of the grains they have been modified or created for. In Oklahoma the grains that are produced for this task are listed below.

Some Aphid resistant varieties:

Wheat: Gallagher

Barley: Post 90

Sorghum: DKS, TX2783, Pioneer

Millet: Millex 32

Oklahoma is home to aphid species, which have been targeted with both with modified crops and pesticides. The most prolific of aphids are the Russian Wheat Aphid (RWA) (*Diuraphis noxia*), Sugarcane Aphid (SCA) (*Melanaphis sacchari*), and the Green Bug Aphid or Wheat Aphid (*Schizaphis graminum*).

Russian Wheat Aphid

The Russian Wheat Aphid (RWA) is an invasive species that was first discovered in the US in Texas around, 1986 ("Diuraphis noxia (Russian wheat aphid)", 2022). RWA originated from Russia where it feeds on wheat and other grasses bordering the crops. By 1988, RWA was found in 15 states in the US, feeding on wheat and barley crops (Kindler and Springer, 1989). Through further investigation, the RWA was discovered on multiple hosts, including both crops and numerous warm/cool season wild grasses around the US

(Kindler and Springer, 1989). This marked the first outbreak of the RWA in the US costing an approximate 800 million in both direct and indirect losses (Haley et al., 2004). Since the beginning of the outbreak, resistant genes in both barley and wheat were used as resilient crop varieties. The resistant genes were given the names D1-D6, with D-4 being one of the most effective. With the help of both natural biological control agents and pesticide usage the spread of RWA was slowed enough to implement the resistant crop varieties in fields. However, by the time it took new resistant genotypes to be implemented, new biotypes of RWA were already being discovered (Botha, Li, and Lapitan, 2005). By 2003-2004, a new RWA biotype was observed in Colorado, which was highly virulent on most, if not all, of the previous resistant genes (Haley et al., 2004). This was followed by a new outbreak. This has led to constant pressure on the new biotypes of host resistant wheat varieties.

RWA causes damage through a toxin that is injected into a host plant. This toxin can greatly reduce the plant rigidity, causing stunted growth and the wilting of leaves. This also leads to yellowing of the leaves and stems ("Russian wheat aphid (Diuraphis noxia)", 2022). It was observed that RWA infested plants have a reduction in growth rate and significant yield loss. Further, leafs become rolled making it difficult for topical pest control measures to reach them (Burd and Burton, 1992).

Sugarcane Aphid

The Sugarcane Aphid (SCA) is an invasive species of aphid primarily found on sugarcane, sorghum and some closely related wild grasses. In the 1970's, the SCA was identified in southern Texas on sugarcane with but was only regarded as a vector of disease which caused damage to these crops (Nibouche et al., 2018). By 2013, the SCA had spread through most of Texas and the other neighboring states attacking sorghum crops, and resulting in a mass economic loss (Nibouche et al., 2018). Because the aphid was not identified on sorghum sooner, there had yet to be resistant varieties of these crops planted, causing the spread to be faster and more devastating. After the outbreak, cross resistant tests were done between lines of sorghum which were also resistant to greenbug biotypes (Armstrong et al., 2015). These tests found that the SCA shared most of the susceptible lines with greenbugs carrying the resistant varieties TX2752/TX2783 (Brewer et al., 2017). After finding the cross resistance, researchers were able to continue using the genes from these plants to test and create more resistant varieties. Most of these varieties are still in study due to the length of time it takes for genotypes to be researched, created, and distributed.

SCA damage can be characterized by leaf and seeding discoloration, with tillers of sorghum becoming purple in color (Singh, Padmaja, and Seetharama, 2004). Most of the host plant's damage depends on the rate of colonization. SCA have a very high fecundity and a high population can lead to the plant becoming chlorotic and showing signs of necrosis (Singh, Padmaja, and Seetharama, 2004). With damages being primary on a plant's leaves and stems, the SCA also causes stunting and low yield production.

Green bug/Wheat Aphid

The greenbug is a cereal grain pest in US, which attacks most staple crops such as sorghum and wheat. The greenbug also feeds on other related wild grass in natural systems and on the edges of crops (Royer et al., 2015). It was first identified as a pest in the US in the late 1800's, feeding on both wheat and sorghum. The first recorded outbreak reported on sorghum was in the early 1900's (Royer et al., 2015). With over 70 different hosts primarily being wild grasses, control of the greenbug has through a crop resistant variety has proven difficult. Greenbugs have an extensive list of biotypes, with the most virulent being biotypes C, E, G, and H (Nicholson and Puterka, 2014).

Feeding damage from the greenbug is apparent on most host species. Damage is presented with a necrotic line, usually ranging from yellow to brown in color, forming down the middle of the leaf (Royer et al., 2015). Studies have shown that most biotypes of greenbugs have a toxin in their saliva that causes stunting and extreme loss in yield (Royer et al., 2015). A large population on one plant will cause death through the toxin or over feeding.

Hedgehog grain aphid

Sipha maydis Passerini (Hemiptera: Aphididae) is a small, black in color aphid, with white hairs protruding from most of the dorsal area (CABI, 2023). This aphid is a known cereal pest around the world specifically in Europe, South Africa, Asia, and the United States (Puterka et al. 2019). *S. maydis* was first found in the continental United States in Argentina in 2002 where it was found mainly living on wild grasses (44). First identification of *S. maydis* state side in the US was in California, 2007, and was classified and an invasive pest (Mornhinweg et al. 2020). From California, this aphid has been sampled around the eastern-central areas of the US consisting of Colorado, New Mexico, Wyoming, and Utah (Puterka et al. 2019). Though these were the states sampled, *S.*

maydis sightings have been found across the US in the states of Florida, Georgia, and South Carolina (Puterka et al. 2019).

S. maydis has not been found to be a consistent threat to the US agricultural system with many sightings only being on wild grasses in sparse locations (Puterka et al. 2019). However, in 2005 in Argentina an outbreak occurred in both commercial barley and wheat that quickly spread around the country (Corrales et al. 2007). Crops used that were cross-resistant to RWA and greenbug were not resistant to *S. maydis* and were needed quickly after to combat the growing invasive pest (Corrales et al. 2007). *S. maydis* is seen to feed on a wide variety of wild grasses and commercial cereal crops around the world. In a list made by Mahmood et al., 2002, there has been around 33 different species that are applicable hosts for this aphid species (Mahmood, Poswal, and Shehzad, 2002). In recent studies, however this list may need revision.

S. maydis is found to colonize these host plant in clusters on the upper and underside of tillers near the base and can be found sporadically around the stem and other tillers (CABI, 2023). Sexually mature aphids will mainly be found in small groups or alone near the tops of tillers or the host plant itself (CABI, 2023). In these clusters *S. maydis* feeds on either the tiller of the host plant or the stems causing yellowing of the plant tissues otherwise known as chlorosis. It has been observed that chlorosis can differ from plant species where a purple/red discoloration has been found on sugarcane or sorghum.

Aphid Biology & Pathogens

Aphids [Hemiptera: Aphididae] are one of the world's leading pests of agricultural crops with high fecundity and extensive damage that can be both direct and indirect. Aphids can either be monophagous or polyphagous feeders meaning they either feed on one host species or can move from one host species to the other while able to complete their life cycle. This feeding habit is one of the reasons that puts them at the top of the pest list for farmers. Around 100 species of aphids are of high significance in agricultural damage around the world (Dedryver et al. 2010).

Aphids have a piercing-sucking mouthpart used for probing and feeding on plants. The proboscis uses intercellular feeding in order to find the phloem veins under plant cells (De Vos et al. 2007). The phloem of a plant carries sugars and other nutrients downward through the leaves and to the rest of the plant, making it a prime target for aphid feeding. Feeding from this nutrient rich highway causes plants to lose significant photosynthates that are needed for correct and continuous plant growth (De Vos et al. 2007).

Due to intercellular feeding, aphids can cause a plethora of indirect damage to plants mainly in the form of disease transmission. Due to the intercellular feeding done by aphids multiple plant cells are probed during the process to find a phloem vein. During the probing of plant cells saliva is excreted along with any disease, the aphid might be carrying. This process can cause the rapid infection of a host plant from a number of viruses. Aphids transmit around 50% of the insect borne viruses known to infect plants (Dedryver et al. 2010). Well known virus families such as *Bromoviridae* and *Potyviridae* are just some of the viruses that can be transmitted by aphids (Ng and Perry 2004).

One aphid on a plant feeding on phloem and the photosynthates traveling through is not a significant loss to the plant. However, due to the aphids high fecundity rates one aphid can turn into tens or hundreds at a steady pace and doom a crop. Aphids are able to reproduce either sexually or asexually (parthenogenesis) (Simon et al. 2010). Parthenogenesis is the main reproduction strategy used in aphids unless there are strict environmental conditions. Sexually reproductive aphids will be found in late spring as day length shortens and are produced from sexuparae females (Simon et al. 2010). These sexuparae females will give produce male and female wingless aphids that will in turn mate with the female laying frost resistant eggs to endure the winter (Simon et al. 2010). However, aphid's high fecundity is mainly due to the reproduction process parthenogenesis.

Aphids cause multiple types of damage with the most prominent being chlorosis, or the discoloration of the fed on tissue of a host plant. This discoloration can vary among hosts with the most popular being an opaque yellow color. Other plant hosts such as sorghum (*Sorghum bicolor*) or related hosts will turn a dark purple color around the feeding site. This discoloration of the tissue may also spread due to a phytotoxic substance in the aphid's saliva (Riedell and Blackmer 1999). Not only will this toxin cause chlorosis but also can cause necrosis that can ultimately lead to death of the host plant (Dedryver et al. 2010). Phototoxic saliva is only one of the ways that aphids can cause damage to a host plant. From feeding on high nutrient content sources, aphids produce excrement known as honeydew that can form molds. The most well-known mold is sooty molds, a fungal disease that can hinder the plants photosynthetic responses and cause economic lose in crops (Dedryver et al. 2010).

REFERENCES

Andrews, D. J., and K. A. Kumar. **1992**. Pearl Millet for Food, Feed, and Forage, pp. 89–139. *In* Sparks, D.L. (ed.), Advances in Agronomy. Academic Press.

Awika, J. M. 2011. Major Cereal Grains Production and Use around the World, pp. 1–13. *In* Awika, J.M., Piironen, V., Bean, S. (eds.), ACS Symposium Series. American Chemical Society, Washington, DC.

Baryeh, E. A. 2002. Physical properties of millet. Journal of Food Engineering. 51: 39–46.

Botha, A.-M., Y. Li, and N. L. V. Lapitan. **2005**. Cereal host interactions with Russian wheat aphid: A review. Journal of Plant Interactions. 1: 211–222.

Brewer, M. J., J. W. Gordy, D. L. Kerns, J. B. Woolley, W. L. Rooney, and R. D. Bowling. **2017**. Sugarcane Aphid Population Growth, Plant Injury, and Natural Enemies on Selected Grain Sorghum Hybrids in Texas and Louisiana. Journal of Economic Entomology. 110: 2109–2118.

Burd, J. D., and R. L. Burton. **1992**. Characterization of Plant Damage Caused by Russian Wheat Aphid (Homoptera: Aphididae). Journal of Economic Entomology. 85: 2017–2022.

Corrales, C. E., A. M. Castro, M. Ricci, and A. F. G. Dixon. **2007**. Sipha maydis: Distribution and Host Range of a New Aphid Pest of Winter Cereals in Argentina. Journal of Economic Entomology. 100: 1781–1788.

De Vos, M., J. H. Kim, and G. Jander. **2007**. Biochemistry and molecular biology of Arabidopsis–aphid interactions. Bioessays. 29: 871–883.

Dedryver, C.-A., A. Le Ralec, and F. Fabre. **2010**. The conflicting relationships between aphids and men: A review of aphid damage and control strategies. Comptes Rendus Biologies, Les pucerons : modèles biologiques et ravageurs des cultures. 333: 539–553.

(Economy of Oklahoma including Oklahoma Agriculture and Manufacturing from NETSTATE.COM) . **2022**. Economy of Oklahoma including Oklahoma Agriculture and Manufacturing from NETSTATE.COM. (https://www.netstate.com/economy/ok_economy.htm#:~:text=Crops,are%20also%20grown%20in%20Oklahoma.).

Emden, H. F. van, and R. Harrington. **2017**. Aphids as Crop Pests, 2nd Edition. CABI.

(Global grain production, 2022) Statista. **2022**. Global grain production, 2022. (https://www.statista.com/statistics/271943/total-world-grain-production-since-2008-2009/).

(Grain Products Market Analysis, Size And Trends Global Forecast To 2022-2030) . **2022**. Grain Products Market Analysis, Size And Trends Global Forecast To 2022-2030. (https://www.thebusinessresearchcompany.com/report/grain-products-global-market-report).

Haley, S. D., F. B. Peairs, C. B. Walker, J. B. Rudolph, and T. L. Randolph. **2004**. Occurrence of a New Russian Wheat Aphid Biotype in Colorado. Crop Sci. 44: 1589–1592.

Kavi Kishor, P. B., S. Anil Kumar, J. Naravula, P. Hima Kumari, D. Kummari, R. Guddimalli, S. Edupuganti, A. R. Karumanchi, P. Venkatachalam, P. Suravajhala, and R. Polavarapu. **2021**. Improvement of small seed for big nutritional feed. Physiol Mol Biol Plants. 27: 2433–2446.

Kindler, S. D., and T. L. Springer. **1989**. Alternate Hosts of Russian Wheat Aphid (Homoptera: Aphididae). Journal of Economic Entomology. 82: 1358–1362.

(Leading barley producing countries worldwide 2021/22) Statista. **2022**. Leading barley producing countries worldwide 2021/22. (https://www.statista.com/statistics/272760/barley-harvest-forecast/).

(Leading U.S. states in sorghum production for grain 2021) Statista. **2022**. Leading U.S. states in sorghum production for grain 2021. (https://www.statista.com/statistics/191471/top-us-states-in-production-of-sorghum-for-grain/).

Lemerle, D., B. Verbeek, R. D. Cousens, and N. E. Coombes. **1996**. The potential for selecting wheat varieties strongly competitive against weeds. Weed Res. 36: 505–513.

(Millets) . **2022**. Millets. (https://hort.purdue.edu/newcrop/afcm/millet.html#:~:text=In%20the%20United%20States%2C%20both,of%20the%20seed%2C%20barnyard%20grass.).

Mornhinweg, D. W., G. J. Puterka, and J. S. Armstrong. **2020**. Resistance in Barley (Hordeum vulgare L.) to New Invasive Aphid, Hedgehog Grain Aphid (Sipha maydis, Passerini) (Hemiptera: Aphididae). American Journal of Plant Sciences. 11: 869–879.

Ng, J. C. K., and K. L. Perry. **2004**. Transmission of plant viruses by aphid vectors. Mol Plant Pathol. 5: 505–511.

Nibouche, S., L. Costet, J. R. Holt, A. Jacobson, A. Pekarcik, J. Sadeyen, J. S. Armstrong, G. C. Peterson, N. McLaren, and R. F. Medina. **2018**. Invasion of sorghum in the Americas by a new sugarcane aphid (Melanaphis sacchari) superclone. PLOS ONE. 13: e0196124.

Nicholson, S. J., and G. J. Puterka. **2014**. Variation in the salivary proteomes of differentially virulent greenbug (Schizaphis graminum Rondani) biotypes. Journal of Proteomics, Special Issue: Proteomics of non-model organisms. 105: 186–203.

Onono, P. A. 2018. Response of Sorghum Production in Kenya to Prices and Public Investments. SAR. 7: 19.

Perring, T. M., D. Battaglia, L. L. Walling, I. Toma, and P. Fanti. **2018**. Chapter 2 - Aphids: Biology, Ecology, and Management, pp. 15–48. *In* Wakil, W., Brust, G.E., Perring, T.M. (eds.), Sustainable Management of Arthropod Pests of Tomato. Academic Press, San Diego.

Poutanen, K. S., A. O. Kårlund, C. Gómez-Gallego, D. P. Johansson, N. M. Scheers, I. M. Marklinder, A. K. Eriksen, P. C. Silventoinen, E. Nordlund, N. Sozer, K. J. Hanhineva, M. Kolehmainen, and R. Landberg. **2022**. Grains – a major source of sustainable protein for health. Nutrition Reviews. 80: 1648–1663.

Prakasham, R. S., D. Nagaiah, K. S. Vinutha, A. Uma, T. Chiranjeevi, A. V. Umakanth, P. S. Rao, and N. Yan. **2014**. Sorghum biomass: a novel renewable carbon source for industrial bioproducts. Biofuels. 5: 159–174.

Puterka, G. J., R. W. Hammon, M. Franklin, D. W. Mornhinweg, T. Springer, S. Armstrong, and M. J. Brown. **2019**. Distribution of a New Invasive Species, Sipha maydis (Heteroptera: Aphididae), on Cereals and Wild Grasses in the Southern Plains and Rocky Mountain States. Journal of Economic Entomology. 112: 1713–1721.

Riedell, W. E., and T. M. Blackmer. **1999**. Leaf Reflectance Spectra of Cereal Aphid-Damaged Wheat. Crop Sci. 39: 1835–1840.

Royer, T. A., B. B. Pendleton, N. C. Elliott, and K. L. Giles. **2015**. Greenbug (Hemiptera: Aphididae) Biology, Ecology, and Management in Wheat and Sorghum. Journal of Integrated Pest Management. 6: 19–19.

(Russian wheat aphid (Diuraphis noxia) - DAWE) . **2022**. Russian wheat aphid (Diuraphis noxia) - DAWE. (https://www.awe.gov.au/biosecurity-trade/pests-diseases-weeds/plant/russian-wheat-aphid#:~:text=Russian%20wheat%20aphid%20has%20a,growth%20and%20loss%20of%20vigour.).

(Russian Wheat Aphid | National Invasive Species Information Center) . **2022**. Russian Wheat Aphid | National Invasive Species Information Center. (https://www.invasivespeciesinfo.gov/terrestrial/invertebrates/russian-wheat-aphid).

Saleh, A. S. M., Q. Zhang, J. Chen, and Q. Shen. **2013**. Millet Grains: Nutritional Quality, Processing, and Potential Health Benefits: Millet grains…. Comprehensive Reviews in Food Science and Food Safety. 12: 281–295.

Scott Armstrong, J., W. L. Rooney, G. C. Peterson, R. T. Villenueva, M. J. Brewer, and D. Sekula-Ortiz. **2015**. Sugarcane Aphid (Hemiptera: Aphididae): Host Range and Sorghum Resistance Including Cross-Resistance From Greenbug Sources. Journal of Economic Entomology. 108: 576–582.

Shewry, P. R. 2009. Wheat. Journal of Experimental Botany. 60: 1537–1553.

Simon, J.-C., S. Stoeckel, and D. Tagu. **2010**. Evolutionary and functional insights into reproductive strategies of aphids. Comptes Rendus Biologies, Les pucerons : modèles biologiques et ravageurs des cultures. 333: 488–496.

Singh, B. U., P. G. Padmaja, and N. Seetharama. **2004**. Biology and management of the sugarcane aphid, Melanaphis sacchari (Zehntner) (Homoptera: Aphididae), in sorghum: a review. Crop Protection. 23: 739–755.

(Sorghum Explorer) . **2022**. Sorghum Explorer. (https://ipad.fas.usda.gov/cropexplorer/cropview/commodityView.aspx?cropid=0459200).

(US farming - top states with barley production) Data Visualization Made Easy | Argon. **2019**. US farming - top states with barley production.

(https://www.getargon.io/posts/economy/agriculture/us-farming/top-states-barley-production/).
(US States That Produce the Most Wheat) WorldAtlas. **2018**. US States That Produce the Most Wheat. (https://www.worldatlas.com/articles/leading-wheat-producing-states-in-the-united-states.html).
(Wheat Production by Country 2022) . **2022**. Wheat Production by Country 2022. (https://worldpopulationreview.com/country-rankings/wheat-production-by-country).

CHAPTER II

DISTRIBUTION RANGE OF A NEWLY INVASIVE SPECIES *SIPHA MAYDIS* [HOMOPTERA: APHIDIDAE] IN THE ROCKY MOUNTAIN RANGE.

Abstract

An introduced cereal grain aphid, *Sipha maydis* Passerini (Hemiptera: Aphididae), has established in the western United States of America. The species was described from individuals collected on many different cool and warm season grasses in Italy (Puterka et al., 2019). It develops on approximately 28 different grass host plant species (Mahmood, Poswal, and Shehzad, 2001). Although its native range has not been determined, is likely native to Europe and Northern Africa. It has spread to most parts of the world including South Africa and North and South America ("Invasive Species Compendium", 2022). The first recorded introduction of *S. maydis* to the Americas was in Argentina in 2002. Although this species was found mainly on wild grass around the area, damage to cereal crops soon occurred (Corrales et al., 2007). Similar to the other introduced aphids such as the Russian wheat aphid (*Diuraphis noxia*), Sugarcane Aphid (*Melanaphis sacchari*), and Greenbug (*Schizaphis graminum*), initial damage and losses can be severe. In this study researchers went to the western Rocky Mountain and Great Plains States Colorado, New Mexico, Oklahoma, Utah, and Wyoming to sample and determine *S. maydis* population

and distribution. Sampling occurred mainly on the side of roadways and in agricultural croplands. *S. maydis* was only found to occur in Utah and Colorado.

Introduction

The small grain hedgehog aphid has the potential to cause extensive damage to cereal crops. During feeding, *S. maydis* injects phytotoxic saliva to breakdown plant materials and defense proteins causing damage to spread further than just then feeding site (Puterka et al., 2019). Feeding by *S. maydis* causes discoloration on plant hosts, including chlorosis (yellowing) and has been noted to cause purpling of leaves in sorghum. This species can form large, closely packed, groups on the underside of plant tillers that rapidly cause damage (Puterka et al., 2019). In addition to causing direct feeding damage, *S. maydis*, has the ability to spread viruses among plant hosts (Katis et al., 2006). The most serious of these viruses is the barley yellow dwarf virus (BDYV) that can cause severe losses to wheat and barley (Miles, 1999).

In 2007, *S. maydis* was first identified on giant wild rye in California, U.S.A. (Puterka et al., 2019). Following this report, it was detected in several other states on a variety of cereal grain and wild grasses. In 2019, sampling was conducted in Texas, New Mexico, Oklahoma, Colorado, Wyoming, and Utah (Puterka et al., 2019). This species was detected in WY, UT, CO, and NM (Puterka et al., 2019), although large populations and economic losses have not been reported. We conducted field surveys in 2021 and

2022 in WY, NM, CO, OK, and UT to characterize distribution and hostplant use as well as analyzing genetics among populations.

Materials and Methods

Field sampling was conducted in 2021 and 2022 to determine if the distribution of *S. maydis* has changed since surveys during 2015-2017 (Puterka et al. 2019). Sites in Oklahoma (OK), New Mexico (NM), Colorado (CO), Utah (UT), and Wyoming (WY) were where surveys were conducted. In OK, we focused on sites in the Northwest region. Sites in NM were mainly in the Northeast and North Central regions. Sampling occurred throughout CO except in the Eastern area near Trinity and Grand Junction. The East and central part of UT was sampled mainly along the CO boarder. The Southcentral and Eastern areas of WY were sampled.

Sites were chosen based on previous records and from similar sites. Areas that were sampled included roadsides with tall grasses, agricultural fields (cereal grains including wheat, sorghum, and barley), areas around USDA research station, residential areas, and previous positive areas (Puterka et al. 2019). At each site, temperature, altitude, and weather conditions were noted.

Sites were sampled with two different distinct methods. These methods included hand searching and sweep netting areas. Hand searching was the main way of sampling, as the sweep net was mostly used for large, over grown areas. Hand searching was conducted by 2-3 individuals spread out 10-20 feet from another (if the area would allow for spread out searching i.e. roadsides). Tillers were searched approximately every 5-10 feet depending on area of search. Large fields were searched in a longer more spread formation to cover more area. Areas that were searched with a sweep were used in a straight line through a 10-15 foot section with 180° sweeps from side to side. These swept areas were done in one direction out into the densest area and then brought back for inspection.

When a positive sample was found during hand searching the aphid(s) were collected on the host plant tissue and placed in a petri dish. There was extra plant material placed in the petri dish along with filter paper soaked in water to prevent desiccation. On the petri dish information such as date, location, and plant, host species was written. These petri dishes were then placed in a cooler to further prevent desiccation and to absorb some shock from travel. At the lab these samples were then given a certain number correlating with the data sheet and placed on a host plant to maintain the colony for future testing.

In the data set collected, there were a total of 59* sites sampled combing the two sampling trips from 2021 to 2022. In May 2021, there was a total of 19 sites found to be positive and an unknown amount (that I will know soon) to be negative. In April 2022, there was 40 sites with 13 found to be positive and 26 found to be negative. From these data points, information such as latitude and longitude, altitude, county or nearby landmark, positive or negative results, flora genus species and common name, and in some cases a picture was taken at each site. From these sites there was around 20* different species of grasses and cereal sampled with 13 found to be positive hosts as well as a new host plant species found in CO. All of these data points were placed in both Google Earth and Gaia.GPS systems for easy to find points and picture access. Other data was placed into an Excel format as seen in Table 1. The reason these were placed into mapping systems is to compare the distribution of this study to the previous data.

Results

Sampling Trip 2021

S. maydis were found in small to large groups usually in the middle areas of grass patches or under the tillers. In some areas, *S. maydis* was found to be tended by ants, but with no indication of any viral transmission or parasitoid activity. None of the colonies that were found in either year were found on cereal crops. All *S. maydis* found and collected were on wild grasses in the areas that were sampled even species closely related to cereal crop targets. CO was the only state that had any positive accounts of *S. maydis* in a centralized area. However, during this trip UT was not sampled as it was in the 2022 sampling trip. All *S. maydis* was found on wild grasses and not found on any cereal crops. Grand junction, CO is mainly a cold semi-arid climate, but during the sampling season, temperatures were in the high 80's during the day and 50's during the night.

Since *S. maydis* was found in only one area and state the areas were broken down in this section of the methods.

In May, the first sighting of *S. maydis* was found at the Colorado State University Extension Office, Grand Junction, CO. Five different positive sites were checked with altitudes ranging from 4,666 to 4,653 feet. These were found on a combination of Foxtail Barley (*Hordeum jubatum*), Downy Brome (*Bromus tectorum*), and Broad leaf. There were two other accounts found in Fruita, CO at another research station containing fruit trees. The two wild grasses colonies were found on were Smooth Brome (*Bromus inermis*) and Hair Barley (*Hordeum murinum*). Altitudes ranged from 4,607 to 4,619 ft. between the two points. This site was seen to have a one colony of *S. maydis* being tended by ants. Two more points were found positive in Orchard Mesa, CO in an area with crop research being conducted. *S. maydis* however was found on wild grasses within these test crop plots. Wild grasses that were found to be positive were Smooth Brome (*Bromus inermis*). Altitudes ranged from 4,692 to 4,697 ft. There were six points found in Palisade, CO in a residential area and in the town park. In the residential area, *S. maydis* was found in a driveway on Downy Brome (*Bromus tectorum*) and Hare Barley (*Hordeum murinum*). Altitude in the residential area was 4,735 ft. In Palisade Park there were *S. maydis* colonies found near a water bank and further inland in the park. Altitudes for this area ranged from 4,692 to 4,697 ft. and relatively close together. Wild grasses these colonies were found on were an Unknown species, Hare barley (*Hordeum murinum*), Stink Grass (*Eragrostis cilianensis*), and Jointed Goat Grass (*Aegilops* *cylindrica*). The last area sampled was a residential area of a fellow researcher with four positive points. The altitudes of these points ranged from 4,777 to 4,800 ft. both next to a water system and in drier areas of the property. Colonies were found on Tall Wheat Grass (*Thinopyrum ponticum*), Hare Barley (*Hordeum murinum*), and Smooth Brome (*Bromus inermis*).

There were no positive sampling points in the other three states, NM, OK, and WY, looking at the same positive host plants and cereal crops. Negative samples were still recorded as well as the plants that were sampled during both years. These points were placed on a map to better record the distribution of *S. maydis*.

Sampling Trip 2022

During the April 2022 study, *S. maydis* were found in previous areas sampled in CO. There were positive sites found around the Grand Junction Colorado State University research station in the same areas on the same species of grass. *S. maydis* was also found in Southwest CO on both private farms and in random roadside areas. *S. maydis* colonies were not found on any cereal crops or other agricultural crops but were found mostly on wild grasses. There was one county record during this sampling trip where a colony was found on Bottle Brush Squirrel Tail (*Elymus elymoides*) next to a home in a residential area. *S. maydis* was found to be tended by ants in this area as well.

S. maydis were also found the in the Eastern areas of UT along roadsides and in a large-scale agricultural crops on wild grasses. In Northeast UT the only areas that *S. maydis* were found was along highway 128 towards Moab, UT at altitudes of 4,115 ft. on wild grasses. Other points were found in Southeast UT near Horse Head Point at altitudes of 6,520 to 6,559 ft. on wild grasses in private cropland. There were no other anomalies found in this states sampling areas.

For the 2022 sampling study there were no positive sampling points for the other 3 states, NM, OK, and WY. Just as the other sampling trips points sampled that were negative had been sampled once before or new sites that were found while traveling. The negative sites were again plotted and plant species were noted. The plotted points are placed into a mapping system with the positive sites to see relative distribution.

State	Elevation	Site #	Area	Date	Latitude	Longitude	Host	
CO	4,666	1	CSU Extension Office	12-May-21	39.034967	-108.539764	Fox Tail Barley	*Hordeum jubatum*
CO	4,666	2	CSU Extension Office	12-May-21	39.034967	-108.539765	Fox Tail Barley	*Hordeum jubatum*
CO	4,663	3	CSU Extension Office	12-May-21	39.03509	-108.5401	Downy Brome	*Bromus tectorum*
CO	4,656	4	CSU Extension Office	12-May-21	39.0354	-108.5401	Downy Brome	*Bromus tectorum*
CO	4,653	5	CSU Extension Office	12-May-21	39.03743	-108.54075	Broad Leaf	
CO	4,619	6	CSU Fruita RS	12-May-21	39.18562	-108.69679	Hare Barley	*Hordeum murinum*
CO	4,607	7	CSU Fruita RS	12-May-21	39.18243	-108.6983	Smooth Brome	*Bromus inermis*
CO	4,779	8	Orchard Mesa	12-May-21	39.04243	-108.46706	Smooth Brome	*Bromus inermis*
CO	4,768	9	Orchard Mesa	12-May-21	39.04168	-108.4696	Smooth Brome	*Bromus inermis*
CO	4,692	10	Palisade Park	12-May-21	39.10164	-108.35833	Unknown	
CO	4,697	11	Palisade Park	12-May-21	39.10144	-108.35823	Hare Barley	*Hordeum murinum*
CO	4,692	12	Palisade Park	12-May-21	39.10165	-108.35886	Stink Grass	*Eragrostis cilianensis*
CO	4,697	13	Palisade Park	12-May-21	39.10144	-108.35806	Jointed Goat Grass	*Aegilops cylindrica*
CO	4,735	14	Town of Palisade	12-May-21	39.10576	-108.35357	Hare Barley	*Hordeum murinum*
CO	4,735	15	Town of Palisade	12-May-21	39.10575	-108.35355	Downy Brome	*Bromus tectorum*
CO	4,800	16	Bob's House	12-May-21	39.05222	-108.43755	Tall Wheat Grass	*Thinopyrum ponticum*
CO	4,780	17	Bob's House	12-May-21	39.05282	-108.4387	Hare Barley	*Hordeum murinum*
CO	4,787	18	Bob's House	12-May-21	39.05241	-108.43842	Smooth Brome	*Bromus inermis*
CO	4,777	19	Bob's House	12-May-21	39.05265	-108.4388	Hare Barley	*Hordeum murinum*
CO	4,679	N/A	CSU Extension Office	26-Apr-22	39.03418	-108.53801	Hare Barley	*Hordeum murinum*
UT	4,115	20	Dewey Bridge	27-Apr-22	38.81104	-109.30872	Downy Brome	*Bromus tectorum*
UT	4,115	21	Dewey Bridge	27-Apr-22	38.81104	-109.30872	Hare Barley	*Hordeum murinum*
UT	6,520	22	Horse Head Point	27-Apr-22	37.70163	-109.17998	Crested Wheat Grass	*Agropyron cristatum*
UT	6,559	23	Horse Head Point	27-Apr-22	37.6842	-109.18934	Downy Brome	*Bromus tectorum*
UT	6,559	24	Horse Head Point	27-Apr-22	37.6842	-109.18934	Crested Wheat Grass	*Agropyron cristatum*
CO	6,467	25	Walter's Farm	28-Apr-22	37.52468	-108.93067	Hare Barley	*Hordeum murinum*
CO	6,346	26	Walter's Farm	28-Apr-22	37.51423	-108.94921	Mutton Grass/Bulbous Blue Grass	*Poa bulbosa*
CO	6,403	27	Walter's Farm	28-Apr-22	37.72623	-108.74938	Hare Barley	*Hordeum murinum*
CO	6,346	28	Walter's Farm	28-Apr-22	37.51809	-108.93982	Crested Wheat Grass	*Agropyron cristatum*
CO	6,467	29	Walter's Farm	28-Apr-22	37.71376	-108.79983	Smooth Brome	*Bromus inermis*
CO	4,782	30	Bob's House	28-Apr-22	39.0528	-108.43861	Hare Barley	*Hordeum murinum*
CO	4,782	31	Bob's House	28-Apr-22	39.0528	-108.43861	Annual Wheat Grass	*Eremopyrum triticeum*
CO	4,782	32	Bob's House	28-Apr-22	39.0528	-108.43861	Bottle Brush Squirrel Tail	*Elymus elymoides*

Table 1: Positive points found from both the 2021 and 2022 distribution studies. State, altitude, area, latitude and longitude, and both scientific name and common name are stated in this chart. Positive points are placed in order of site number with one not being collected but found to be negative.

Discussion/Conclusion

S. maydis do not follow a direct correlation of dispersion patterns especially since the first known discovery in 2007 in California. The two states that *S. maydis* was found out of the five were CO and UT. In these areas there was heavy infestation on host plants. Though most plants were not commercial cereal crops, the wild grasses that were documented are closely related. However, there could be a different biotype in the future that would make it possible for *S. maydis* to target cereal crops. This has been seen in other invasive aphid species such as the Russian Wheat Aphid (*Diuraphis noxia*). During this study there was a new county record with *S. maydis* being found on a new species of host plant in CO. This new host will be added to the list of wild grass hosts that *S. maydis* has been found on.

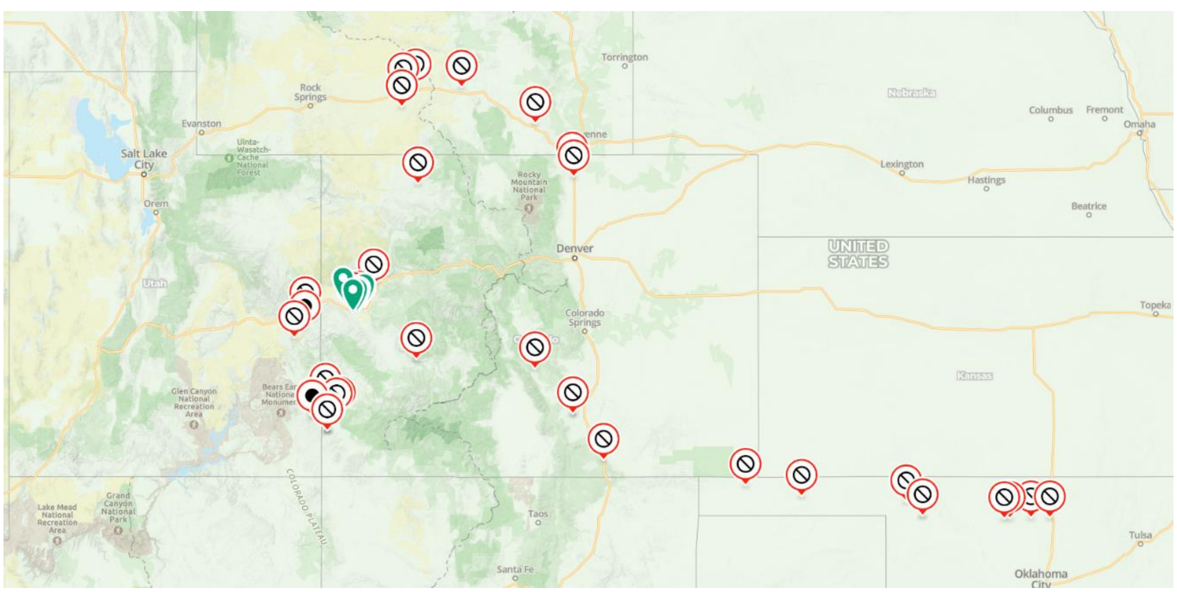


Image 1: Total points from both the 2021 and 2022 distribution studies. Green map markers are positive sights from the 2021 study. Red map markers are from the study in 2022. The makers with a circle and line are points found negative and filled circles are found positive.

Other observations that are notable were the ant tending lines found in colonies. In a book by Blackman and Eastop (2000), ant tending is a normal find when observing the *S. maydis* colonies in nature. A more in-depth paper for this information is from Addicott (1979), describing several species of ants found in the Rock Mountain area of CO doing the same thing. The reason for this aphid tending behavior would be for the ants to have sustained nutrients off the honeydew produced. In return, the ants provide the aphids with protection from natural enemies.

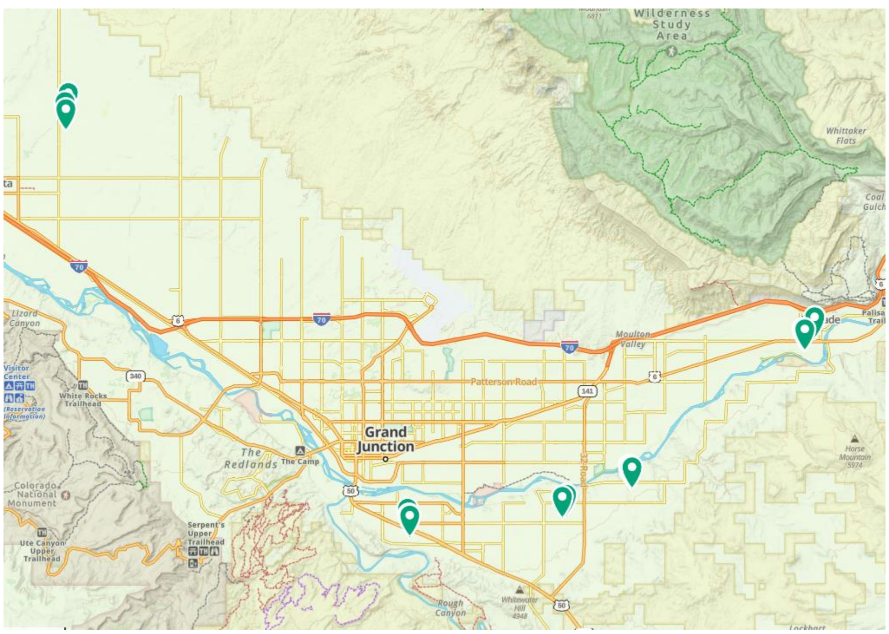


Image 2: Positive sites found in 2021 distribution study around the Grand Junction, CO area.

Sites that were used in the two-year period of 2021 and 2022 had a variety of sites. Though some sites were used from the past study, there were also positive sites that were not rechecked in this study. There were only 1-2 sites in NE NM found to be negative, where as positive sites further south were not checked. This was also the case in

WY with sites further north of new sites were not checked or found to be positive. This could skew the distribution data with a smaller radius used in this study, but will be or should be checked in future studies. Other possibilities for the negative results in WY

could be due to a snowstorm during the sampling time.

Images 3 & 4: Negative (left image) and positive (right image) site found during the 2022 distribution study in the Rocky Mountain States.

In summary, from the distribution data collected it can be determined that there is no cause for concern this aphid will become a highly invasive pest in the US. Though there is no immediate threat, studies should continue to keep relevant data on dispersion. Compared to other studies showing the drastic change in land area inhabited such as Greenbug wheat aphid and Russian wheat aphids, the movement is not seen as a threat at this time. However, there has been cases in Argentina that would suggest otherwise as *S. maydis* was found to take over wheat fields with great intensity. This brings to light that there might be new biotypes found in the US in the next coming years that could prove to be a problem as seen in other species. There should be continuing of studies in this manner to keep an eye on this species as they are a possible threat to the cereal agriculture economy.

REFERENCES

Addicott, J. F. 1979. A multispecies aphid–ant association: density dependence and species-specific effects. Can. J. Zool. 57: 558–569.

Blackman, R.L., Eastop, V.F., 2000. Aphids on the world's crops: an identification and information guide. John Wiley & Sons Ltd.

Corrales, C. E., A. M. Castro, M. Ricci, and A. F. G. Dixon. **2007**. Sipha maydis: Distribution and Host Range of a New Aphid Pest of Winter Cereals in Argentina. Journal of Economic Entomology. 100: 1781–1788.

Goggin, F. L. 2007. Plant–aphid interactions: molecular and ecological perspectives. Current Opinion in Plant Biology. 10: 399–408.

(Invasive Species Compendium) . **2022**. Invasive Species Compendium. (https://www.cabi.org/isc).

Katis, N. I., J. A. Tsitsipis, D. P. Lykouressis, A. Papapanayotou, J. T. Margaritopoulos, G. M. Kokinis, D. Ch. Perdikis, and I. N. Manoussopoulos. **2006**. Transmission of Zucchini yellow mosaic virus by Colonizing and Non-colonizing Aphids in Greece and New Aphid Species Vectors of the Virus. J Phytopathol. 154: 293–302.

Mahmood, R., M. A. Poswal, and A. Shehzad. **2001**. Distribution, Host Range and Seasonal Abundance of Sipha Sp. (Homoptera: Aphididae) and Their Natural Enemies in Pakistan. Pakistan J. of v Biological Sciences. 5: 47–50.

Miles, P. W. 1999. Aphid saliva. Biological Reviews. 74: 41–85.

Puterka, G. J., R. W. Hammon, M. Franklin, D. W. Mornhinweg, T. Springer, S. Armstrong, and M. J. Brown. **2019**. Distribution of a New Invasive Species, Sipha maydis (Heteroptera: Aphididae), on Cereals and Wild Grasses in the Southern Plains and Rocky Mountain States. Journal of Economic Entomology. 112: 1713–1721.

CHAPTER III

EFFECT OF TEMPERATURE AND SUPERCOOLING POINTS ON *SIPHA MAYDIS* PASSERINI (HETEROPTERA: APHIDDIDAE)

ABSTRACT

The impact of temperature on the development, survival and reproduction of the hedgehog grain aphid (HGA), *Sipha maydis*, is crucial for understanding its population dynamics, potential distribution and management strategies. In this study, we investigated the effects of different temperatures on demographic parameters of HGA and determined the supercooling point (SCP) for first instar nymphs, apterous, and winged adults. Our findings revealed that temperatures between 20°C and 25°C were optimal for HGA development and reproduction, leading to rapid population growth on wheat. However, HGA development is hindered below and above the temperature thresholds of 10°C and 35°C, respectively. The SCP was statistically similar among life stages with -16.55 ± 2.04°C for nymphs, -16.25 ± 0.45°C for apterous adults and -15.30 ± 0.46°C for winged adults. By expanding our knowledge of temperature-dependent development, reproduction and mortality of *S. maydis*, we can better predict and manage future HGA populations.

INTRODUCTION

The hedgehog grain aphid (HGA), *Sipha maydis* Passerini (Hemiptera: Aphididae), is a cereal pest in many regions of the world. It feeds on a variety of wild grasses and cereal crops and has been documented to use 52 plant host species worldwide (Puterka et al., 2019). In the United States, it was first identified in 2007 in California, feeding on giant wild rice *Leymus condensatus* (Sorensen and Center, 2007). Since this first observation, *S. maydis* has been monitored because of its capacity to cause damage to crops. Damage occurs in two ways: through direct injury caused by its feeding habits, and by transmitting cucumber mosaic *cucumovirus* and barley yellow dwarf *luteovirus* (Blackman and Eastop, 2000; Wieczorek and Bugaj-Nawrocka, 2014).

Because of unique aspects of aphid biology, particularly their cyclic parthenogenesis reproduction and dispersal strategies (through active or passive flight), controlling pest species of aphids poses a significant challenge (Wieczorek and Bugaj-Nawrocka, 2014). Moreover, the extensive distribution and diverse host range of *S. maydis*, combined with its presence in the field during both the cereal seedling stage and the maturation stage, make controlling this pest especially challenging (Corrales et al., 2007).

Temperature plays a crucial role in determining aphid populations, as external environmental conditions influence all aspects of their biology. The impact of temperature on aphid fitness can be observed through reproduction, growth, survival, and rate of population increase (Sun et al., 2022; Wang et al., 2021). There is an optimal temperature in which the aphids can reach the highest rate of population increase, and this temperature varies depending on the species of aphid and the origin of the population (Sun et al., 2022).

Knowing the optimal temperature, as well as thresholds for survival, and reproduction of HGA, is essential for predicting future outbreaks and determining potential distribution limits. In addition, documenting the supercooling point (SCP), can allow prediction of HGA distribution, versus areas where recolonization determines presence. The SCP is defined as the temperature at which a liquid, like the insect's body fluids, transitioning into a solid state or crystallizing and the heat of crystallization is released (Armstrong et al., 1998; Lee, 2010). Ice formation within cells causes damage, and if a significant number of cells are affected, the insect will not survive upon thawing.

Thus, the objectives of this study were to investigate the effects of temperature on the development and reproduction of HGA, and to determine the SCP for first instar nymphs, as well as apterous and winged HGA adults.

MATERIALS AND METHODS

Aphid Culture

The *S. maydis* specimens utilized in both experiments were obtained from Taos, New Mexico in 2019 and were kept on "Jagger" wheat in 4.4-liter pots, which were fitted with Lexan™ cylinders (45 cm tall x 16 cm diameter; SABIC Polymershapes, Tulsa, OK) and organdy cloth tops to prevent the escape of aphids. The aphid colonies were maintained in a biologically secure laboratory located on the premises of the USDA-ARS station in Stillwater, OK to prevent any external contamination, as *S. maydis* had not been documented in wild populations in Oklahoma (Puterka et al., 2019). To ensure the colony's health and survival, aphids were transferred to new plants every week or as necessary. The laboratory was maintained at a consistent temperature of 21±2°C and 14:10 L:D photoperiod with lighting provided by seven TS 32W Ecolux® daylight fluorescent lamps (Fairfield, Connecticut, USA).

HGA Development Under Different Temperatures

Six identical growth chambers (Percival® Model E30B, Perry IA) that provide temperature, light, and humidity control were used in this experiment. Each growth chamber was considered a treatment, and in each was set to a temperature of 10, 15, 20, 25, 30 and 35°C with the same photoperiod of 14:10 L:D, provided by a light rack equipped with eight, fluorescent grow lights (Philips Inc., Guadalajara, Mexico).

The wheat "Jagger" used in this experiment was planted in Cone-tainers™ (model SC10; S7S Greenhouse Supply, Tangent, OR) containing three layers of media: potting soil, fitting clay, and sand (bottom to top, respectively). Each Cone-tainer™ was fit- ted with an 8-cm-diameter Lexan sleeve, 45 cm in height and ventilated with organdy cloth. After the plant reached approximately 5 cm of height, 10 sexually mature adult aphids were placed on each plant using a horsehair paintbrush.

Aphids were left on plants for 24 hours to produce offspring. After this period, all aphids except for one, 1st instar, were removed from the plant. The remaining aphid was checked every 24 hours until reproduction, to estimate the pre-reproduction period (d). After the aphid has become sexually mature, each nymph produced was counted and removed. Data were collected every 24-h, until the death of each female. Each plant with a single female was considered a replication, and there were twelve replications for each temperature (treatment).

Supercooling Point Experiment

To determine the supercooling point (SCP) of the HGA, individual was placed in a 30-gauge, copper-constant thermocouple coated with a thin layer of petroleum jelly (Walmart Inc, Bentonville, AR). The thermocouple was attached to a CR12x micrologger (Campbell Scientific, Logan, UT) and placed inside a Pyrex® test tube measuring 2.5 x 15 cm. The test tube was held erect and partly submerged in a bath containing dry ice (≈0.5 kg) and 70% ethyl alcohol (≈0.75 L) following the methodology described by Armstrong et al. (1998). The bath temperature was maintained between -30°C and -35°C, and the exposure was done through vertical movement of the thermocouple down into the test tube. The temperature of the thermocouple was recorded every 0.2 seconds using a data logger. The lowest body temperature of each individual was identified by plotting its temperature time series, and determining the release of the latent heat during crystallization, also known as the SCP. Individuals of each developmental stage (1st instar nymphs, apterous adults, and winged adults) were used in the study, and each individual was considered as one replication. A total of twelve supercooling points were measured for each developmental stage.

Statistical Analysis

All trials were carried out in a completely randomized design. The parameters evaluated for development were: fertility, longevity (days), pre-reproductive time (d), number of progeny produced (Md) and intrinsic rate of increase (rm) calculated from the equation: rm = 0.74 (logeMd)/d (Wyatt and White, 1977). For the SCP experiment, the super cooling points of each individual were averaged and compared by life stage.

Analyses were performed in the R computing environment, using the "AgroR" package (Shimizu et al., 2022) and graphs were created using the "ggplot2" package (Wickham, 2016). Data were submitted to exploratory analysis to verify the assumptions of normality of residuals (Shapiro and Wilk, 1965) and homogeneity of variances (Burr and Foster, 1972) prior to the means analyzed using ANOVA (a = 0.05). Post-hoc Tukey tests were used when significance was identified.

RESULTS

HGA Development Under Different Temperatures

Temperatures of 10ºC and 35ºC did not allow the development for *S. maydis* until reproduction. Development and reproduction occurred among the temperatures of 15°C, 20°C, 25°C and 30°C (Table 1). Aphids reared under temperatures of 25°C and 30°C had similar pre-reproductive period (d; $p < 0.001$, $F=175.64$, $DF_{residuals}= 41$) requiring approximately 10.5 days to produce their first offspring (Figure 2). At temperature of 25°C the females showed the highest intrinsic rate of increase (rm) of 0.245 ± 0.011, and those reared under 15°C showed the lowest rm, with 0.085 ± 0.005 (Figure 5).

Females at 30°C exhibited a significant reduction in offspring production during the duration of d (Md), producing approximately one-third the offspring compared to those at 20°C and 25°C. Furthermore, these females demonstrated the lowest fertility, producing a markedly smaller number of offspring throughout their lifespans, approximately one-sixth that of females maintained at 20°C and 25°C (refer to Table 1). Moreover, females at 30°C experienced the lowest longevity among the viable temperature conditions, with a mean lifespan of 18.50 ± 1.70 days (Table 1).

Supercooling Point

The supercooling point (SCP) for the HGA was similar among tested life stages. Nymphs and apterous adults were -16.55 ± 2.04°C and -16.25 ± 0.45°C respectively, while winged adults had a SCP of -15.30 ± 0.46°C (Figure 1). The differences were not statistically significant ($p=0.758$, $F= 0.278$, $DF_{residuals}= 57$).

DISCUSSION

In the present study, we investigated the effects of temperature on the development, reproduction, and life history of the HGA, *S. maydis,* as well as determined the supercooling points for various developmental stages. Our findings contribute to a deeper understanding of the temperature-dependent life history traits of this aphid species and shed light on its potential range and survival in temperate areas in the U.S.

We found that temperatures of 10°C and 35°C did not allow reproduction. Although HGA is known from a wide range of regions, hosts and edaphoclimatic conditions (Corrales et al., 2007; Wieczorek and Bugaj-Nawrocka, 2014), during the winter period, the HGA population becomes scarce (Corrales et al., 2007), possibly because of its long exposures to low temperatures (Renault et al., 2002).

The temperature of 30°C was also highly detrimental to HGA development, survival, and reproduction. Previous aphid studies have found that at this temperature, the fitness of species such as the English grain aphid *Macrosiphum avenae* (Elliott et al., 1988), and corn aphids *Sitobion avenae* and *Metopolophium dirhodum* (Asin and Pons, 2001) was considerably affected, with some experiencing 100% mortality.

Prolonged exposure to temperatures below and above their thermal tolerance can result in cold and heat stress for aphids. This can adversely impact their metabolism, energy reserves, and subsequently lead to reduced growth and reproductive rates. Additionally, temperatures outside the optimal range can cause higher mortality rates and ultimately contribute to a decrease in populations (Chown et al., 2004; Colinet et al., 2015; Renault et al., 2002).

Our findings indicate that temperatures between 20°C and 25°C are most suitable for HGA development and reproduction. Within this range, HGA females had higher fertility, longevity, number of progeny (Md) and intrinsic rate of natural increase (rm), as well as lower pre-reproductive time (d). These demographic parameters suggest that within this temperature range, HGA has a faster life cycle and can reproduce rapidly, potentially leading to large infestations in a short amount of time and potentially causing damage to crops. These results can aid researchers in developing a predictive model for its distribution. On a farm-level, these models can also help predict potential populations and identify the appropriate time to adopt management strategies aimed at minimizing crop loss should such treatments become necessary.

Determination of the SCP also aids in modeling distribution and minimum survivable temperatures. At the SCP, the hemolymph in an insect's body undergoes a phase change from liquid to solid forming ice crystals within its cells and tissues (Armstrong et al., 1998; Armstrong and Nielsen, 2000; Lee, 2010). Although some groups of insects are able survival even at temperatures below the SCP (Chown et al., 2004; Renault et al., 2002), the formation of ice in tissues is usually lethal, as it causes cellular damage and disrupts physiological processes.

The SCP is determined by an organism's inherent traits, such as body composition, which is closely related to its physiological state (e.g., feeding status, diapause, life stage, metamorphosis) (Renault et al., 2002). Our results indicate that 1st instars, apterous adults and winged adults of HGA have equivalent SCPs, despite their physiological differences.

Under field conditions, however, HGA employs various overwintering tactics to improve its survival, even when temperatures fall below its SCP. One strategy involves seeking refuge under a protective layer of snow, which insulates the aphids from severe cold temperatures. Furthermore, a mutualistic relationship between HGA and ants can contribute to the aphids' survival during winter. Ants gather HGA nymphs and adults and carry them to their subterranean nests, shielding them from harsh winter conditions (Way, 1963).This symbiotic relationship aids in the survival of both species throughout the winter months and positively impacts their respective populations.

It is also important to consider the limitations of this study, including the focus the controlled laboratory conditions. We determined the effects of temperature on life history using simple growth chambers for each temperature rather than the replicated chambers. Previous studies (Higley et al.) have demonstrated potential limitation of this approach and additional assessment and refinement of temperature requirements is warranted. In addition, tests of HGA on different grass hots under different temperatures is warranted.

Future research should address these limitations by investigating the impact and interactions of other abiotic factors, the influence of different plant hosts (Sarkar et al., 2022; Souza et al., 2019), the differences between HGA individuals reared in the laboratory and those found in the field (Armstrong and Nielsen, 2000), as well the potential differences between HGA populations (Sun et al., 2022).

The study of SCP has several practical implications, such as for studying the insect's distribution and population fluctuation (Bale, 1987; Vrba et al., 2022). It is also important for pest management programs. For example, the adoption of the bacteria *Pseudomonas syringae* as a management tool can lower aphids' SCP, thereby reducing the temperature at which they freeze (Armstrong et al., 1998). Additionally, the use of the active fungus *Fusarium* sp. Has been effective for larvae of the rice stem borer (Lee, 2010).

REFERENCES

Armstrong, J.S., Lee, R.E., Peairs, F.B., 1998. Application of ice-nucleating active bacteria decreases the supercooling capacity of the Russian wheat aphid (Homoptera: Aphididae). Response Model an Introd. Pest-the Russ. Wheat Aphid. Entomol. Soc. Am. Maryl. 248–257.

Armstrong, J.S., Nielsen, D.C., 2000. Supercooling points for Russian wheat aphid and winter wheat tissue as indicators of cold acclimation. Southwest. Entomol. 25, 265–272.

Asin, L., Pons, X., 2001. Effect of high temperature on the growth and reproduction of corn aphids (homoptera: Aphididae) and implications for their population dynamics on the Northeastern Iberian Peninsula. Environ. Entomol. 30, 1127–1134. https://doi.org/10.1603/0046-225X-30.6.1127

Bale, J.S., 1987. Insect cold hardiness: Freezing and supercooling-An ecophysiological perspective. J. Insect Physiol. 33, 899–908. https://doi.org/10.1016/0022-1910(87)90001-1

Blackman, R.L., Eastop, V.F., 2000. Aphids on the world's crops: an identification and information guide. John Wiley & Sons Ltd.

Burr, I.W., Foster, L.A., 1972. A test for equality of variances, Mimeo Series No. 282. University of Purdue.

Chown, S.L., Chown, S.L., Nicolson, S.W., 2004. Insect physiological ecology: mechanisms and patterns, Choice Reviews Online. Oxford University Press. https://doi.org/10.5860/CHOICE.42-5272

Colinet, H., Sinclair, B.J., Vernon, P., Renault, D., 2015. Insects in fluctuating thermal environments. Annu. Rev. Entomol. 60, 123–140. https://doi.org/10.1146/annurev-ento-010814-021017

Corrales, C.E., Castro, A.M., Ricci, M., Dixon, A.F.G., 2007. *Sipha maydis*: distribution and host range of a new aphid pest of winter cereals in Argentina. J. Econ. Entomol. 100, 1781–1788. https://doi.org/10.1093/jee/100.6.1781

Elliott, N.C., Kieckhefer, R.W., Walgenbach, D.D., 1988. Effects of constant and fluctuating temperatures on developmental rates and demographic statistics for the corn leaf aphid (Homoptera: Aphididae). J. Econ. Entomol. 81, 1383–1389. https://doi.org/10.1093/jee/81.5.1383

Lee, R.E., 2010. A primer on insect cold-tolerance, Low Temperature Biology of Insects. https://doi.org/10.1017/CBO9780511675997.002

Puterka, G.J., Hammon, R.W., Franklin, M., Mornhinweg, D.W., Springer, T., Armstrong, S., Brown, M.J., 2019. Distribution of a new invasive species, *Sipha maydis* (Heteroptera: Aphididae), on cereals and wild grasses in the southern plains and rocky mountain states. J. Econ. Entomol. 112, 1713–1721. https://doi.org/10.1093/jee/toz068

Renault, D., Salin, C., Vannier, G., Vernon, P., 2002. Survival at low temperatures in insects: What is the ecological significance of the supercooling point? Cryo-Letters 23, 217–228.

Sarkar, N., Murray, M.J., Stout, M.J., Davis, J.A., 2022. Impact of host plant resistance on emergence, body parameters, and supercooling point of *Cylas formicarius elegantulus* (Coleoptera: Brentidae). Florida Entomol. 105, 65–70. https://doi.org/10.1653/024.105.0110

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normailty (complete samples). Biometrika 52, 591–611. https://doi.org/10.2307/1267427

Shimizu, G.D., Marubayashi, R.Y.P., Goncalves, L.S.A., 2022. AgroR: Experimental statistics and graphics for agricultural sciences.

Sorensen, J.T., Center, P.P.D., 2007. Sipha maydis Passerini: A new grass/cereal aphid in North America. Plant Pest Diagnostic Center. Calif. Dep. Food Agric.

Souza, M.A.D.M.A. De, Armstrong, J.S., Hoback, W.W., Mulder, P.G., Paudyal, S., Foster, J.E., Mark, E., 2019. Temperature dependent development of sugarcane aphids *Melanaphis sacchari,* (Hemiptera: Aphididae) on three different host plants with estimates of the lower and upper threshold for fecundity. Curr. Trends Entomol. Zool. Stud. 2. https://doi.org/10.29011/2690-0114.100011

Sun, J., Tan, X., Li, Q., Francis, F., Chen, J., 2022. Effects of different temperatures on the development and reproduction of *Sitobion miscanthi* from six different regions in China. Front. Ecol. Evol. 10, 1–15. https://doi.org/10.3389/fevo.2022.794495

Vrba, P., Sucháčková Bartoňová, A., Andres, M., Nedvěd, O., Šimek, P., Konvička, M., 2022. Exploring cold hardiness within a butterfly clade: Supercooling ability and polyol profiles in European Satyrinae. Insects 13, 1–20. https://doi.org/10.3390/insects13040369

Wang, Y., Yan, J., Sun, J., Shi, W., Harwood, J.D., Monticelli, L.S., Tan, X., Chen, J., 2021. Effects of field simulated warming on feeding behavior of *Sitobion avenae*

(Fabricius) and host defense systems. Entomol. Gen. 41, 567–578. https://doi.org/10.1127/entomologia/2021/1271

Way, M.J., 1963. Mutualism between ants and honeydew producing homoptera. Annu. Rev. Entomol. 8, 307–344.

Wickham, H., 2016. Programming with ggplot2, in: Ggplot2. Springer, pp. 241–253.

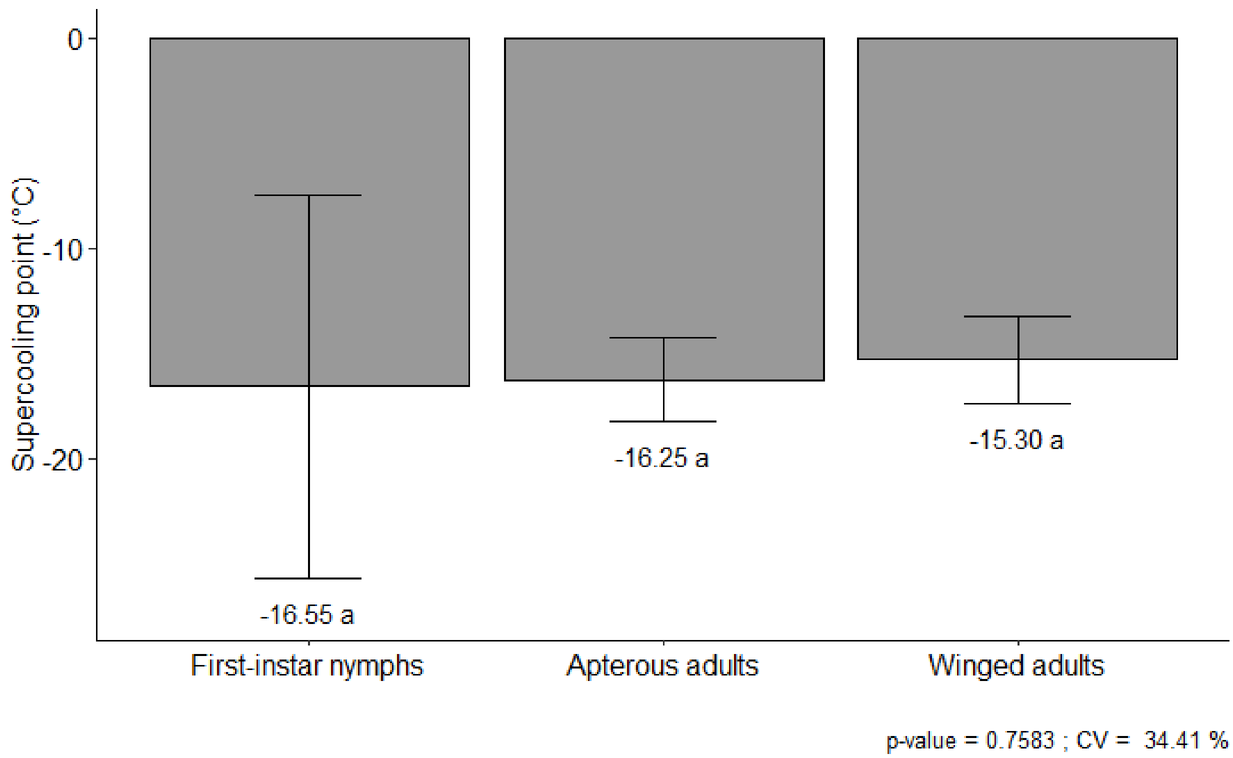
Wieczorek, K., Bugaj-Nawrocka, A., 2014. Invasive aphids of the tribe Siphini: A model of potentially suitable ecological niches. Agric. For. Entomol. 16, 434–443. https://doi.org/10.1111/afe.12073

Wyatt, I.J., White, P.F., 1977. Simple estimation of intrinsic increase rates for aphids and tetranychid mites. J. Appl. Ecol. 14, 757. https://doi.org/10.2307/2402807

Table 1. Reproductive parameters of the hedgehog grain aphid, *Sipha maydis* reared on different temperatures under growth chamber at photoperiod of 14:10 (L:D).

Temperature (°C)	**Fertility**	**Pre-reproductive time (d)**	**Number of progeny (Md)**	**Intrinsic rate of natural increase (rm)**	**Longevity**
10	-	-	-	-	-
15	31.46 ± 3.60 b	28.18 ± 1.15 a	26.09 ± 2.42 a	0.085 ± 0.005 c	63.91 ± 4.71 a
20	65.67 ± 4.30 a	14.08 ± 0.43 b	32.67 ± 2.63 a	0.183 ± 0.009 b	57.58 ± 3.14 a
25	63.58 ± 7.39 a	10.50 ± 0.23 c	33.00 ± 2.83 a	0.245 ± 0.011 a	39.83 ± 3.76 b
30	11.00 ± 1.89 c	10.50 ± 0.22c	10.20 ± 1.57 b	0.153 ± 0.018 b	18.50 ± 1.70 c
35	-	-	-	-	-
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
F	27.40	175.64	17.45	35.72	30.59
$DF_{residual}$	41	41	41	41	41

Figure 1. Supercooling point ± SE (°C) of different development stages of the hedgehog grain aphid, *Sipha maydis*, reared under laboratory conditions (21±2°C and 14:10 L:D).



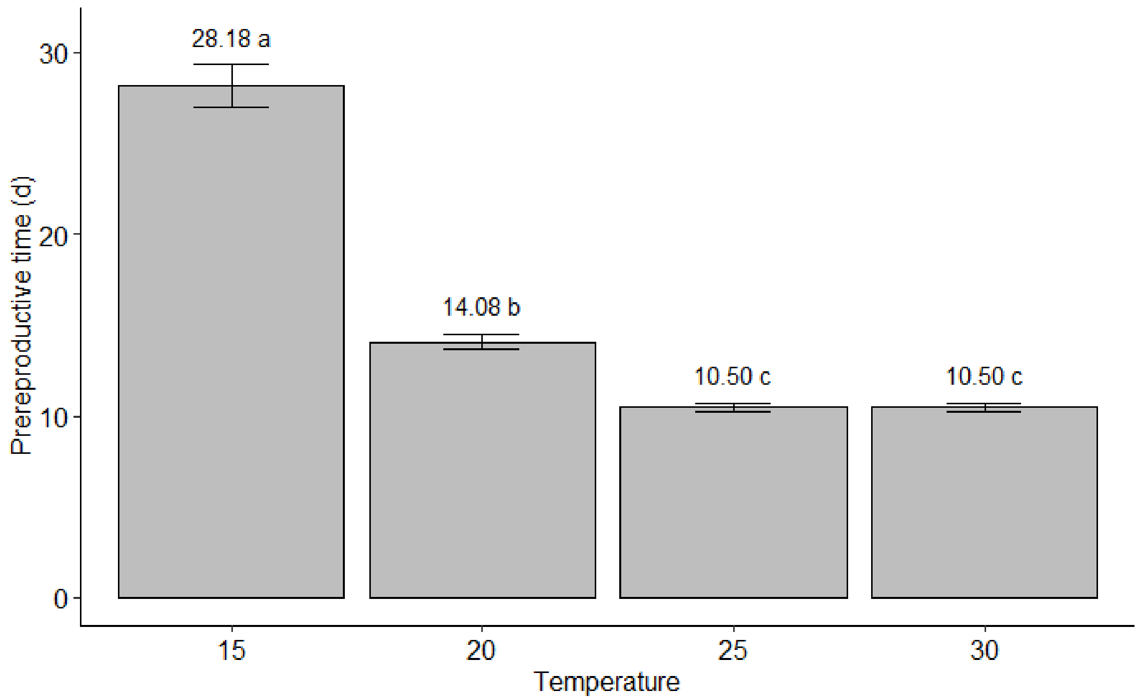


Figure 2. Pre-reproductive days (d) for each temperature excluding 10 and 35°C. Average number of days before reproduction shown for each temperature.

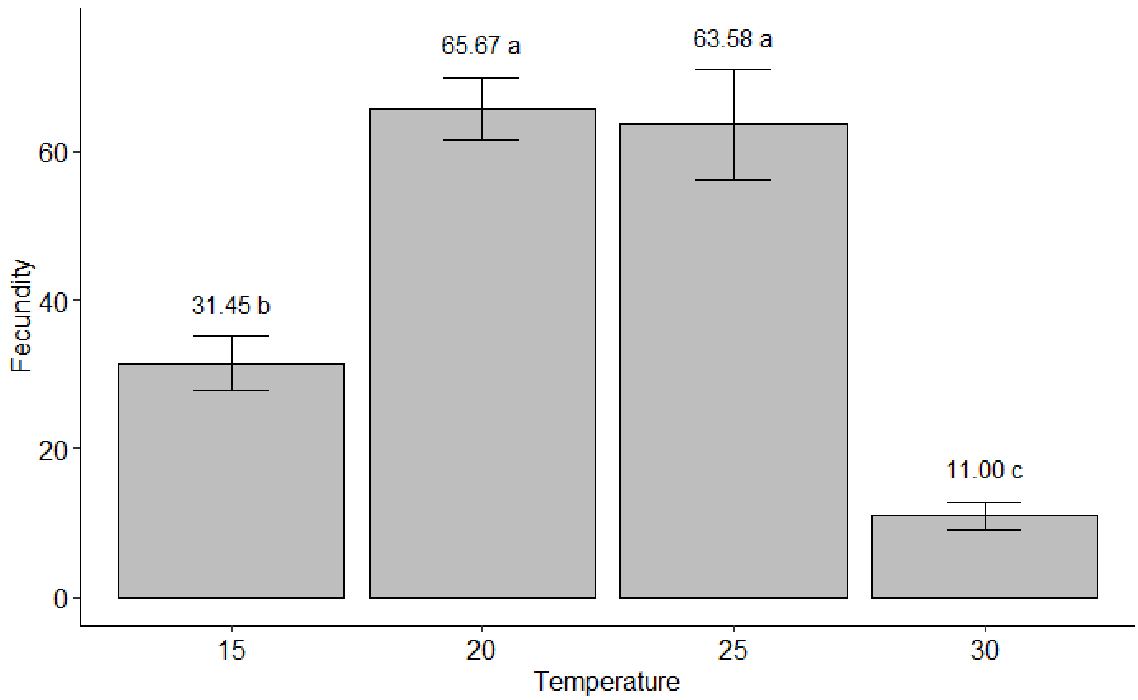


Figure 3. Total average fecundity or aphids produced until death for each temperature excluding 10 and 35°C.

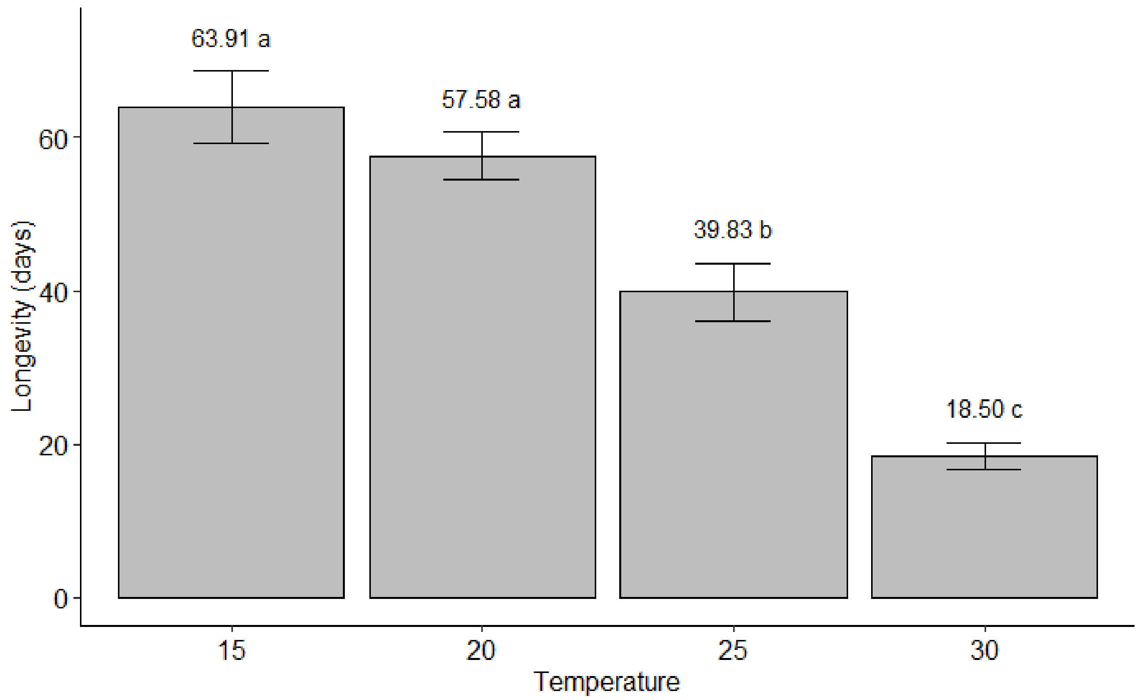


Figure 4. Total average days survived or longevity for each temperature excluding 10 and 35°C.

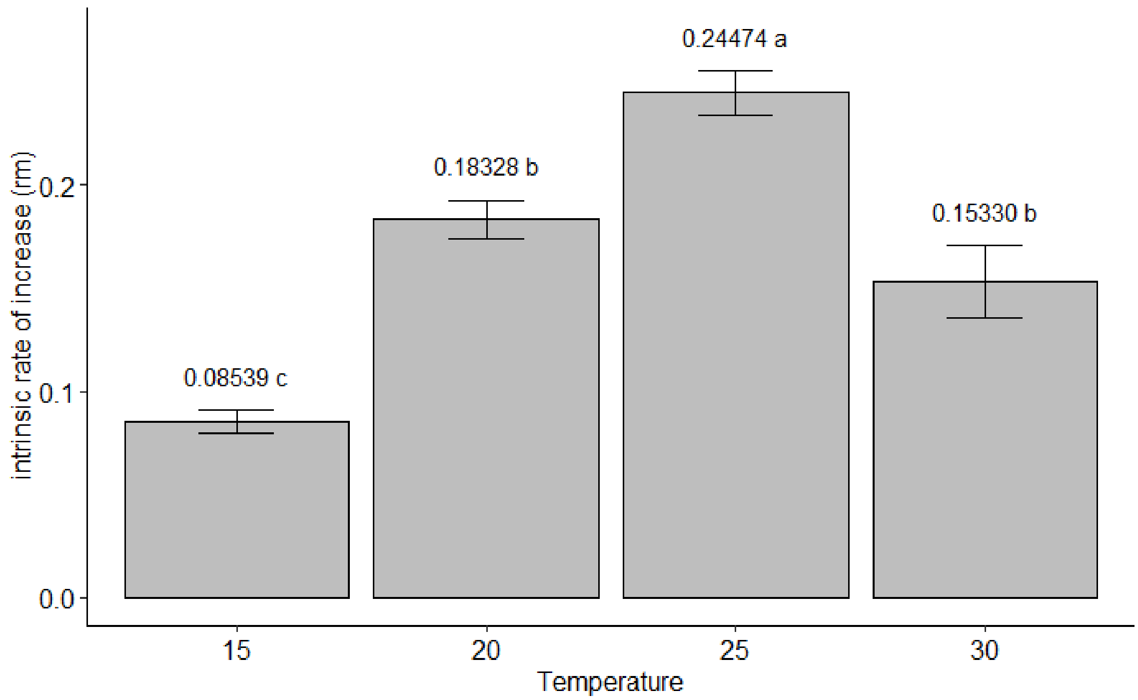


Figure 5. Intrinsic rate of increase (rm) shown as the average mean for each temperature excluding temperatures 10 and 35°C.

CHAPTER IV

COMPARISON OF LIFE HISTORY OF *SIPHA MAYDIS* [HEMIPTERA: APHIDIDAE] AND *MELANAPHIS SORGHI* ON DIFFERENT HOST PLANTS

Introduction

The hedgehog grain aphid (HGA), Sipha maydis Passerini (Homoptera: Aphididae) was first found in the new world in Argentina in 2002 (Delfeno, 2002). The HGA had a major outbreak in the country's commercial wheat and barley crops with no cross resistant strains having been planted in the area (Correlles 2007). It was then found in 2007 in California with multiple sightings since in both the eastern and western parts of the U.S. (Puterka et al., 2019). HGA has been found on a multitude of monocot host plants ranging from wild grasses to wheat, barley and sorghum (Puterka et al., 2019). With over 33 different known host plants, the HGA is continually being monitored for distribution status and potential to become a pest (Mahmood, 2002, Puterka et al., 2019).

The sorghum aphid, *Melanaphis sorghi,* followed a similar pattern of occurrence outside of its native range prior to becoming a serious annual pest of sorghum beginning in 2012. Since this time, resistant lines of sorghum have been tested and developed. As part of the initial screening for resistance, cultivars known to be resistant to greenbug were also used. The SA is one of the most prolific pests of sorghum in the US in only a matter of years (Zapata et al. 2018).

HGA was tested with the sorghum aphid (SA) *Melanaphis sorghi* (Zehntner) (Hemiptera: Aphididae), on multiple cereal grain hosts in order to determine if sorghum resistant to SA would also be cross-resistant to HGA.

This experiment tested performance of HGA and SA on sorghum varieties TX7000, TX2783, KS585, Millet variety Millex32, and wheat variety Custer in a controlled laboratory study. HGA has been observed to feed on all five of these with plants while SA has only been found to feed on the first three. SA has only been observed to feed on sorghum and the close relative Johnson grass.

Material and methods

Experiments were conducted in Stillwater, OK at the USDA-ARS labs with the two aphid species. The *S. maydis* colony has been maintained with specimens collected in 2019 from Taos, New Mexico that were kept on Custer wheat. *S. maydis* are kept in a biologically contained lab where they are maintained on wheat in 3” to 6” pots with a plastic cone sheath for containment. Plants were changed weekly and watering happened when needed. This room was kept at a constant 20 to 23°C with humidity varying from 30-50% RH.

Colonies of *M. sorghi* are maintained in a green house and growth chambers at the USDA-ARS laboratories. *M. sorghi* colonies were originally collected in Matagorda County, Texas in 2015. These colonies are maintained on the susceptible sorghum variety

TX7000. Plants were refreshed every week to 2 weeks and watered when needed. Sorghum was planted in 3” to 6” pots with a plastic sheath for containment. Green house temperatures and humidity ranged depending on climate outside.

For this study *S. maydis* populations were placed on 5-7 6” pots of the wheat variety Custer to start the boosting of colony numbers. Due to a much slower reproduction time than many other aphids *S. maydis* has to be placed in much more colonies. Around 2-3 weeks were taken for *S. maydis* populations to be around 250-400 sexually mature female aphids for the experiment. *M. sorghi* had less need for as many extra pots planted due to the sizes of existing colonies and less time it took for them to reproduce. Only 2-3 6” pots were planted with the sorghum variety TX7000 and left to reproduce for around a week.

<u>Seed varieties and Planting</u>

There were 5 different seed varieties used during this experiment and 3 different plant types. Three sorghum genotypes (TX7000, TX2783 and KS585), a wheat (Custer) and a millet (Millex 32) were used for assessing the biological parameters of SCA and HGA. These seeds were planted in yellow containers call “conetainers” with a cone like shape around 6” long. These contaniers had 3 slots close to the bottom and one at the very bottom for ventilation and drainage of excess water. Containers were filled with a mixture of potting soil at the bottom, clay above, and fine sand the rest of the way full. Colored and numbered strips of tape were placed on the outside of each container designating what each cone had and how data would be contained. When seeds were ready to be planted, the sand was first moistened then depressed around a ¼" into the sand. Seeds were then placed in numbers of around 3-5 for guaranteed growth or sprouting the sand was placed over top and watered again. When seeds were starting to emerge and were at the least ½" above the surface they were thinned to only one sprout per container and ready to be infested.

Infesting and Growth Chambers

To infest the grass specimens, aphids from rearing colonies were placed on plants one aphid at a time. To placed aphids on plants we used horse hair paintbrushes dipped in water to help aphids adhere easier. Each of the plant species were infested first with 3-5 adult reproductive females and left on for 24 hours. After this 24 hour mark, all aphid adults and all but one nymph aphid were taken off of the plants. Aphids were then placed in 2 of the exact same make and model growth chambers (Percival® Model E30B, Perry IA). These growth chambers were set at a constant temperature of 23°C, a 14:10 day: night cycle, and an RH of around 70%. Aphids were observed every 24 hours and data was collected from the day they began to produce until they died.

Parameters

The biological aphid parameters assessed were fecundity (number of offspring per female), longevity (days), pre-reproductive period (days). Intrinsic rate of increase (rm) was calculated using the formula: $rm = 0.074(1og_e M_d)/d$ (Wyatt and White, 1977).

Data analysis

All parameters were submitted to exploratory analysis to verify the assumptions of normality of residuals, homogeneity of treatment variance, and additivity of the model before the analysis of variance (ANOVA). When significant differences were detected, averages were compared by the post-hoc Tukey test at 5% probability. The analysis and graphs were made using the statistical analysis program R (Shimizu *et al.*, 2022).

Results

Pre Reproduction Days

The wheat, Custer, and millet, Millex32, were not suitable for SA, with only one individual reaching adult stage in each plant (Table 1). Thus, for the SA, the biological parameters were compared between the sorghum genotypes (TX7000, TX2783 and KS585).

In this experiment pre reproduction days (PRD) were cataloged to look at and compare how long it took HGA and SA to produce offspring. We had observed that only on the sorghum varieties SA had

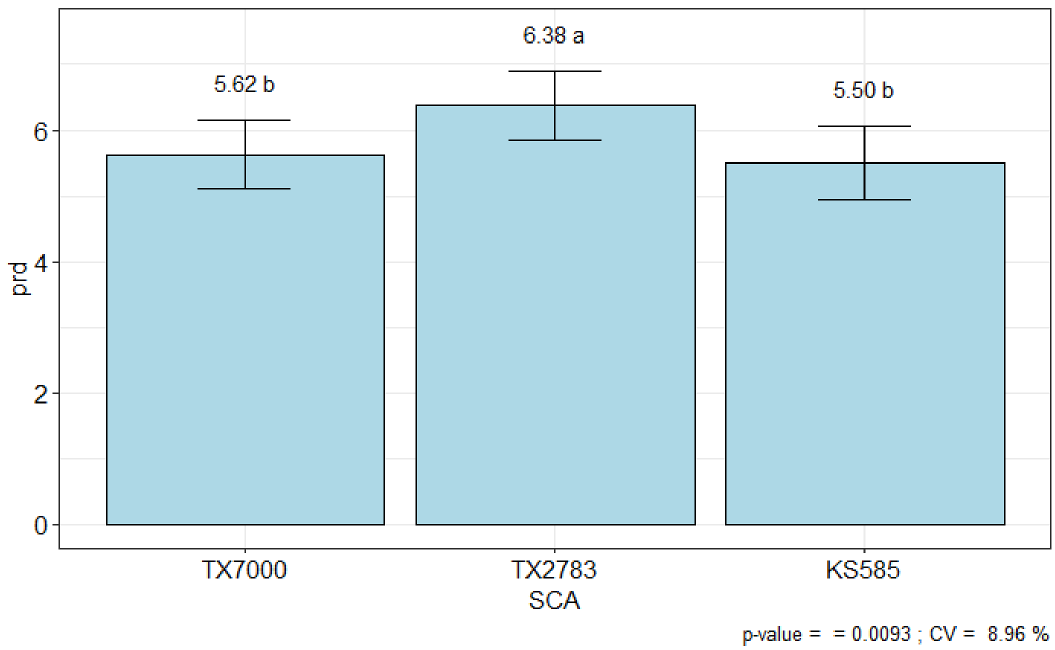


Figure 1. Pre reproduction days (prd) for SA on the 3 sorghum varieties TX7000, TX2783, and KS585. The wheat variety, Custer, and millet variety, Millex32, did not have any reproduction from SA due to these plant species not being in SA diet.

fast developmental and reproduction time. Seen in Table 1, SA had a significance between TX7000 and KS585 reproducing on average 5.62 and 5.50 days. TX2783 was found to have the longest average pre reproduction days of 6.38 due to a slight resistance to SA. The other two varieties planted, Millex32 and Custer, did not have any SA survive long enough to reproduce.

For HGA however, it took twice as long or longer for them to reach a sexually mature state after being introduced to the plants. HGA was found to have to significance in any of the 5 different plant varieties tested. Though on the wheat variety Custer, HGA had the least average days to reproduce with 11.0 as seen in Table 2. From other calculations found in Table 2, we observed them to have the most amount of average days until reproduction on the sorghum variety KS585 with 13.4. The three sorghum varieties came very close to one another with TX7000 having 12.2 and TX2783 having 12.8 average days as well. Millet and wheat were the two with lowest days of reproduction and with Millex32 having an average of 11.6 days until reproduction.

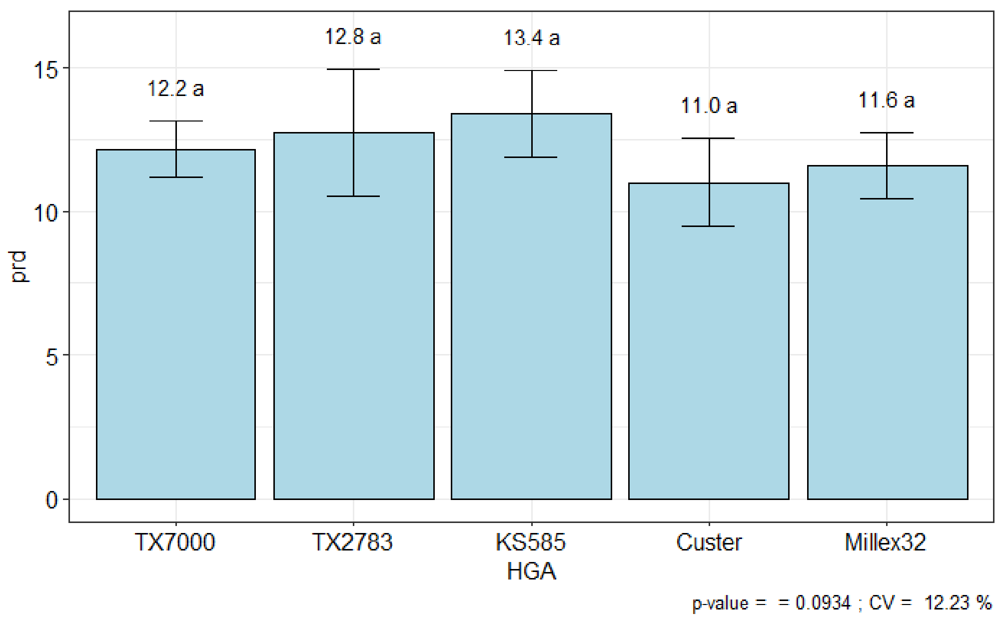


Figure 2. Pre reproduction days for HGA shown on 5 different host plant varieties. For the sorghum varieties, TX7000, TX2783, and KS585, for wheat, Custer, and for millet, Millex32, were used in this experiment. All 5 of the varieties were able to be shown as there have been observed to be in the HGA diet.

<u>Offspring</u>

For this experiment, total offspring or fecundity was measured for each aphid species at the end of the experiment. This gives a better look at how well each species did on the 5 varieties that were tested in combination with the other data points given. In most cases a higher fecundity or total offspring means that there is a better chance that the species will be able to survive in an area or on a species of plant.

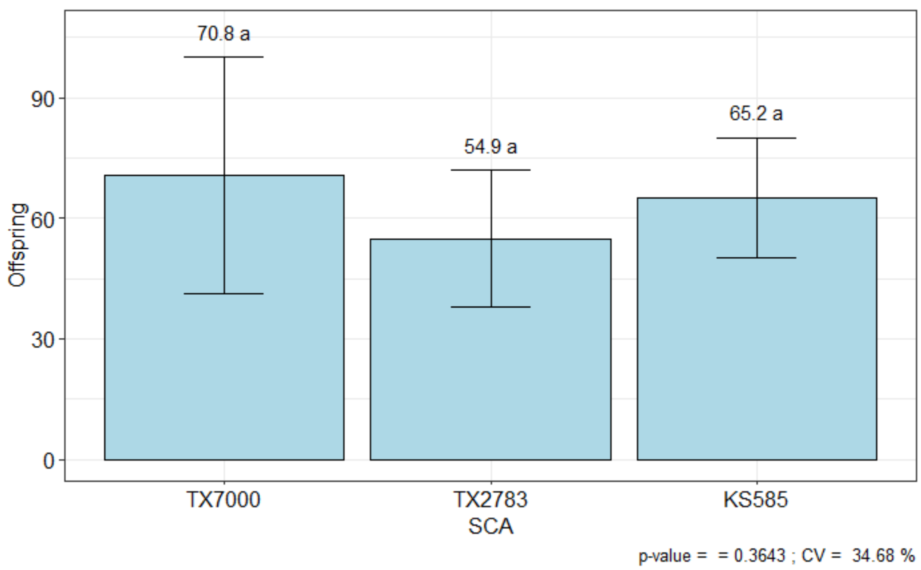


Figure 3. Total average offspring or fecundity of SA from the first reproduction day until death of the female aphid. This graph shows the 3 sorghum varieties, TX7000, TX2783, and KS585, that SA were able to reproduce on.

From Table 3, we found that there was no significant difference between the 3 different sorghum varieties. However, there was a difference from the universally susceptible TX7000 variety with total average offspring of 70.8 nymphs produced. This was the most out of all of the varieties that were tested in both species of aphid. The sorghum variety KS585 had the second highest average total offspring recorded for the SA aphid species with 65.2 nymphs produced. The last and lowest sorghum variety, TX2783, had an average total offspring of 54.9 nymphs produced. This variety could be the lowest due to a known slightly resistant gene. These 3 data points were the highest recorded compared to the HGA species.

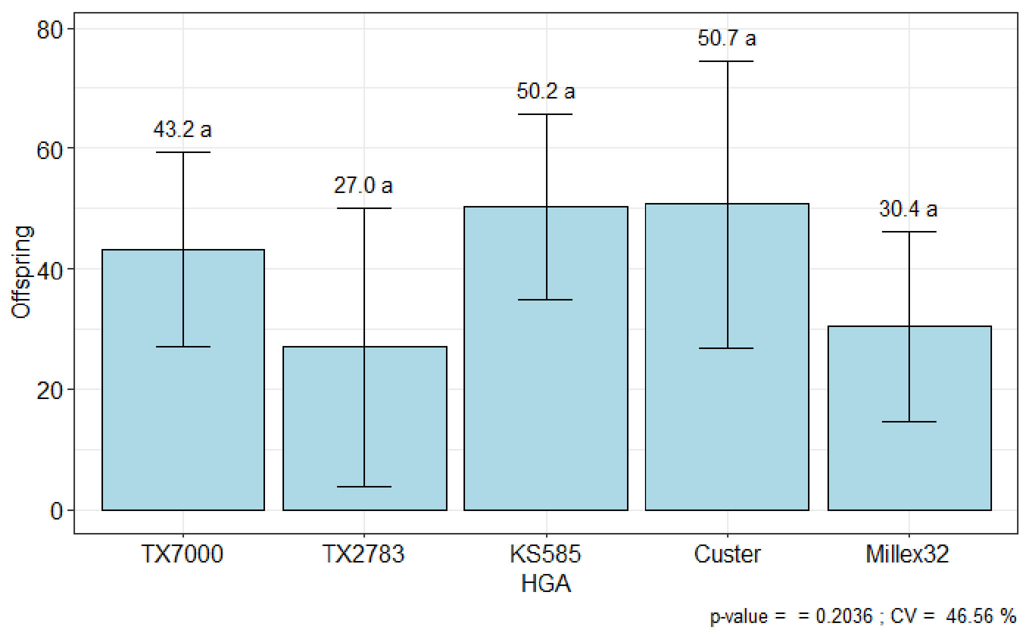


Figure 4. This is the average offspring or fecundity for HGA with the 5 different varieties. For the sorghum varieties, TX7000, TX2783, and KS585, for wheat, Custer, and for millet, Millex32, were used in this experiment. Unlike the SA experiment, HGA was able to all 5 of the varieties due to their expansive diet.

For the average total offspring or fecundity of HGA on the 5 different varieties of small grains there was no significant different observed. However, there was an observable difference between the varieties used and which were better for survivability. The 2 varieties that were found to have the highest average offspring or fecundity were varieties from sorghum, KS585, and wheat, Custer. These varieties had an observed average total offspring or fecundity of 50.2 and 50.7 nymphs. The 2 that were next were a sorghum variety, TX7000, and millet, Millex32, with average total offspring or fecundity of 43.2 and 30.4 nymphs. The lowest of these was the last sorghum variety, TX2783, with an average total offspring or fecundity of 27.0 nymphs. This was found to be the lowest of both of the species used possibly due to the slightly resistant gene found in this variety.

Longevity

Longevity was a data point used to show how long a female aphid stayed alive and reproducing on a variety of plant. This point was calculated from when the nymph female was introduced to the plant until the female was observed to be deceased. Data found on Table 5 is the average total amount of days from each aphid on one variety of small grain plant and separated by species of aphid.

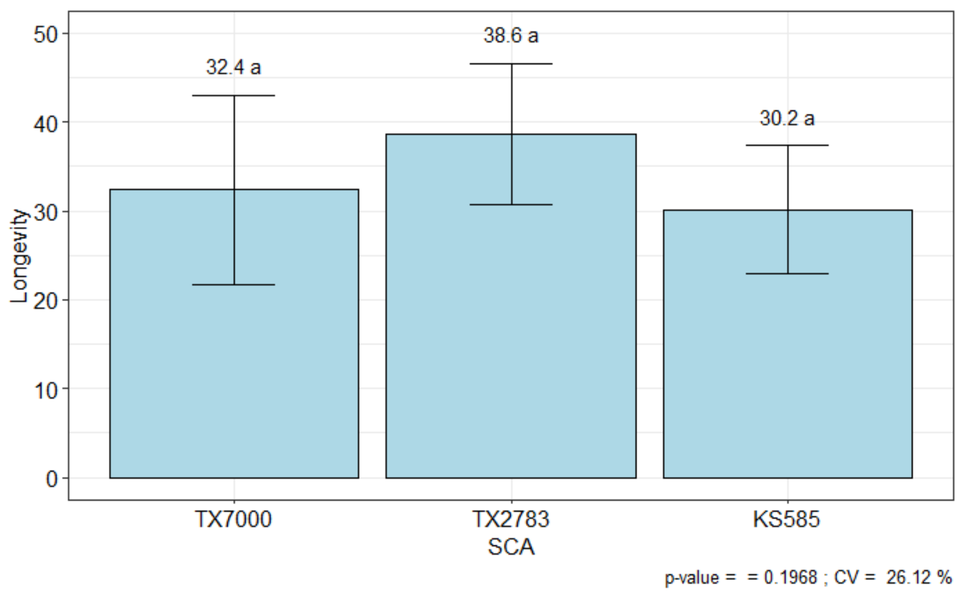


Figure 5. Longevity of the SA species calculated by the average amount of days the aphid survived from the introduction of the nymph to a plant until the death of the female aphid. This graph shows the 3 sorghum varieties, TX7000, TX2783, and KS585, that SA were able to reproduce on.

From the data on Table 5, there is no observable significant difference from any of the data points or varieties. However, there is a small difference between each of the data points and how long the aphids lasted on each of the varieties. The longest average days the SA species lasted was on TX2783 with 38.6 days on average. With a higher known resistance than the other two, it was surprising to see that SA was found to survive the longest on this variety. The other two varieties were our universally susceptible TX7000 with 32.4 average days until death and KS585 with 30.2 average days until death.

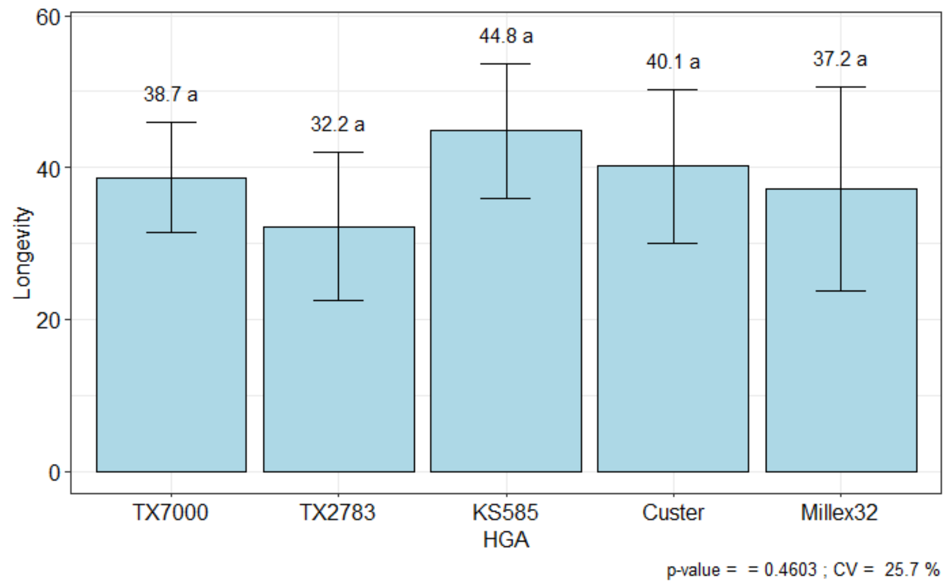


Figure 6. Longevity of the HGA species calculated by the average amount of days the aphids survived from introduction to the plant until their death. This graph shows the sorghum varieties, TX7000, TX2783, and KS585, for wheat, Custer, and for millet, Millex32, were used in this experiment.

From the data in Table 6, there is no significant difference that was observed using the data collected. However, there were some points that were closer and further away from one another that should be noted. The longest amount of average days survived was on the sorghum variety KS585 with 44.8 days. The other two varieties closer together and were wheat, Custer, and millet, Millex32 with 40.1 and 37.2 days on average. The other two sorghum varieties TX7000 and TX2783 were in the lower ends with 38.7 and 32.2 days on average.

<u>Intrinsic rate of increase (rm)</u>

Intrinsic rate of increase (rm) is the main data point that was being observed in this study. All of the other data entries were used to calculate this figure using the formula rm = 0.074($1og_eM_d$)/d. This output shows how well the aphids survived overall on each of the varieties that were used. From this data output we can find a significant curve, if there is one on which variety and aphid will most likely colonize and feed on amongst other flora.

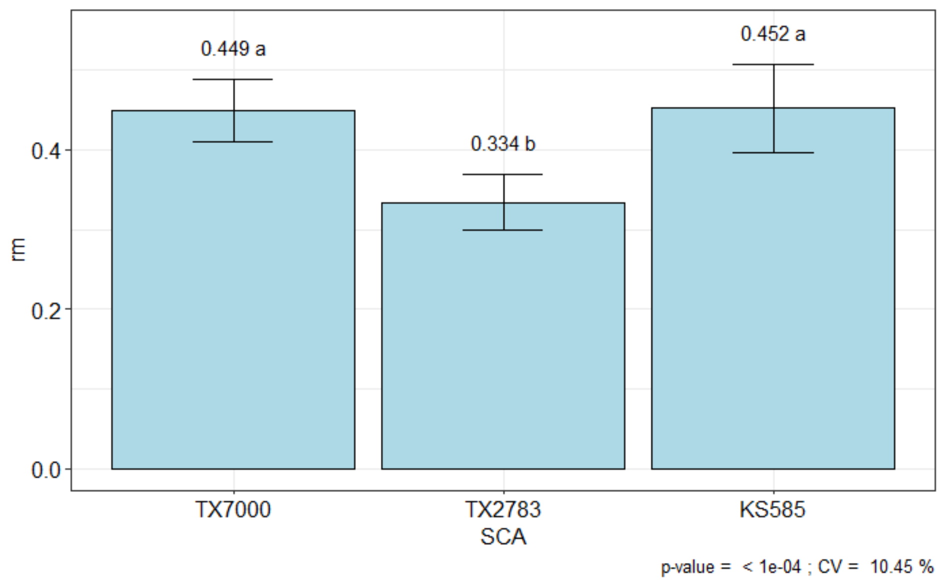


Figure 7. Intrinsic rate of increase (rm) or output average mean of the formula used found for SA in this study on 3 different varieties of sorghum. The varieties that were used were TX7000, TX2783, and KS585.

For SA there was one data point found to be have significant difference from the other two calculated. In table 7, TX2783 had a significantly lower intrinsic rate of increase than the other two sorghum varieties with an average mean of 0.334. This still shows that there is a slight resistance to SA found in TX2783 as seen in the other data points as well. The other two sorghum varieties however were very close and had a much higher average mean. The highest found was that of KS585 with an average mean of 0.452 and TX7000 shortly behind with an average mean of 0.449.

From table 8 we can see that there was no significant differentness between the 5 different varieties of small grains used for this experiment. However, there are some observable differences between the high and low data points presented for HGA's intrinsic rate of increase. The data point with the highest average mean or was found to be the best host for HGA in this experiment was the wheat variety Custer. This variety had an average mean of 0.214, though lower than any rm for SA. Two of the sorghum varieties, TX7000 and KS585, came next and very close to one another with average means of 0.188 and 0.180. Due to a lower fecundity than most, the millet variety, Millex32, was the second lowest of the average means measured at 0.168. The sorghum variety, TX2783, was the lowest of the average means calculated at 0.148 suggesting that there may be some cross resistance in this variety.

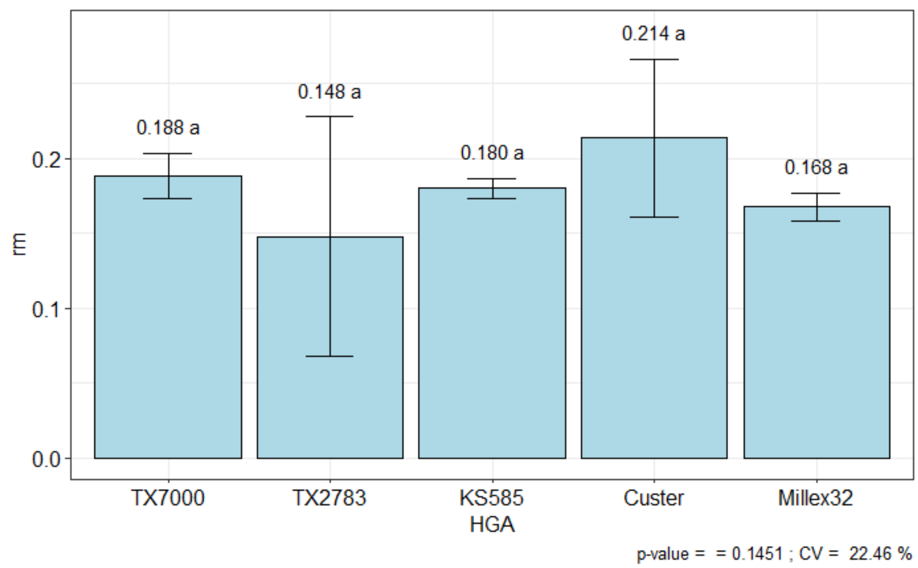


Figure 8. This graph shows the intrinsic rate of increase (rm) by using the average mean calculated from the other 3 data points observed. On the graph are the sorghum varieties, TX7000, TX2783, and KS585, for wheat, Custer, and for millet, Millex32, were used in this experiment.

<u>Discussion</u>

From the results of this experiment, it was found that there is a very obvious difference between an aphid that has been recorded as a highly invasive pest (SA) compared to our newly introduced aphid species (HGA). There are many factors that can prove a species to be invasive or successful at becoming a virulent pest in an environment. Using the data above we were able to obtain the outcomes to help provide insight and come to a concussion as to if HGA has the possibility of becoming one. However, there was no data available to compare for the two varieties of millet, Millex32, and wheat, Custer. This should not be too much of a problem due to the data shown above not being nearly as high in any of the other plants as it was in the 3 that were shown for SA. HGA's numbers overall stayed vastly different from the SA's in almost every observable data point that was used.

Comparing the SA and HGA numbers throughout this experiment shows that these two aphids respond differently to plants that have been proven susceptible. Beginning with the pre reproductive days (prd) found in tables 1 and 2 we see that HGA takes double, if not more, the amount of time it takes SA to reproduce. For the sorghum varieties TX7000, TX2783, and KS585, SA was much quicker to reach sexual maturity after being introduced to the plants. Even on plants that HGA was able to reproduce on, it still took them almost twice as long to reach as sexually mature state and begin reproducing.

From how long HGA and SA took to breed we can also look at how many total offspring were produced by both. This offspring total average, or fecundity total average, was dominated by the SA species once again. For SA the highest observed average was 70.8 nymphs on the sorghum variety TX7000 compared to the HGA highest total at 50.7 nymphs produced on the wheat variety Custer. To make this a clearer picture we will also compare the average total amount of days the aphids stayed alive and breeding from first nymph to death. For SA's TX7000 test, female aphids produced 70.8 aphids within an average of 32.4 days before death. For HGA, 50.7 aphids were produced over an average of 40.1 days. Though HGA lasted longer on all of the plants that were screened they had a much lower output of aphids than the SA. Due to HGA not having a higher number of nymphs, it would take them much longer to spread and have a harder time to build a population.

The main output that we were calculating with all of these other data points is the intrinsic rate of increase (rm) measured by the average mean. This was found to be almost twice as high in the SA study as the HGA study. The highest rm for SA was found in KS585 with an average mean of 0.452, and for HGA the highest average mean was 0.214 in the wheat variety Custer. Comparing each of the rm or intrinsic rate of increase average means, SA shows that it is much better at surviving and reproducing on small grains that they are able to feed on. Even with the small window of species that the SA can survive on, they became of the most prolific aphid pests in the US at one time. With a smaller rm on all of the plants that were tested, HGA seems to not be very fit to become a wide spread problem in the US especially with the new more resistant varieties that are being used in crops now. However, the versatility they have to feed on all types of plants in multiple areas of the world is still a concerning factor. Though they might not be reproducing fast or have the highest fecundity, HGA can survive on a wide variety of small grains and wild grass species around the world.

REFERENCES

Corrales, C.E., Castro, A.M., Ricci, M., Dixon, A.F.G., 2007. *Sipha maydis*: distribution and host range of a new aphid pest of winter cereals in Argentina. J. Econ. Entomol. 100, 1781–1788. https://doi.org/10.1093/jee/100.6.1781

Delfino, M.A. 2002. Two aphid species of agricultural interest in Argentina (Hemiptera: Aphididae), p. 123. In: Proceeding XI Jornadas Fitosanitarias, 18–22 September 2002, Rio Cuarto Córdoba, Argentina.

Mahmood, R., M. A. Poswal, and A. Shehzad. **2001**. Distribution, Host Range and Seasonal Abundance of Sipha Sp. (Homoptera: Aphididae) and Their Natural Enemies in Pakistan. Pakistan J. of Biological Sciences. 5: 47–50.

Puterka, G.J., Hammon, R.W., Franklin, M., Mornhinweg, D.W., Springer, T., Armstrong, S., Brown, M.J., 2019. Distribution of a new invasive species, *Sipha maydis* (Heteroptera: Aphididae), on cereals and wild grasses in the southern plains and rocky mountain states. J. Econ. Entomol. 112, 1713–1721. https://doi.org/10.1093/jee/toz068

Zapata, S. D., R. Dudensing, D. Sekula, G. Esparza-Díaz, and R. Villanueva. **2018**. ECONOMIC IMPACT OF THE SUGARCANE APHID OUTBREAK IN SOUTH TEXAS. J. Agric. Appl. Econ. 50: 104–128.

APPENDICES

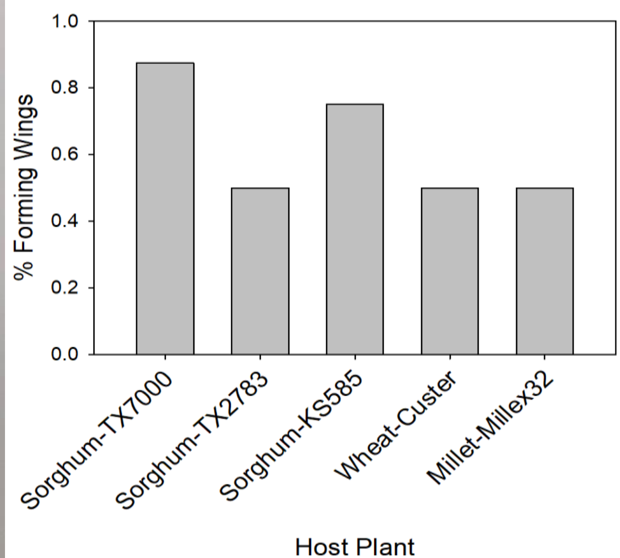
Figure 1: Barley experiment 1. *S. maydis* was placed onto multiple varieties of barley to test for cross resistance or if it preferred this small grain over the others. This was the first barley experiment that we tested with 30 different varieties ranging from susceptible, tolerant, and resistant to other species of aphid. This test came up inconclusive.

Figure 2: Barley experiment 2 infested. This was tested in a new environment with less varieties being tested at one time. Planted in the same reservoirs as barley experiment one but placed in a sealed growth chamber within our biological control room. Tests came back inconclusive as well with no good data. There was no correlation as well as thrip damage on most plants.

Figure 3: Barley experiment 3 infested. We used again less varieties of barely and a completely different containment and test effort. Smaller resivour as well as being completely sealed in with a plastic box covering in the biological control room with no growth chamber. Data again came back inconclusive with no correlations or significance to one variety or another.

Figure 4: Winged adult with fungus. Due to the spine like growths on the dorsal area of the HGA honey dew refuse would being to collect atop the spines in tight groups of aphids. Due to the high sugar content and humidity, this honeydew would culture mold or fungus effectively sticking aphids to a host plant and killing them. In winged adults however, an aphids own honeydew would often get caught in wings and have the same effect. They would also spread growing mold or fungus where they would walk around the host plant.

Figure 5 & 6: Winged HGA adults. HGA seemed to produce a mass of winged females when on host plants. This can be seen on any host that they were transferred to. Normally this means the habitat, temperature, host or other factor was in play that they did not find favorable. However, in this case HGA would produce winged females even when they were on a host plant they had high intrinsic rates of increase on.



VITA

Mason Alexander Taylor

Candidate for the Degree of

Master of Science

Thesis: LIFE HISTORY, DISTRIBUTION, AND TEMPERATURE TOLERANCE OF THE INTRODUCED HEDGEHOG GRAIN APHID *SIPHA MAYDIS* PASSERINI [HEMIPTERA: APHIDIDAE]

Major Field: Entomology

Biographical:

Education:

Completed the requirements for the Master of Science in Entomology and Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in May, 2023.

Completed the requirements for the Bachelor of Science in Entomology at Oklahoma State University, Stillwater, OK in 2020.

Experience:

Professional Memberships:
Entomological Society of America, Southwestern Branch