# INHERITANCE OF RESISTANCE TO THE COTTON APHID IN *Gossypium herbaceum*

by

JUSTIN DEAN ALEXANDER, B.S.

A THESIS

IN

ENTOMOLOGY

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

MASTER OF SCIENCE

# ACKNOWLEDGMENTS

I would like to thank Dr. John Gannaway for his guidance and input throughout my graduate career. Also I thank Dr. Harlan Thorvilson and Dr. Don Rummel for their input and assistance in the preparation of this manuscript.

To my wife, Tamara, I owe special thanks for her love and continued motivation throughout this time in my life. Without her support none of this would be possible.

Thanks to Lyndon Schoenhals, Leslie Wells, Monica Bellows, Valerie Morgan, and each of the student workers who played a valuable part in the collection, maintenance, and analysis of research data.

TABLE OF CONTENTS

# LIST OF TABLES

LIST OF FIGURES

# CHAPTER I

# INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), is a small, slow-moving, soft-bodied insect. First described in 1876 by Townend Glover (Palmer 1952), the cotton aphid has been placed in the order Homoptera, suborder Sternorrhyncha, superfamily Aphidoidea, family Aphididae, subfamily Aphidinae, tribe Aphidini, and subtribe Aphidina. Two common names for *A. gossypii* have been recognized by the Entomological Society of America: the cotton aphid and the melon aphid. These names reflect two of the most important agricultural crops affected by *A. gossypii*.

The cotton aphid was once considered only a minor and occasional pest of cotton. Since 1975, however, it has become a serious threat to cotton producers across the cotton belt including the Texas High Plains. Infestations by the cotton aphid may cause delayed growth, yield loss and reduced fiber quality. In addition, "honeydew" secreted by cotton aphids may result in "sticky cotton" which causes problems in textile mills.

Control of the cotton aphid has become increasingly difficult. Aphid populations have developed resistance to many insecticides that were once highly effective in their control. To add to this problem, pyrethroids, which are heavily relied upon for the control of many other cotton pests, have been found to promote aphid reproduction (Kidd 1995).

## Physical Description

Adult cotton aphids are oval or pear-shaped and are generally about one millimeter in length. Cotton aphids are polymorphic with considerable variation in both size and color (Rosenheim et al. 1995). They may range in color from yellow to green to near black in some cases. According to Kring (1959), the yellow aphid forms are the aestivating form of the species and are usually present under less favorable environmental conditions. Yellow aphids are generally small and characteristic of dense populations and unfavorable hosts (Wall 1933). Dark forms seem to be triggered by optimal environmental conditions, such as lower summer temperatures and shorter day lengths (Rosenheim et al. 1995).

Adult cotton aphids may be either alate (winged) or apterous (wingless). O'Brien et al. (1993) reported that winged aphids may form in

response to overcrowding in heavily infested areas. Winged forms are able to move to new, more favorable host plants and colonize, giving rise to wingless individuals (Freeman and Smith 1999). Cotton aphids have a pair of cornicles which arise from either the fifth or sixth abdominal segment. These two projections are used to excrete defensive fluids. Both adult and immature stages (nymphs) of the cotton aphid have stylet-like mouthparts which are used to suck fluids from the host plant.

Eight aphid species are known to colonize cotton in the United States: *Aphis gossypii* Glover, cotton aphid; *Aphis craccivora* Koch, cowpea aphid; *Aphis fabae* Scopoli, bean aphid; *Aphis maidiradicus* Forbes, corn root aphid; *Myzus persicae* (Sulzer), green peach aphid; *Macrosiphum euphoribiae* (Thomas), potato aphid; *Rhopalosiphum rufiabdominalis* (Sasaki), rice root aphid; and *Smynthurodes betae* Westwood (Stoetzel et al. 1996). Many of these species are similar in appearance to the cotton aphid, and sometimes are difficult to distinguish. Several characteristics, however, may allow for species identification. The antennae of cotton aphids are usually six-segmented with the terminal process straight and tubercles weakly developed. The cauda is shorter than the cornicles and generally pale to dusky in color with three pairs of lateral setae.

## Life Cycle

The life cycle of the cotton aphid is unusual and sometimes very complex. In northern portions of the United States, the cotton aphid is holocyclic, reproducing sexually by laying eggs (Slosser et al. 1989). However, in the cotton belt, most cotton aphids are female, and relatively few males have ever been identified (Slosser et al. 1989, Freeman and Smith 1999). The females are able to reproduce asexually through a process called parthenogenesis.

Cotton aphids are distributed worldwide and are known to infest at least 64 different plant species including cotton, cucurbits, citrus, ornamentals and vegetable crops (Akey and Butler 1989, Slosser et al. 1989). O'Brien et al. (1992) collected cotton aphids from 24 non-cultivated host plants in Mississippi. Young and Garrison (1949) reported the collection of cotton aphids from 17 additional non-cultivated hosts in Louisiana. Kring (1959) reported *Catalpa bignonioides* Walt. (common catalpa) and *Hibiscus syriacus* L. (rose of Sharon) as primary hosts and numerous secondary hosts for the cotton aphid in Connecticut.

When utilizing primary hosts, the cotton aphid undergoes a holocyclic stage in which male and female aphids mate and eggs are produced.

Overwintering takes place in the egg stage, and young hatch the following spring.

Populations of cotton aphids feeding on cotton and other secondary hosts are thought to consist almost exclusively of parthenogenetic females (Kring 1959, Slosser et al. 1989, O'Brien et al. 1992). These viviparous females are able to reproduce asexually and give birth to live young. Overwintering takes place in the adult stage.

The collection of male cotton aphids on cotton by O'Brien et al. (1990) and Kidd (1995) suggests the presence of a holocyclic life cycle in cotton as well. Two male cotton aphids were collected on cotton in Stoneville, Mississippi in 1988 (O'Brien et al. 1990). Kidd (1995) reported the collection of 13 male cotton aphids (six in cotton and seven by suction trap) in Lubbock, Texas. All of these collections were made in November and December. This late season production of males may be a result of certain environmental stimuli. Shortening of day length is responsible for production of males in other aphid species (Hales and Mittler 1986, Puterka and Slosser 1983). Whatever the responsible stimulus, Kidd (1995) suggested the purpose of male cotton aphid production was for mating with oviparous females, which would allow for overwintering in either adult or

egg stages. Sexual reproduction would also allow for genetic recombination and therefore increase the size of the gene pool (Kidd 1995).

Cotton aphids undergo four larval instars before reaching maturity. Under normal conditions, sexual maturity can be reached in less than 10 days with the reproductive period lasting about three weeks (Drees 1999). Akey and Butler (1989) found that with an optimal temperature of 27.5°C, development can be completed in as little as five days.

The average life of an adult cotton aphid is about one month (Drees 1999). Females are capable of producing 80-85 young in a lifetime (Freeman and Smith 1999, Drees 1999, Slosser et al. 1989), or as many as 2.85 nymphs per day (Akey and Butler 1989). Dark morphs are characteristic of favorable conditions and tend to develop more quickly. These forms are generally responsible for rapid population outbreaks. Yellow aphid forms develop slowly and produce few offspring (Rosenheim et al. 1995). Wall (1933) and Kring (1959) attribute these small, yellow aphids to dense populations and unfavorable host plants. Isely (1946) reported the development of yellow morphs under hot and dry conditions. Once yellow aphids are produced, reproduction may cease until favorable conditions resume (Kring 1959).

## Feeding and Damage

Adult and immature cotton aphids feed by sucking juices from phloem tissue in the host plant. A specialized digestive system allows the cotton aphid to filter out unwanted liquids, to digest only necessary amino acids and plant sugars, and to excrete honeydew (Chapman 1982). With the help of this system, cotton aphids are capable of ingesting up to 133% of their own body weight in one hour (Slosser et al. 1989, Kennedy and Stroyan 1959, Auclair 1963). They may feed on tender stems and fruit but are most often found on the undersides of leaves. In addition, higher numbers are generally found on lower portions of the plant canopy (Hardee et al. 1994).

Heavy infestations by cotton aphids reduce plant vigor and may cause leaves to crinkle and cup downward. Frequently, plants are able to compensate for the physical damage caused by cotton aphids. However, in severe cases, plants are stunted, and leaves and fruit may be shed. The accepted economic threshold for aphids in Texas High Plains cotton is 50 aphids per leaf. Treatment may be justified at this level; however, other factors such as beneficial arthropod populations should be taken into consideration.

As aphids feed, they excrete a sticky substance called "honeydew." This anular secretion is generally a mixture of water and sugar, including fructose, glucose and sucrose (Auclair 1963). Honeydew may fall onto leaves and open bolls resulting in "sticky cotton." This may cause serious problems in textile mills by sticking to the machinery. In open boll cotton, treatment may be justified when aphid numbers reach an average of 10-15 per fifth mainstem node leaf to prevent possible sticky problems. A black fungus known as "sooty mold" also may develop on honeydew deposits, resulting in inhibition of photosynthesis and reduced fiber quality. In addition, honeydew may make plants more attractive to other cotton pests (Slosser et al. 1989).

## Pest Status

Although the cotton aphid has long been a pest in cotton, it was not considered a major pest in many areas until recent years. The High Plains region of Texas suffered its first major outbreak during the 1975 growing season. According to Rummel (1975), infestations were heavy and widespread throughout the area. Since then, infestations have been sporadic and varied considerably from year to year.

The entire cotton belt has experienced major infestations, and in 1991 the cotton aphid was deemed the most severe pest of cotton (Hardee et al. 1993, Hardee and Herzog 1992). An estimated 10 million of the 13 million acres harvested were considered infested, resulting in the loss of more than 360,000 bales, including over 333,000 in Texas alone (Kidd et al. 1996). The problems encountered during the 1991 season caused much attention to be devoted to the cotton aphid. As a result, a Cotton Aphid Task Force was established to develop management suggestions to aid in aphid control (Godfrey and Leser 1999). Since 1991, moderate infestations on the Texas High Plains have occurred in 1993, 1995, 1996 and 1997. In some instances, problems due to "sticky cotton" have occurred in textile mills, and as a result, the marketability of cotton from certain areas was threatened. During the 1995 season, the High Plains experienced some sticky cotton problems. The presence of cotton aphids late in the season coupled with the absence of September rains allowed honeydew-contaminated lint to remain through harvest (Godfrey and Leser 1999). To compound the problem, high plant sugar levels occurred on lint due to an early plant-killing freeze in the area (Godfrey and Leser 1999). Fortunately, no sticky cotton problems have occurred in Texas since 1995.

## Control Measures

Many different insect predators may feed upon cotton aphids. Key predators include lady beetle larvae and adults, primarily *Hippodamia* spp.; minute pirate bugs, *Orius* spp.; lacewing larvae, *Chrysopa* spp.; big-eyed bugs, *Geocoris* spp.; damsel bugs, *Nabis* spp.; and syrphid fly larvae, *Syrphus* spp. An important parasite is the parasitic wasp, *Lysiphlebus testaceipes*. Often times these predators and parasites may suppress aphid numbers when not disturbed by insecticides, however, due to their high reproductive capacity occasional aphid outbreaks will occur (Kidd and Rummel 1997).

Certain fungal pathogens responsible for control of the cotton aphid also have been identified. According to Sanchez-Pena (1993), heavy aphid infestations in Arkansas have been greatly reduced by *Neozygites fresenii*. This fungus, along with *Arthrobotrys* spp. and *Verticillium lecanii*, has been observed in cotton fields in the Texas High Plains (Sanchez-Pena 1993). Though effective at times, these pathogens have been unable to consistently prevent aphid levels from exceeding economic thresholds.

Pesticides were once considered very effective in aphid control. Many times these chemicals were considered the only control option and

may have been too heavily relied upon. Since the heavy infestations of the mid seventies, cotton producers began applying insecticides to light, subeconomic populations (Rummel et al. 1995). While control was successful at first, a population of resistant aphids began to develop. Difficulty in controlling aphid populations was noted in 1989 (Allen et al. 1990) and again during the 1991 season. In 1991, signs of resistance to both organophosphate and pyrethroid insecticides were reported (Kidd 1995). Due to this development of resistance, very few insecticides exist today that are highly effective on the cotton aphid. In addition, pyrethroids, which are commonly used for control of the bollworm, *Helicoverpa zea* (Boddie), may promote aphid reproduction (Kidd et al. 1996). Kidd et al. (1996) suggested that rapid increases in aphid numbers should be expected in fields treated with pyrethroids.

Several cultural practices also have been studied for their effects on cotton aphid numbers. Rummel et al. (1995) reported fewer aphids on cotton planted in wheat straw mulch as compared to conventional cultivation plots. The same authors also suggested that early planting and uniform plant spacing may be helpful in aphid control. Two to four plants per foot for dryland production or three to five plants per foot for irrigated production

was recommended. Avoiding the use of excessive nitrogen may also benefit in preventing aphid population build-ups.

## Host Plant Resistance

The failure of natural, insecticidal, and cultural practices to consistently control the cotton aphid has led to a search for alternative control measures. One such control is host plant resistance. Host plant resistance can be defined as any inherited characteristic of a host plant which lessens the effects of parasitism (Russell 1978). While host resistance has always existed in nature, many producers and researchers have failed to take advantage of it. In recent years, however, it has gained popularity and has been successfully incorporated into many crops.

Reed et al. (1999) reported the screening of several cotton genotypes for aphid resistance. In the study, *Gossypium arboreum*, a wild diploid species, displayed a high level of resistance. These findings offer promise for the future development of aphid resistant cotton varieties. *G. arboreum* or other wild genotypes could serve as sources of an aphid resistant trait.

Painter (1951) divided the causes of resistance into three main groups: nonpreference, antibiosis, and tolerance. If host plant resistance is to be

incorporated into a cotton breeding program, an understanding of these mechanisms is necessary.

Nonpreference, sometimes referred to as antixenosis, may be influenced by plant allelochemic and morphological defenses (Metcalf and Luckman 1994). Many insects display feeding and oviposition preferences and avoid certain host plants for these reasons. Some glabrous cotton cultivars are less attractive to aphids (Weathersbee et al. 1994).

Antibiosis refers to the adverse effects on insect life history when a resistant plant variety is used for food (Painter 1951). Examples of these effects may be irregular growth rates and behavior, malformation, decreased fecundity, reduced fertility, and even death (Metcalf and Luckman 1994). Metcalf and Luckman (1994) also suggested that improper diets or the presence of toxic metabolites are possible explanations for these symptoms.

The third class of resistance is tolerance. Tolerance can be described as a plant's ability to compensate for insect injury that would otherwise cause greater damage to susceptible plants under the same conditions. Reasons for tolerance include plant vigor and regrowth of damaged tissues (Metcalf and Luckman 1994).

Whatever the mechanism utilized, it is clear that host plant resistance in cotton could be extremely beneficial as a contol measure for the cotton aphid. While it is possible for host resistance to serve as the principal control means, resistant varieties would most likely be used as an adjunct to other measures in an integrated pest management program. Insect resistant cultivars may require less frequent treatments as well as lower rates of pesticides. Reduced pesticide use also might decrease the chances for further development of insecticide resistant aphid populations.

Host plant resistance is not without its problems. The development of new insect biotypes is common. Biotypes are insect populations capable of damaging and surviving on plants previously known to be resistant to populations of the same species (Metcalf and Luckman 1994). Because of their high reproductive rate and rapid development, aphids frequently develop new biotypes.

Environmental factors sometimes affect the expression of resistance as well. Extreme temperatures, excessive shading, and changes in soil fertility have all caused loss of resistance expression in some crops (J. S. Armstrong, personal communication).

Despite these problems, host plant resistance should not be overlooked as a possibility for aphid control. Successful incorporation of aphid resistance into many other crops suggests that the same success should be achievable with cotton. The development of aphid-resistant cotton genotypes could benefit the entire cotton industry by eliminating many of the problems associated with heavy aphid infestation.

## Objectives

Several genotypes of cotton have been screened for aphid resistance at the Texas Agricultural Experiment Station in Lubbock, Texas, during recent years (Reed et al. 1999). These include several varieties of *Gossypium hirsutum* as well as *G. arboreum* and *G. herbaceum*. *G. arboreum* showed some resistance to the cotton aphid and other genotypes displayed varying levels of susceptibility (Reed et al. 1999).

While it is cultivated in some parts of Asia, *G. arboreum* is considered a wild cotton and possesses very poor agronomic properties. Bolls are extremely small, and when open, the lint and seed may fall to the ground. *G. arboreum* displays undesirable fiber properties as well. Even with an aphid-resistance trait, it could not compete with the high-yielding and stormproof

*G. hirsutum* cultivars grown in the United States. Genetic improvement of these agronomic properties within *G. arboreum* is unrealistic and would take far too long. In addition, *G. arboreum* is an Old World diploid species containing only 26 chromosomes and will not directly cross with the New World tetraploid species, including *G. hirsutum*, which have 52 chromosomes. Though it remains a possibility, incorporation of the *G. arboreum* aphid-resistance trait into modern commercial cultivars using traditional breeding methods is probably not worth the time and trouble it would take.

Today, however, the creation of transgenic plants has become a common practice. If the gene or genes responsible for conferring aphid resistance in *G. arboreum* could be identified, they could possibly be transferred into the DNA of cultivars that already possess the agronomic properties desired by cotton producers. This process, if successful, could minimize the time required to develop and release aphid resistant cotton varieties.

To aid in this process, the following research objectives were adopted:

1. To evaluate several wild cotton genotypes for aphid resistance,

2. To determine the inheritance of the aphid-resistance trait in resistant lines, and
3. To examine possible sources of aphid resistance.

# CHAPTER II

# MATERIALS AND METHODS

## General Information

A greenhouse served as the site for all research conducted in this study. To ensure adequate environmental conditions for both cotton plants and aphid populations, temperature was maintained at approximately 26.7°C (80°F) year-round. This was accomplished with a gas heater in the winter and an evaporative cooling system during the summer. Plant growth medium consisted of a 1:1 mixture of soil and potting soil. Plants received approximately 0.25 centimeters (0.1 inch) of water twice daily with a Rain Bird™ (Rain Bird Corporation, Glendora, CA) automatic watering system. Plants were grown in 15.24-cm (6-inch) pots for all tests. Plants for cross-pollination and seed increase were grown in 30.48-cm (12-inch) pots.

## Cotton Genotypes

Several cotton genotypes were evaluated in this study. These included commercial *G. hirsutum* varieties commonly grown on the Texas High Plains as well as varieties known to have certain leaf characteristics.

Several wild lines of the diploid species *G. arboreum* and *G. herbaceum* also were utilized. These were obtained from the National Collection of *Gossypium* Germplasm in College Station, Texas. Table 2.1 shows a list of each genotype tested and its origin.

## Aphid Populations

Cotton aphids for infestation tests were collected from cotton fields at the Texas Agricultural Experiment Station in Lubbock, Texas. Aphids were placed in predator-exclusion cages (Kidd and Rummel 1997) and reared year-round in the greenhouse. The cages consisted of a wooden frame covered with a fine 32x32 Lumite® (Style # 50060, Lumite Division of Synthetic Industries, Gainsville, GA) synthetic mesh screen. The Lumite® screen allowed 100% light penetration and did not adversely affect the insect or plant populations inside. Two cages in separate sections of the greenhouse were utilized to ensure the availability of a back-up aphid population if needed. Caged aphids were reared on commercial varieties 'Paymaster HS26' and 'Paymaster HS200'. Fresh plants were added to the cages as necessary.

Table 2.1. Designation and origin of all cotton genotypes tested.

| Cotton Genotype | Origin |
|---|---|
| 1. Herbaceum A1-20 | Afganistan |
| 2. Herbaceum A1-29 | Turkey |
| 3. Herbaceum A1-52 | India |
| 4. Herbaceum A1-63 | India |
| 5. Herbaceum A1-68 | India |
| 6. Herbaceum A1-84 | PRC China |
| 7. Herbaceum A1-124 | Uzbek SSR |
| 8. Herbaceum A1-127 | Switzerland |
| 9. Arboreum A2-19 | India |
| 10. Arboreum A2-29 | India |
| 11. Arboreum A2-20 | India |
| 12. Arboreum A2-34 | India |
| 13. Arboreum A2-40 | India |
| 14. Arboreum A2-42 | Korea |
| 15. Arboreum A2-45 | Pakistan |
| 16. Arboreum A2-55 | Pakistan |
| 17. Arboreum A2-61 | Japan |
| 18. Arboreum A2-63 | India |
| 19. Arboreum A2-71 | Pakistan |
| 20. Arboreum A2-75 | PRC China |
| 21. Arboreum A2-88 | India |
| 22. Arboreum A2-128 | unknown |
| 23. Arboreum A2-170 | India |
| 24. Arboreum A2-171 | Holland |
| 25. Deltapine 50 | Commercial variety |
| 26. Paymaster HS-26 | Commercial variety |
| 27. Paymaster 2326RR | Commercial variety |
| 28. Paymaster 145 | Commercial variety |
| 29. Paymaster 2145RR | Commercial variety |
| 30. Paymaster HS-200 | Commercial variety |
| 31. Paymaster 2200RR | Commercial variety |

## Test One

Twenty-eight cotton genotypes were planted in the greenhouse in a randomized complete block design (RBD). The cotton genotypes tested consisted of sixteen lines of the diploid species *G. arboreum*, eight lines of the diploid species *G. herbaceum*, and four commercial *G. hirsutum* varieties (Table 2.2).

Pots were fertilized at planting with 9.0 grams of Osmocote® 14-14-14 (Scotts-Sierra Horticultural Products Company, Marysville, OH.). Due to poor seed germination only three replications were utilized.

## Sugar Analysis

At the time of this study, ongoing research at the Texas Agricultural Experiment Station in Lubbock suggested that high leaf sugar levels might be detrimental to cotton aphids. To test if differences in sugar levels existed between the cotton genotypes in our study, the fifth true leaf was removed from each plant when the majority of all plants reached the pinhead square stage. This was accomplished by cutting the petiole at the base of the leaf with scissors. Leaves were placed in plastic bags, stored on dry ice and

Table 2.2. Cotton genotypes included in test one.

| *Arboreum* | *Herbaceum* | *Hirsutum* |
|---|---|---|
| A2-19 | A1-20 | Paymaster HS-26 |
| A2-20 | A1-29 | Paymaster 145 |
| A2-29 | A1-52 | Paymaster 2326 |
| A2-34 | A1-63 | Deltapine 50 |
| A2-40 | A1-68 | |
| A2-42 | A1-84 | |
| A2-45 | A1-124 | |
| A2-55 | A1-127 | |
| A2-61 | | |
| A2-63 | | |
| A2-71 | | |
| A2-75 | | |
| A2-88 | | |
| A2-128 | | |
| A2-170 | | |
| A2-171 | | |

transported to the International Textile Center in Lubbock for analysis. Leaves were then cut into quarters, and a wet weight was recorded. Half of the leaf was placed in hot 0.1% aqueous Triton X-100 solution and then placed in a stomacher machine for ten seconds. The prepared sample was then analyzed using a Dionex DX 500 High Performance Liquid Chromatograph (Dionex AG, Switzerland). The remaining half of the leaf was dried to obtain a dry leaf weight. Results were recorded as percent sugar based on dry leaf weight. Analysis of variance was performed using Agrobase® (Agronomix Software, Inc., Winnipeg, MB., Canada). The accepted level of error was determined to be $P$=0.05.

## Test Two

Twenty-four of the 28 cotton genotypes used in test one were planted in test two. *G. Herbaceum* A1-68 and A1-127 and *G. arboreum* A2-55 and A2-63 were not included due to the lack of seed. Because of the poor germination of seeds in test one, all seeds planted in test two were hot water treated. Seed were wrapped in cheesecloth and immersed in a hot water bath (80°C) for 1.5 minutes. Seed were allowed to dry and then planted. Pots

were fertilized at planting with 9.0 grams of Osmocote® (14-14-14). Layout was a randomized complete block design with four replications.

Sugar Analysis

When a majority of the plants reached the pinhead square stage, the fifth true leaf was clipped for sugar analysis. Leaf samples were stored, transported, and prepared in the same manner as in test one. HPLC analysis was performed at the International Textile Center in Lubbock. Analysis of variance with an accepted error level of $P$=0.05 was performed using Agrobase®.

Aphid Infestation

When the majority of plants reached the 1/3-grown-square stage (approximately one week after sugar analysis), all plants were infested with aphids. This was accomplished by manually placing five aphids on the terminal of each plant. Aphid counts were recorded at 7, 12, 17, 22, and 27 days after infestation. Results were recorded and ANOVA and LSD were performed using Agrobase® (critical p-value=0.05). Correlations between sugar levels and aphid numbers were also calculated.

## Test Three

Test three was designed to examine trichome (leaf hair) densities as a possible cause of aphid resistance in the tested genotypes. The three most resistant genotypes and the three most susceptible genotypes in test two were planted in test three. These included the following diploid lines: resistant- *herbaceum* A1-52 and A1-124 and *arboreum* A2-40; susceptible- *arboreum* A2-61, A2-19, and A2-128. Three commercial *G. hirsutum* varieties were also planted as checks: 'Paymaster 2145' (a pubescent variety), 'Paymaster 2200' (a glabrous variety), and 'Paymaster 2326' (a commercial standard for the Texas High Plains). Experimental design was a randomized complete block design (RBD) with four replications. Seed was hot-water treated and allowed to dry prior to planting. Each pot was fertilized at planting with 9.0 g of Osmocote® (14-14-14).

### Trichome Densities

Leaf samples were taken for trichome density counts when the plants reached the stage of first bloom. A 0.38 $cm^2$ hole punch was used to take two samples from the fifth and eighth true leaves of each plant. One sample was taken from the distal end of the leaf, and the other sample was taken

proximal to the petiole. These samples were placed on ice immediately after removal and stored until trichome counts could be taken. Trichomes were counted on the top and underside of each leaf sample using a 30X dissecting microscope. After counting, data were converted to obtain the number of trichomes per square centimeter of leaf area. Analysis of variance with an error level of P=0.05 was performed using Agrobase®.

Aphid Infestation

Immediately following removal of all trichome samples, each plant was artificially infested with five aphids. Aphids were manually placed on the terminal of each plant. Aphid counts were recorded at 10, 17, and 24 days after infestation. Analysis of variance was performed using Agrobase® and a significant level of error was defined to be P=0.05. Correlations between aphid levels and trichome densities were calculated.

## Test Four

*Herbaceum* lines A1-52 and A1-124 performed well in both aphid infestation tests and were chosen as resistant lines for an inheritance study. To determine the inheritance of the aphid-resistance trait, crosses were made

between resistant and susceptible lines. *Arboreum* lines A2-128, A2-61, and A2-19 performed poorly in the infestation tests and were chosen as susceptible lines. Seed from each of these genotypes were hot-water treated and planted in 30.48 cm (12 inch) pots. After seedling emergence, pots were fertilized on a weekly basis with Miracle Grow® 15-30-15 (Scotts Miracle-Gro Products, Inc., Port Washington, N.Y.). After the appearance of blooms, Miracle Grow Bloom Booster® 10-52-10 (Scotts Miracle-Gro Products, Inc., Port Washington, N.Y.) was used.

Crosses and reciprocal crosses were made between *herbaceum* A1-52 and all three susceptible lines. Crosses and reciprocals were also made between *herbaceum* A1-124 and each susceptible line. Flowers to be used as females were emasculated, covered with folded paper straws to prevent pollination, and flagged prior to 8:30 a.m. each morning. Male flowers were glued shut with selfing glue (acetone and cellulose acetate) and flagged prior to 8:30 a.m. each day as well. Crosses were made around noon each day, or when pollen sacs fully opened. Each cross was tagged, recorded and allowed to mature.

The seed and lint from each cross was hand-harvested, ginned and acid-delinted. Seed was then hot-water treated and replanted for increase.

Fertilization was repeated in the same manner as before. Flowers for increase were tagged and selfed to prevent cross pollination. When enough seed was available, parental seed, as well as F1 and F2 seed from each cross, were hot-water treated and planted in 15.24-cm (6-inch) pots for the final aphid infestation test. Three commercial varieties, 'Paymaster 2200', 'Paymaster 2326', and 'Paymaster 2145' were planted as checks. Pots were fertilized at planting with nine grams of Osmocote® (14-14-14) and arranged in a completely randomized design (CRD).

Final Infestation

Table 2.3 is a listing of each cotton genotype and the number of replications available for the final aphid infestation. When a majority of the plants reached the third true-leaf stage, five aphids were placed on the terminal of each plant. Aphid counts were taken at five, ten, and fifteen days after infestation. Maximum aphid population densities during the counting period were recorded for statistical analysis. To test differences between parental *herbaceum* and *arboreum* lines and the three commercial varieties, ANOVA and LSD were performed using SAS® (SAS Institute, Cary, N.C.). To determine if inheritance of the aphid resistance trait was influenced by

Table 2.3. List of each cotton genotype and the number of replications available for the final aphid infestation, Test Four.

| Cotton Genotype | No. Plants |
|---|---|
| A1-52 | 11 |
| A1-124 | 7 |
| A2-61 | 7 |
| A2-19 | 6 |
| A2-128 | 12 |
| Paymaster 2200 | 6 |
| Paymaster 2326 | 7 |
| Paymaster 2145 | 7 |
| A1-52XA2-61 (F1) | 19 |
| A2-61XA1-52 (F1) | 8 |
| Pooled F2 | 93 |
| A1-52XA2-19 (F1) | 0 |
| A2-19XA1-52 (F1) | 13 |
| Pooled F2 | 68 |
| A1-52XA2-128 (F1) | 15 |
| A2-128XA1-52 (F1) | 7 |
| Pooled F2 | 32 |
| A1-124XA2-61 (F1) | 13 |
| A2-61XA1-124 (F1) | 8 |
| Pooled F2 | 72 |
| A1-124XA2-19 (F1) | 16 |
| A2-19XA1-124 (F1) | 10 |
| Pooled F2 | 80 |
| A1-124XA2-128 (F1) | 22 |
| A2-128XA1-124 (F1) | 11 |
| Pooled F2 | 88 |

either parent, F1 reciprocals were compared using ANOVA. Chi-square analysis of aphid densities on the F2 plants was performed to test segregation ratios. The critical p-values in each statistical test was 0.05.

# CHAPTER III

# RESULTS AND DISCUSSION

## Test One

### Sugar Analysis

Figure 3.1 shows the mean leaf sugar composition of all cotton genotypes in test one. Sucrose levels were higher than any other sugar, averaging 0.86% of dry leaf weight, and accounted for approximately 59% of the total sugar in the leaves. Inositol comprised 23% of the total sugar and averaged 0.33% dry leaf weight. Glucose made up 11% total sugar at 0.16% dry leaf weight. Fructose accounted for 6% (0.09% dry leaf weight), maltose 1.0% (0.009% dry leaf weight), and trehalose just over 0 % (0.0018% dry leaf weight).

At the time of this study, ongoing research suggested that varying levels of leaf sugar might have an effect on the development of aphid populations in certain genotypes. Test One was performed with the hope of finding differences in leaf sugar amounts among the genotypes tested. Total sugar as a percent dry leaf weight for each genotype is shown in table 3.1. Significant differences did exist between genotypes (P=0.0063). Leaf sugar

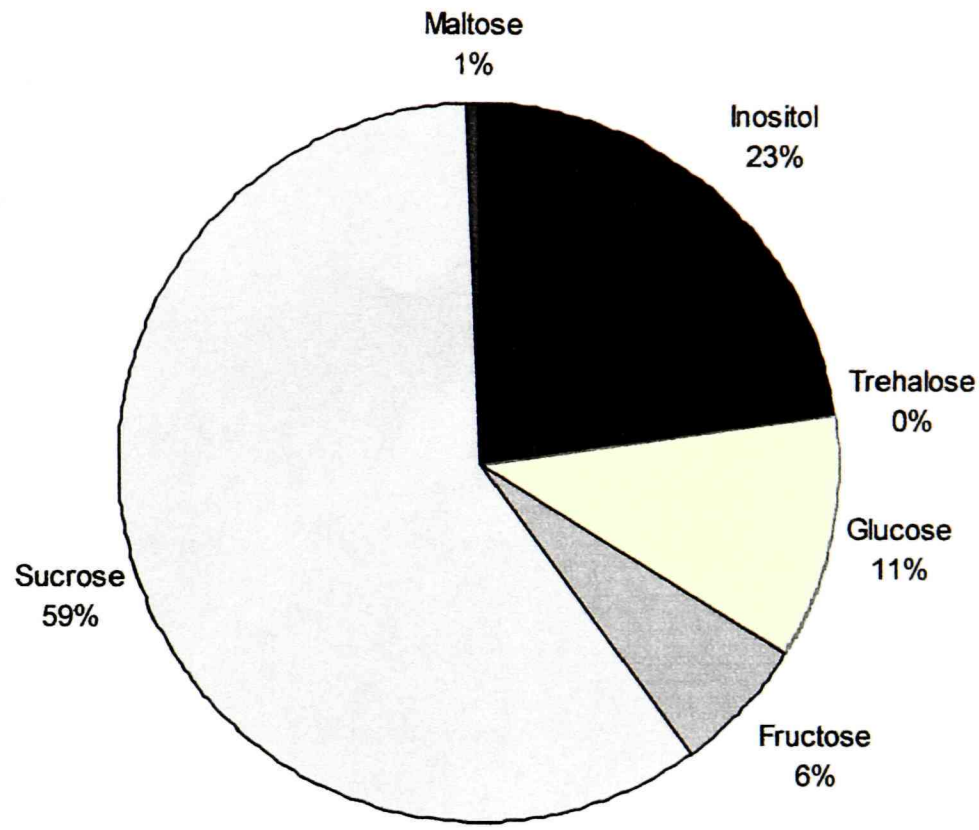


Figure 3.1. Mean leaf sugar composition of all cotton genotypes, Test one.

Table 3.1: Mean percent total sugar based on dry leaf weight. Means within a column followed by the same letter are not significantly different (P>0.05). Test One.

| Cotton Genotypes | Mean Percent Total Sugar |
|---|---|
| 1. Paymaster HS-26 | 2.65 a |
| 2. Herbaceum A1-63 | 2.01 ab |
| 3. Herbaceum A1-124 | 1.97 b |
| 4. Deltapine 50 | 1.85 bc |
| 5. Arboreum A2-45 | 1.83 bcd |
| 6. Arboreum A2-128 | 1.80 bcde |
| 7. Arboreum A2-88 | 1.69 bcde |
| 8. Herbaceum A1-68 | 1.69 bcde |
| 9. Arboreum A2-40 | 1.68 bcde |
| 10. Arboreum A2-171 | 1.64 bcdef |
| 11. Herbaceum A1-52 | 1.63 bcdef |
| 12. Arboreum A2-71 | 1.60 bcdefg |
| 13. Arboreum A2-19 | 1.57 bcdefg |
| 14. Herbaceum A1-20 | 1.54 bcdefgh |
| 15. Arboreum A2-29 | 1.43 bcdefghi |
| 16. Paymaster 2326 | 1.42 bcdefghi |
| 17. Herbaceum A1-127 | 1.38 bcdefghi |
| 18. Arboreum A2-63 | 1.24 cdefghi |
| 19. Arboreum A2-61 | 1.16 defghi |
| 20. Arboreum A2-42 | 1.14 efghi |
| 21. Arboreum A2-34 | 1.14 efghi |
| 22. Paymaster 145 | 1.13 efghi |
| 23. Arboreum A2-75 | 0.99 fghi |
| 24. Arboreum A2-55 | 0.95 ghi |
| 25. Arboreum A2-170 | 0.95 ghi |
| 26. Arboreum A2-20 | 0.93 ghi |
| 27. Herbaceum A1-84 | 0.88 hi |
| 28. Herbaceum A1-29 | 0.84 i |

amounts ranged from 2.65% total sugar in Paymaster HS-26 down to 0.84% total sugar in herbaceum A1-29. Paymaster HS-26 was significantly higher in leaf sugar than all other genotypes except herbaceum A1-63. Herbaceum A1-124 (1.97% total sugar) and Deltapine 50 (1.85% total sugar) had the third and fourth highest sugar levels. Herbaceum A1-29 (0.84% total sugar) and A1-84 (0.88% total sugar) had numerically less sugar than all other genotypes but were not significantly different from genotypes 14-26.

Sucrose levels ranged from 1.30 to 0.42% of dry leaf weight (Table 3.2). Significant differences did exist (P= 0.0088). 'Paymaster HS-26', arboreum A2-45, and herbaceum A1-63 ranked highest at 1.3% sucrose but were not statistically different from the top 14 genotypes. Arboreum A2-170 contained the least amount of sucrose in the leaves at 0.42%, but was not significantly different from the bottom 15 genotypes tested.

Inositol levels were not significantly different among the genotypes tested. Levels ranged from 0.53 to 0.15% of dry leaf weight. Arboreum A2-40 and herbaceum A1-124 had numerically higher inositol levels than other genotypes; whereas, the commercial varieties were low in inositol, ranking 24, 25, 26, and 28 out of 28 genotypes analyzed.

Table 3.2. Mean percent sucrose based on dry leaf weight. Means within a column followed by the same letter are not significantly different (P>0.05), Test One.

| Cotton Genotype | Mean Percent Sucrose |
|---|---|
| 1. Paymaster HS-26 | 1.30 a |
| 2. Arboreum A2-45 | 1.30 a |
| 3. Herbaceum A1-63 | 1.30 a |
| 4. Herbaceum A1-68 | 1.25 ab |
| 5. Arboreum A2-128 | 1.14 abc |
| 6. Arboreum A2-88 | 1.13 abc |
| 7. Herbaceum A1-124 | 1.12 abcd |
| 8. Herbaceum A1-20 | 1.11 abcd |
| 9. Deltapine 50 | 1.05 abcde |
| 10. Arboreum A2-19 | 1.02 abcdef |
| 11. Herbaceum A1-52 | 1.02 abcdef |
| 12. Arboreum A2-29 | 0.94 abcdefg |
| 13. Arboreum A2-40 | 0.92 abcdefgh |
| 14. Arboreum A2-71 | 0.86 abcdefghi |
| 15. Arboreum A2-171 | 0.82 bcdefghi |
| 16. Herbaceum A1-127 | 0.81 bcdefghi |
| 17. Paymaster 2326 | 0.79 bcdefghi |
| 18. Arboreum A2-34 | 0.72 cdefghi |
| 19. Paymaster 145 | 0.69 cdefghi |
| 20. Arboreum A2-61 | 0.67 defghi |
| 21. Arboreum A2-63 | 0.60 efghi |
| 22. Arboreum A2-75 | 0.57 fghi |
| 23. Arboreum A2-20 | 0.56 ghi |
| 24. Arboreum A2-42 | 0.54 ghi |
| 25. Herbaceum A1-84 | 0.48 hi |
| 26. Herbaceum A1-29 | 0.47 hi |
| 27. Arboreum A2-55 | 0.46 i |
| 28. Arboreum A2-170 | 0.42 i |

'Paymaster HS-26' was extremely high in glucose level with 0.82% of dry leaf weight (Table 3.3). This was significantly higher than all other genotypes ($P < .05$, F-value=4.61, df=27). 'Deltapine 50', 'Paymaster 2326', and 'Paymaster 145' were among the highest at 0.32, 0.29, and 0.17% glucose, respectively. Herbaceum A1-84 and arboreum A2-20 ranked at the bottom with only 0.03% glucose, but were not significantly different than the bottom 21 genotypes.

Significant differences also existed in fructose levels ($P = 0.0212$), (see Table 3.4). 'Paymaster HS-26' (0.29) had significantly higher levels than all other genotypes except 'Deltapine 50' (0.26% fructose). Herbaceum A1-124 ranked third at 0.17% fructose while A1-29, A2-29, A1-84, A2-170, and A2-20 had the lowest fructose levels with only 0.02% detected.

Maltose levels were non-significant among all genotypes tested. Levels ranged from 0.06% of dry leaf weight in 'Deltapine 50' down to no detection of maltose in several genotypes. All four commercial varieties contained at least 0.01% maltose.

No significant differences existed in trehalose levels. 0.01% was detected in A1-52, A2-42, A2-88, A2-29, and A2-19. No trehalose was detected in any other genotype tested.

Table 3.3. Mean percent glucose based on dry leaf weight. Means within a column followed by the same letter are not significantly different (P>0.05). Test One.

| Cotton Genotypes | Mean Percent Glucose |
|---|---|
| 1. Paymaster HS-26 | 0.82 a |
| 2. Deltapine 50 | 0.32 b |
| 3. Paymaster 2326 | 0.29 bcd |
| 4. Arboreum A2-171 | 0.25 bcdefg |
| 5. Arboreum A2-128 | 0.22 bcdefgh |
| 6. Herbaceum A1-124 | 0.21 bcdefghi |
| 7. Herbaceum A1-52 | 0.20 bcdefghij |
| 8. Arboreum A2-71 | 0.19 bcdefghijk |
| 9. Paymaster 145 | 0.17 bcdefghijk |
| 10. Herbaceum A1-63 | 0.16 bcdefghijk |
| 11. Herbaceum A1-127 | 0.14 cdefghijk |
| 12. Arboreum A2-75 | 0.14 cdefghijk |
| 13. Arboreum A2-19 | 0.14 cdefghijk |
| 14. Herbaceum A1-20 | 0.13 defghijk |
| 15. Arboreum A2-45 | 0.13 defghijk |
| 16. Arboreum A2-40 | 0.12 efghijk |
| 17. Herbaceum A1-68 | 0.12 efghijk |
| 18. Arboreum A2-88 | 0.11 fghijk |
| 19. Arboreum A2-34 | 0.11 fghijk |
| 20. Arboreum A2-63 | 0.10 ghijk |
| 21. Arboreum A2-42 | 0.10 ghijk |
| 22. Arboreum A2-55 | 0.08 hijk |
| 23. Arboreum A2-61 | 0.08 hijk |
| 24. Arboreum A2-170 | 0.05 ijk |
| 25. Herbaceum A1-29 | 0.04 jk |
| 26. Arboreum A2-29 | 0.04 jk |
| 27. Herbaceum A1-84 | 0.03 k |
| 28. Arboreum A2-20 | 0.03 k |

Table 3.4 Mean percent fructose based on dry leaf weight. Means within a column followed by the same letter are not significantly different (P>0.05). Test One.

| Cotton Genotype | Mean Percent Fructose |
|---|---|
| 1. Paymaster HS-26 | 0.29 a |
| 2. Deltapine 50 | 0.26 ab |
| 3. Herbaceum A1-124 | 0.17 bc |
| 4. Arboreum A2-71 | 0.14 cd |
| 5. Arboreum A2-171 | 0.13 cde |
| 6. Arboreum A2-128 | 0.13 cde |
| 7. Arboreum A2-42 | 0.11 cde |
| 8. Herbaceum A1-63 | 0.10 cde |
| 9. Herbaceum A1-52 | 0.10 cde |
| 10. Arboreum A2-40 | 0.09 cde |
| 11. Arboreum A2-75 | 0.09 cde |
| 12. Paymaster 2326 | 0.09 cde |
| 13. Arboreum A2-19 | 0.09 cde |
| 14. Arboreum A2-88 | 0.08 cde |
| 15. Herbaceum A1-127 | 0.07 cde |
| 16. Arboreum A2-45 | 0.07 cde |
| 17. Arboreum A2-63 | 0.07 cde |
| 18. Herbaceum A1-68 | 0.06 cde |
| 19. Arboreum A2-61 | 0.06 cde |
| 20. Paymaster 145 | 0.06 cde |
| 21. Herbaceum A1-20 | 0.06 cde |
| 22. Arboreum A2-55 | 0.05 cde |
| 23. Arboreum A2-34 | 0.03 de |
| 24. Herbaceum A1-29 | 0.02 e |
| 25. Arboreum A2-29 | 0.02 e |
| 26. Herbaceum A1-84 | 0.02 e |
| 27. Arboreum A2-170 | 0.02 e |
| 28. Arboreum A2-20 | 0.02 e |

## Test Two

### Sugar Analysis

Average sugar composition of all genotypes in test two is shown in Figure 3.2. Sucrose again comprised the largest portion of total sugar but only accounted for 33% (0.30% of dry leaf weight) of the total sugar. Glucose ranked second at 29% (0.27% of dry leaf weight), an increase of 18% over test one. Inositol remained about the same, comprising 21% of total sugar (0.19% of dry leaf weight) while fructose jumped by 10% to 16% (0.15% of dry leaf weight) in test two. Maltose and trehalose remained approximately the same at 1.0% (0.01%) and 0%, respectively.

Unlike test one, no significant differences existed in test two for total sugar. 'Paymaster 2326' ranked highest at 1.5% dry leaf weight, followed by arboreum A2-170 (1.31), A2-29 (1.19), and A2-19 (1.19). Herbaceum A1-29, arboreum A2-45, and herbaceum A1-20 ranked the lowest in total sugar at 0.50, 0.56, and 0.57%, respectively.

Sucrose levels were much lower in test two. 'Paymaster 2326' had the highest amount of sucrose in the leaves with 0.53% of its dry leaf weight. Arboreum A2-34 and A2-29 ranked second and third with 0.45% and 0.43% sucrose. Leaves of herbaceum A1-63 and arboreum A2-61 and A2-45

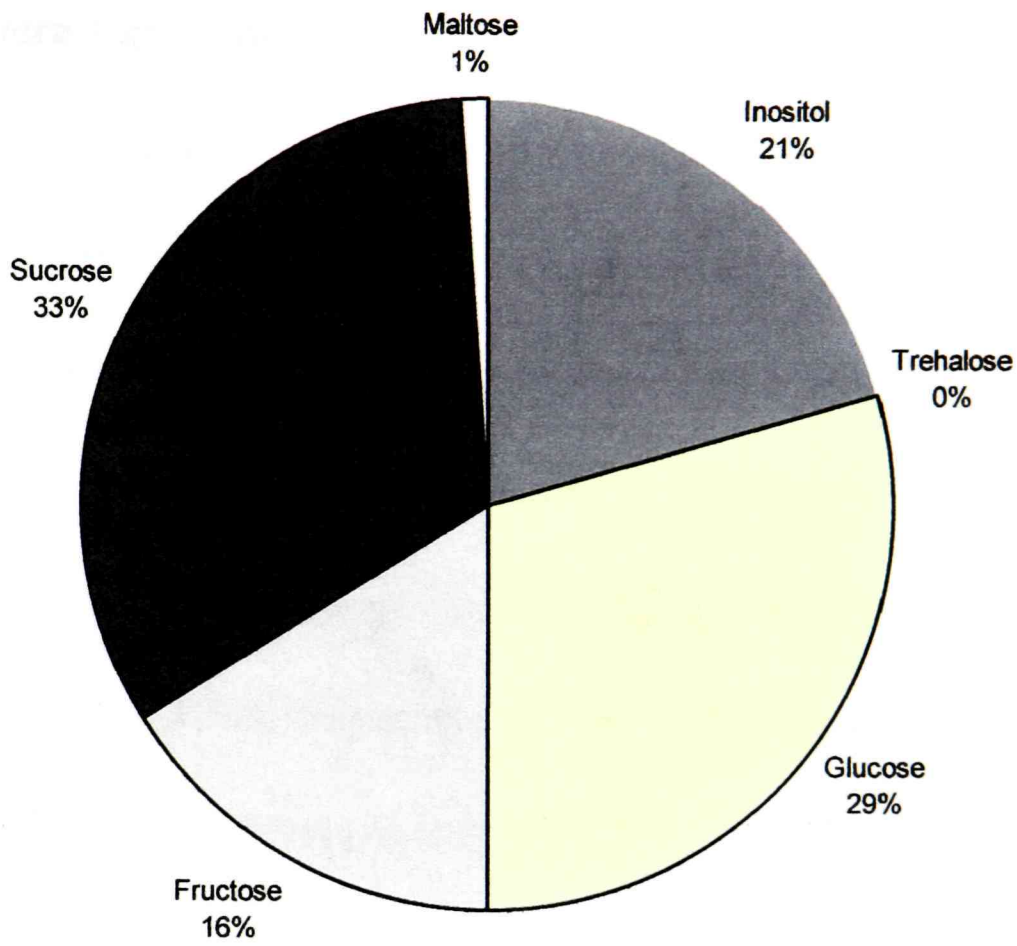


Figure 3.2. Mean leaf sugar composition of all cotton genotypes, Test two.

contained the least amount of sucrose at 0.18% and 0.19%. However, no differences were significant.

Inositol levels were again non-significant. A2-29, A1-84, and A2-71 had the highest levels with 0.28%, 0.27%, and 0.26% inositol. The commercial varieties were again low in inositol with 'Paymaster HS-26' and 'Deltapine 50' containing the least amounts of inositol (0.08% and 0.10%).

Glucose levels were not statistically different among the genotypes tested. Arboreum A2-170, 'Paymaster 2326', and 'Paymaster HS-26' did have numerically higher levels at 0.56%, 0.55%, and 0.39% glucose. Herbaceum lines A1-52, A1-20, and A1-29 were lowest containing 0.11%, 0.11%, and 0.09%.

Significant differences did exist in fructose levels (P= 0.0057; Table 3.5) . Arboreum A2-170 (0.36% fructose) was higher than all other genotypes except arboreum A2-40 (0.25% fructose) and A2-88 (0.24% fructose). Herbaceum lines A1-20 (0.05% fructose), A1-29 (0.06% fructose), and A1-52 (0.07% fructose) were lowest, but were not significantly different from the bottom 18 genotypes.

Significant differences did exist in maltose levels in test two (P= 0.0067). 'Paymaster 2326' was significantly higher than all other genotypes,

Table 3.5. Mean percent fructose based on dry leaf weight, Test two. Means within a column followed by the same letter are not significantly different (P>0.05).

| Cotton Genotype | Mean Percent Fructose |
|---|---|
| 1. Arboreum A2-170 | 0.36 a |
| 2. Arboreum A2-40 | 0.25 ab |
| 3. Arboreum A2-88 | 0.24 ab |
| 4. Arboreum A2-19 | 0.22 bc |
| 5. Paymaster 2326 | 0.19 bcd |
| 6. Arboreum A2-71 | 0.19 bcd |
| 7. Arboreum A2-171 | 0.18 bcde |
| 8. Arboreum A2-42 | 0.17 bcdef |
| 9. Arboreum A2-29 | 0.17 bcdef |
| 10. Arboreum A2-20 | 0.16 bcdefg |
| 11. Arboreum A2-61 | 0.15 bcdefg |
| 12. Paymaster 145 | 0.15 bcdefg |
| 13. Herbaceum A1-124 | 0.15 bcdefg |
| 14. Arboreum A2-34 | 0.14 bcdefg |
| 15. Arboreum A2-75 | 0.12 cdefg |
| 16. Herbaceum A1-84 | 0.12 cdefg |
| 17. Herbaceum A1-63 | 0.10 defg |
| 18. Arboreum A2-128 | 0.10 defg |
| 19. Paymaster HS-26 | 0.09 defg |
| 20. Arboreum A2-45 | 0.07 efg |
| 21. Deltapine 50 | 0.07 efg |
| 22. Herbaceum A1-52 | 0.07 efg |
| 23. Herbaceum A1-29 | 0.06 fg |
| 24. Herbaceum A1-20 | 0.05 g |

averaging 0.06% maltose (Table 3.6). Arboreum A2-128 and 'Paymaster 145' ranked second and third with 0.02%. No maltose was detected in arboreum A2-45 and herbaceum lines A1-29, A1-52 and A1-63.

No trehalose was detected in any of the genotypes analyzed in test two.

Aphid Infestation

Plants in test two were infested with aphids to test if differences in levels of resistance or susceptibility existed among genotypes (Figure 3.3). Aphid populations slowly began to increase after infestation. The grand mean number of aphids per plant increased from 7.96 aphids per plant at day 7 to 17.79 aphids per plant at day 12. A more dramatic increase was noticed at day 17 (50.68 aphids per plant) and continued throughout the check period (172.01 aphids per plant 22 days after infestation and 349.61 aphids per plant at day 27).

At 27 days after infestation (Table 3.7), differences in aphid numbers per plant as a result of genotype were significant at the probability level of 0.0409 (F-value=1.83, df=23). Herbaceum A1-52 had numerically fewer total aphids (9.67 per plant) than all other genotypes but was not statistically

Table 3.6. Mean percent maltose based on dry leaf weight, Test two. Means within a column followed by the same letter are not significantly different (P>0.05).

| Cotton Genotype | Mean Percent Maltose |
|---|---|
| 1. Paymaster 2326 | .06 a |
| 2. Arboreum A2-128 | .02 b |
| 3. Paymaster 145 | .02 b |
| 4. Paymaster HS-26 | .01 b |
| 5. ArboreumA2-171 | .01 b |
| 6. Deltapine 50 | .01 b |
| 7. Arboreum A2-170 | .01 b |
| 8. Arboreum A2-61 | .01 b |
| 9. Arboreum A2-40 | .01 b |
| 10. Arboreum A2-29 | .01 b |
| 11. Arboreum A2-75 | .01 b |
| 12. Arboreum A2-42 | .01 b |
| 13. Arboreum A2-19 | .01 b |
| 14. Arboreum A2-20 | .01 b |
| 15. Arboreum A2-34 | .01 b |
| 16. Arboreum A2-88 | .01 b |
| 17. Herbaceum A1-84 | .01 b |
| 18. Herbaceum A1-124 | .01 b |
| 19. Herbaceum A1-20 | .01 b |
| 20. Arboreum A2-71 | .01 b |
| 21. Arboreum A2-45 | .00 c |
| 22. Herbaceum A1-29 | .00 c |
| 23. Herbaceum A1-52 | .00 c |
| 24. Herbaceum A1-63 | .00 c |

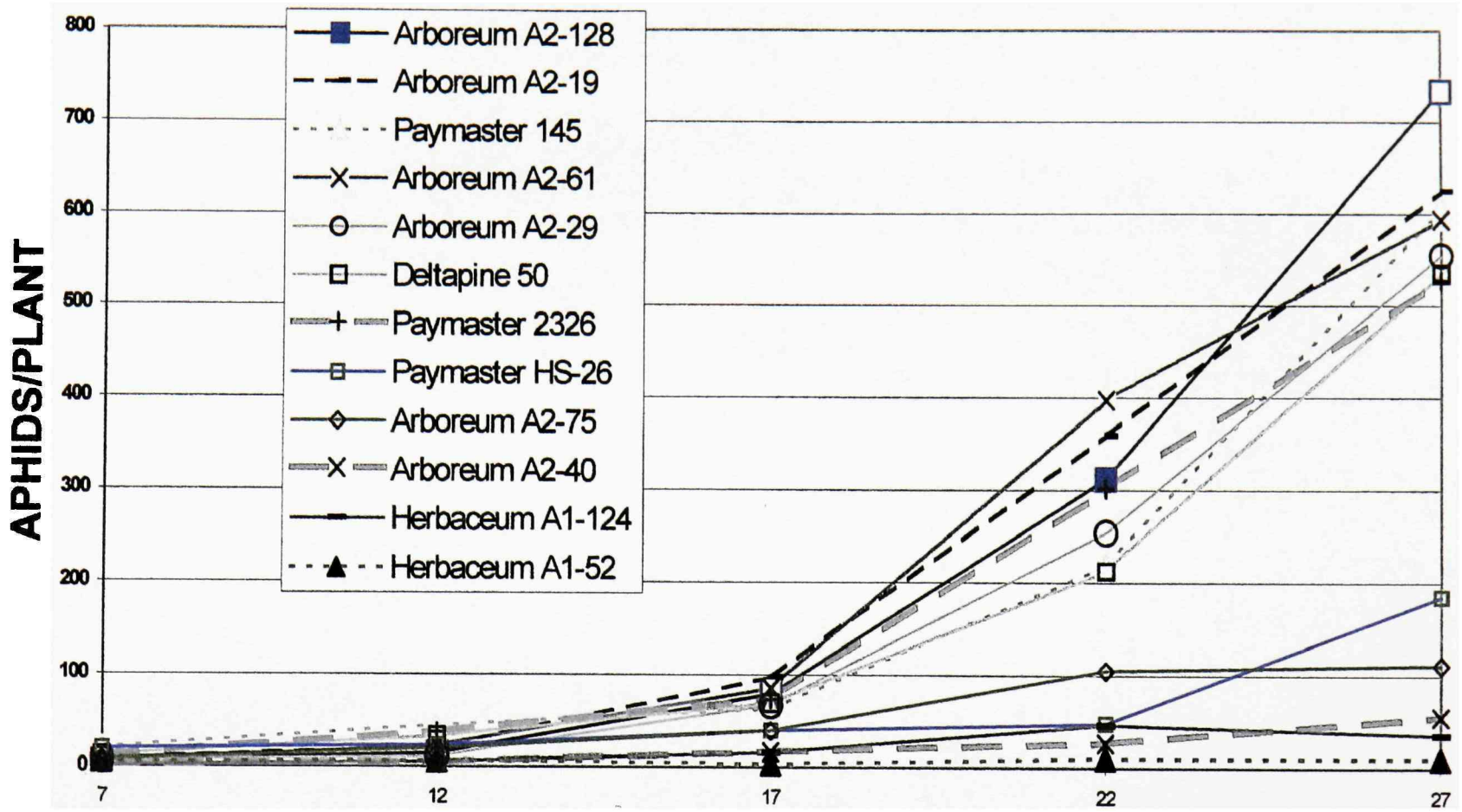


Figure 3.3. Aphid levels throughout the check period, Test two.

Table 3.7. Mean aphid levels at 27 days after infestation, Test two. Means within a column followed by the same letter are not significantly different (P>0.05).

| Cotton Genotype | Mean Aphids/Plant |
|---|---|
| 1. Herbaceum A1-52 | 9.67 h |
| 2. Herbaceum A1-124 | 35.67 gh |
| 3. Arboreum A2-40 | 55.33 gh |
| 4. Arboreum A2-75 | 111.33 fgh |
| 5. Arboreum A2-45 | 140.00 fgh |
| 6. Herbaceum A1-63 | 156.33 efgh |
| 7. Paymaster HS-26 | 184.33 defgh |
| 8. Arboreum A2-170 | 204.33 defgh |
| 9. Herbaceum A1-84 | 205.33 defgh |
| 10. Arboreum A2-88 | 229.67 cdefgh |
| 11. Arboreum A2-34 | 300.67 bcdefgh |
| 12. Arboreum A2-20 | 313.67 bcdefgh |
| 13. Arboreum A2-42 | 370.00 abcdefgh |
| 14. Arboreum A2-71 | 403.33 abcdefg |
| 15. Herbaceum A1-29 | 444.33 abcdef |
| 16. Arboreum A2-171 | 525.33 abcde |
| 17. Paymaster 2326 | 527.33 abcde |
| 18. Herbaceum A1-20 | 528.33 abcde |
| 19. Deltapine 50 | 535.00 abcd |
| 20. Arboreum A2-29 | 554.00 abcd |
| 21. Arboreum A2-61 | 593.67 abc |
| 22. Paymaster 145 | 606.00 abc |
| 23. Arboreum A2-19 | 623.67 ab |
| 24. Arboreum A2-128 | 733.33 a |

different than the top 13 performing genotypes, including 'Paymaster HS-26' (184.33 aphids per plant). Herbaceum A1-124 ranked second numerically with an average of only 35.67 aphids per plant. Arboreum lines A2-128 and A2-19 had more aphids per plant than all other genotypes averaging 733.33 and 623.67 aphids, but were not significantly different than several other lines, including 'Paymaster 2326' (527.33 aphids per plant), 'Deltapine 50' (535.00 aphids per plant), and 'Paymaster 145' (606.00 aphids per plant).

Sugar/Aphid Correlation

Correlations between leaf sugar levels and number of aphids per plant were recorded for test two (Table 3.8). A significant positive correlation existed between leaf maltose level and number of aphids per plant for all check dates. Based on these results, one would expect higher aphid populations to develop on plants with high leaf maltose levels. No other sugars tested showed a significant correlation with aphid numbers per plant.

Table 3.8. Correlations between leaf sugar levels and number of aphids per plant, Test two.

| | Inositol | Trehalose | Glucose | Fructose | Sucrose | Maltose | Total Sugar |
|---|---|---|---|---|---|---|---|
| Day 7 | -0.1944 | -0.2597 | 0.2085 | -0.1250 | 0.0704 | 0.3285 | 0.0592 |
| p-value | 0.1068 | 0.0299 | 0.0833 | 0.3027 | 0.5623 | 0.0055 | 0.6263 |
| Day 12 | -0.1273 | -0.0962 | 0.1837 | -0.1601 | 0.0639 | 0.4582 | 0.0562 |
| p-value | 0.2938 | 0.4281 | 0.1279 | 0.1854 | 0.5991 | 0.0001 | 0.6437 |
| Day 17 | 0.0506 | 0.0276 | 0.1433 | -0.0210 | 0.1374 | 0.2393 | 0.1286 |
| p-value | 0.6777 | 0.8208 | 0.2366 | 0.8631 | 0.2567 | 0.0461 | 0.2886 |
| Day 22 | 0.0400 | 0.0964 | 0.1439 | 0.0188 | 0.0974 | 0.2755 | 0.1204 |
| p-value | 0.7420 | 0.4271 | 0.2346 | 0.8773 | 0.4223 | 0.0210 | 0.3206 |
| Day 27 | -0.0267 | 0.0114 | 0.1325 | -0.0522 | -0.0415 | 0.3432 | 0.0307 |
| p-value | 0.8264 | 0.9254 | 0.2742 | 0.6679 | 0.7329 | 0.0036 | 0.8011 |
| Season Mean | -0.0055 | 0.0358 | 0.1505 | -0.0346 | 0.0210 | 0.3443 | 0.0724 |
| p-value | 0.9639 | 0.7684 | 0.2135 | 0.7762 | 0.8628 | 0.0035 | 0.5515 |

## Test Three

### Trichome Density

Leaf pubescence was studied in an attempt to determine possible sources of aphid-resistance. Because some glabrous varieties are known to display some aphid-resistance, trichome density counts were taken to determine if differences in numbers of leaf trichomes might exist among the genotypes tested. Leaf trichomes were counted for both the top and underside of the fifth and eighth true leaves of each plant. 'Paymaster 2145', a pubescent variety, and 'Paymaster 2200', a glabrous variety, were included as commercial standards. Trichome densities ranged from a very "smooth" leaf in 'Paymaster 2200' to an extremely "hairy" leaf in arboreum A2-19. Significant differences did exist among genotypes for all leaf locations tested ($P < 0.05$). Results can be seen in Table 3.9.

Arboreum A2-19 had significantly more trichomes than all other genotypes except arboreum A2-128, averaging 274.23 trichomes per centimeter$^2$ on the underside of the fifth true leaf ($P<0.001$, F-value=64.23, df=8). Herbaceum A1-124, A1-52, and arboreum A2-128 displayed a significantly higher trichome density than all other genotypes except A2-19, with 179.73, 167.65, and 162.45 trichomes per centimeter$^2$, respectively.

Table 3.9 Trichome densities per $cm^2$ on the under-side of the fifth true leaf, Test three. Means within a column followed by the same letter are not significantly different (P>0.05).

| Cotton Genotype | Trichome Density |
|---|---|
| 1. Arboreum A2-19 | 274.23 a |
| 2. Arboreum A1-124 | 179.73 b |
| 3. Herbaceum A1-52 | 167.65 b |
| 4. Herbaceum A2-128 | 162.45 b |
| 5. Arboreum A2-61 | 123.10 c |
| 6. Paymaster 2145 | 99.98 cd |
| 7. Arboreum A2-40 | 83.23 d |
| 8. Paymaster 2326 | 20.18 e |
| 9. Paymaster 2200 | 6.68 e |

These counts were significantly higher than the pubescent commercial standard, 'Paymaster 2145', which averaged 99.98 trichomes per centimeter$^2$. 'Paymaster 2326' (20.18 trichomes per centimeter$^2$) and 'Paymaster 2200' (6.68 trichomes per centimeter$^2$) had statistically fewer trichomes than any other genotype.

Average number of trichomes per centimeter$^2$ followed the same pattern for the topside of the fifth true leaf as well as both sides of the eighth true leaf (Figure 3.4). Differences in trichome density among leaf locations tested were not significant.

Aphid Infestation

Aphid numbers increased dramatically during test three (Figure 3.5). Significant differences existed among genotypes at each post-infestation check date. Average number of aphids per plant increased from 244 at 10 days after infestation to 1087 at 24 days after infestation.

Herbaceum A1-52 proved to be the most resistant to the cotton aphid at each count date throughout the season. A1-52, however, was not significantly different than herbaceum A1-124 and arboreum A2-19. At the third check date (Table 3.10), A1-52 averaged 215.25 aphids per plant, A1-

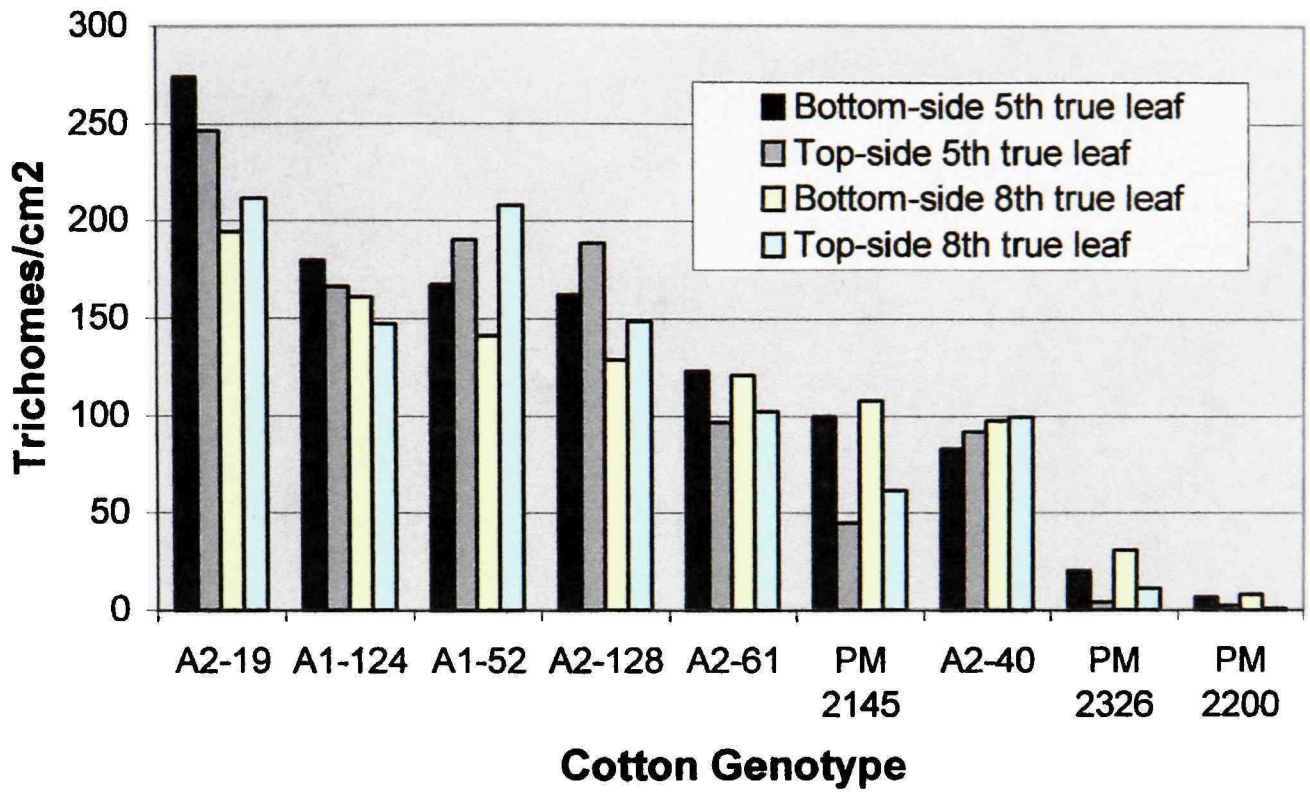


Figure 3.4. Trichome densities for each leaf location tested, Test three.

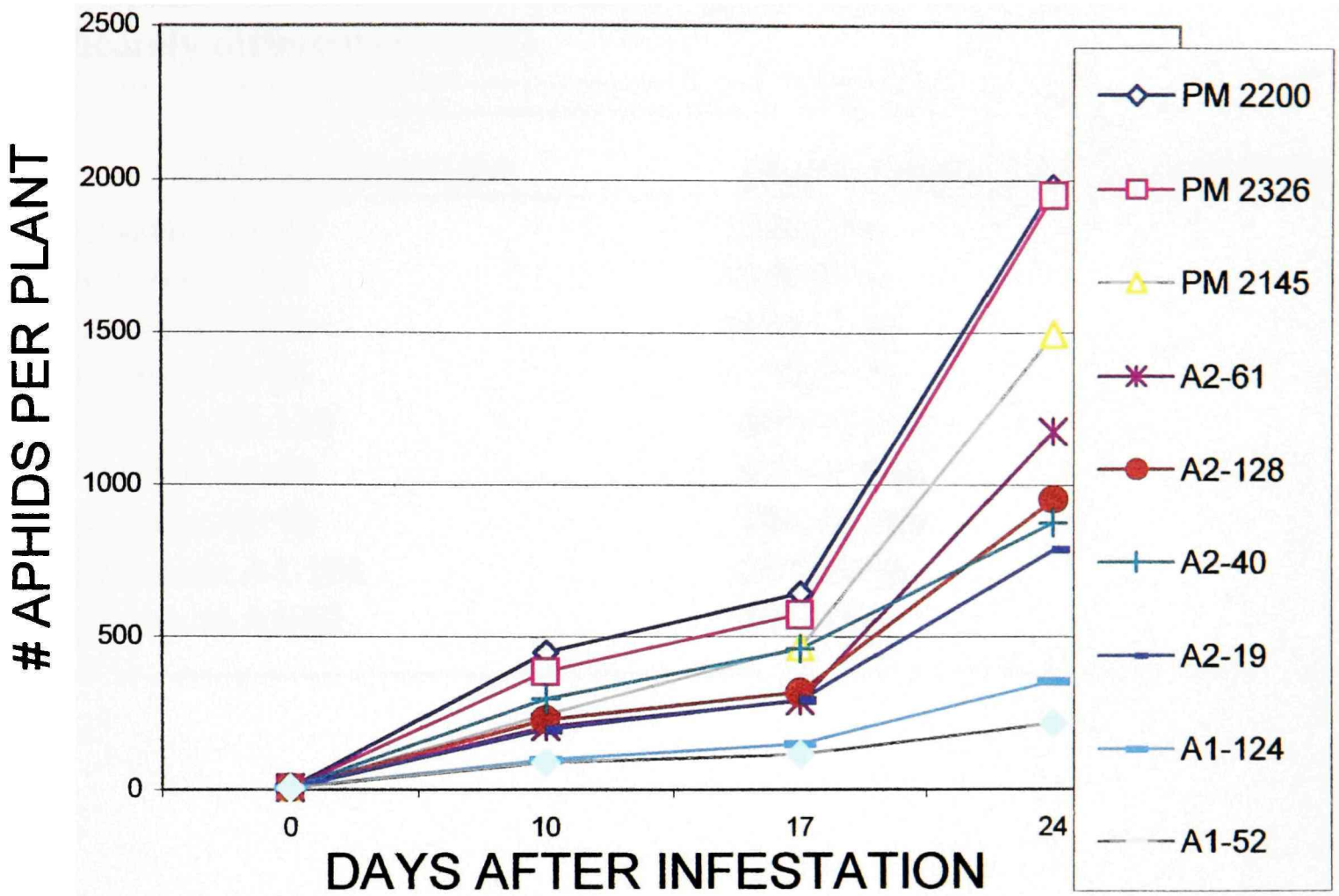


Figure 3.5. Aphid levels at each check date, Test three.

Table 3.10. Mean aphid population levels at 24 days after infestation, Test three. Means within a column followed by the same letter are not significantly different (P>0.05).

| Cotton Genotype | Mean Number of Aphids/Plant |
|---|---|
| 1. Paymaster 2200 | 1980.25 a |
| 2. Paymaster 2326 | 1950.00 a |
| 3. Paymaster 2145 | 1494.75 ab |
| 4. Arboreum A2-61 | 1175.00 bc |
| 5. Arboreum A2-128 | 950.00 bcd |
| 6. Arboreum A2-40 | 875.00 bcd |
| 7. Arboreum A2-19 | 785.25 cde |
| 8. Herbaceum A1-124 | 356.75 de |
| 9. Herbaceum A1-52 | 215.25 e |

124 averaged 356.75 aphids per plant, and arboreum A2-19 averaged 785.25 aphids per plant. 'Paymaster 2200' and 'Paymaster 2326' had more total aphids per plant than any other genotype except 'Paymaster 2145'. Aphid numbers were similar on 'Paymaster 2200' and 'Paymaster 2326' averaging 1980.25 and 1950.00, respectively. 'Paymaster 2145' (1494.75 aphids per plant) was not statistically different than the bottom six most susceptible genotypes tested.

Aphid/Trichome Correlation

Correlations between number of trichomes per leaf and number of aphids per plant were analyzed. Some smooth leaf (glabrous) varieties are thought to be less attractive to cotton aphids. Based on that knowledge, it was expected that a positive correlation would exist between trichome density and aphid numbers. However, a significant negative correlation existed between seasonal mean number of aphids per plant and trichome density ($P < 0.0001$). These results were opposite of what was expected (Figure 3.6). Large numbers of aphids were found on the two genotypes with the lowest trichome densities. Herbaceum A1-52 and A1-124, the two

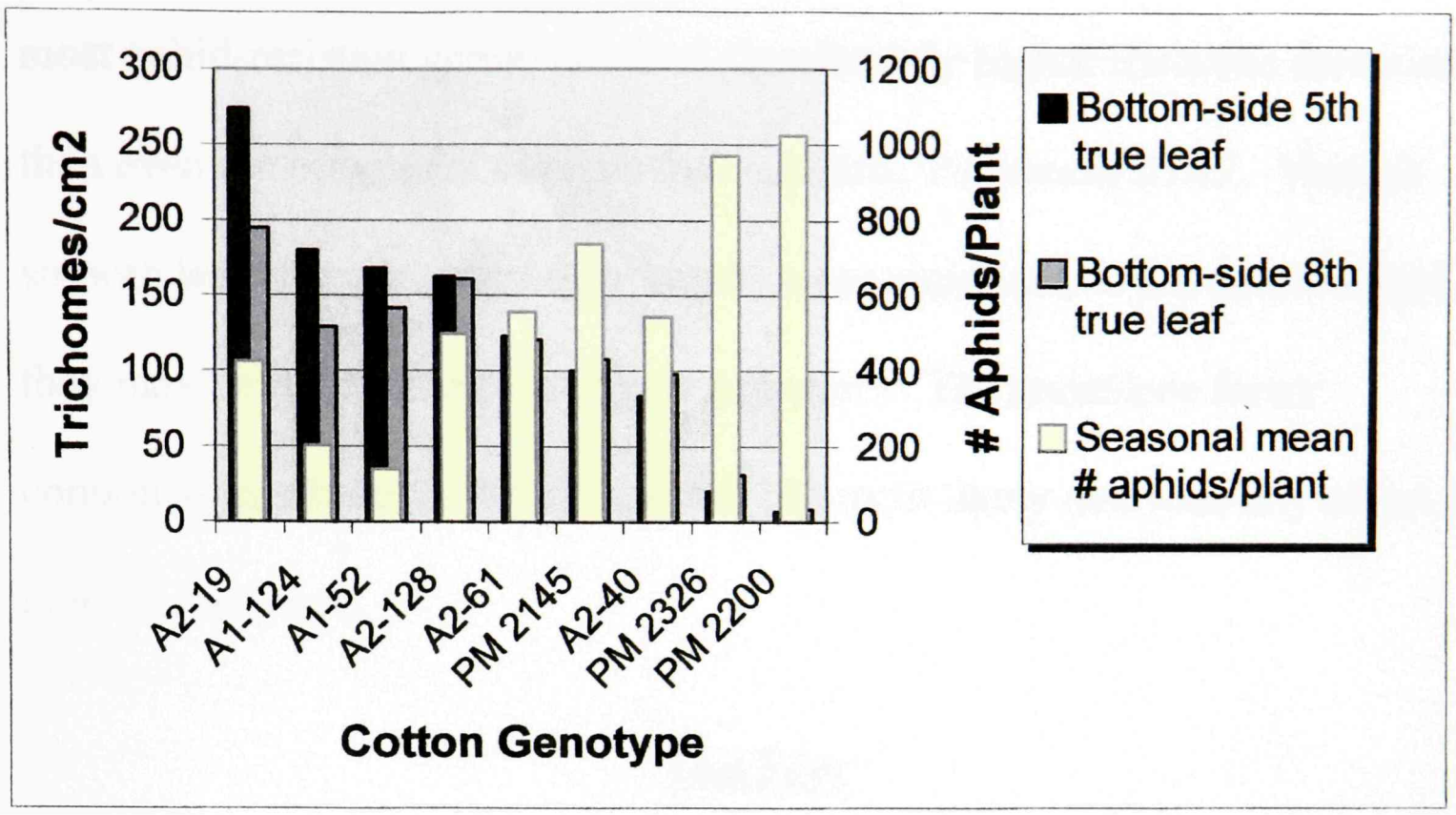


Figure 3.6. Mean trichome density levels on the bottom-side of the fifth and eighth true leaves and seasonal mean aphid levels for each cotton genotype, ($P<0.05$), Test three.

most aphid-resistant genotypes, had significantly higher trichome densities than even the pubescent commercial standard, 'Paymaster 2145'. Though smooth leaf characteristics may impart some resistance to the cotton aphid, they may be easily overcome by other factors. The resistance factor contained in arboreum A1-52 and A1-124 most likely overrode any effect of plant pubescence.

## Test Four

### Aphid Infestation

Peak aphid densities per plant were compared for the five parental lines and the three commercial varieties in test four. Results can be seen in Table 3.11. Significant differences existed among genotypes ($P < .0001$, F-value=7.81, df=7). Herbaceum A1-124 out-performed all other genotypes averaging only 23.71 aphids per plant. Herbaceum A1-52 ranked second with only 29.00 aphids per plant followed by arboreum A2-19 (37.67 aphids per plant). Number of aphids per plant on these genotypes was significantly different from all other genotypes ($P < 0.05$). The commercial varieties averaged more total aphids per plant than all genotypes, but were not statistically different from arboreum A2-128 and A2-61. 'Paymaster 2145'

Table 3.11. Aphid levels, Test four. Means within a column followed by the same letter are not significantly different (P>0.05).

| Cotton Genotype | Aphids/Plant |
|---|---|
| 1. Paymaster 2145 | 176.71 a |
| 2. Paymaster 2200 | 170.17 a |
| 3. Paymaster 2326 | 169.14 a |
| 4. Arboreum A2-128 | 139.00 a |
| 5. Arboreum A2-61 | 130.71 a |
| 6. Arboreum A2-19 | 37.67 b |
| 7. Herbaceum A1-52 | 29.00 b |
| 8. Herbaceum A1-124 | 23.71 b |

averaged 176.71 aphids per plant, followed by 'Paymaster 2200' (170.17 aphids per plant) and 'Paymaster 2326' (169.14 aphids per plant). Arboreum A2-128 and A2-31 were slightly lower with 139.00 and 130.71 aphids per plant, respectively.

Inheritance Study

Peak numbers of aphids per plant were recorded for F1 plants derived from reciprocal crosses between resistant and susceptible genotypes. No significant differences existed among reciprocals for any combination ($P>0.05$), see Table 3.12. Therefore, the expression of aphid resistance in progeny from crosses made in this study was not maternally influenced. In addition, results indicate that neither resistance nor susceptibility is completely dominant.

Peak number of aphids per plant were also recorded for the F2 plants in test four. Because the aphid resistance trait is not maternally influenced, F2 progeny for each cross were pooled together. It was hoped that progeny would segregate into distinct groups, based on varying levels of aphid resistance. Chi-square analysis would then be used to test those groups against certain ratios for goodness of fit. Instead, however, there seemed to

Table 3.12. Mean aphid populations for F1 plants derived from reciprocal crosses (P>0.05), Test four.

| Cotton Genotype | Mean # Aphids/Plant | P>F |
|---|---|---|
| A1-52XA2-61 (F1) | 106.84 | |
| A2-61XA1-52 (F1) | 118.00 | 0.7729 |
| A1-52XA2-19 (F1) | 76.67 | |
| A2-19XA1-52 (F1) | 54.92 | 0.362 |
| A1-52XA2-128 (F1) | 55.67 | |
| A2-128XA1-52 (F1) | 107.67 | 0.1471 |
| A1-124XA2-61(F1) | 119.54 | |
| A2-61XA1-124(F1) | 82.50 | 0.2288 |
| A1-124XA2-19 (F1) | 86.31 | |
| A2-19XA1-124 (F1) | 98.30 | 0.7004 |
| A1-124XA2-128 (F1) | 57.14 | |
| A2-128XA1-124 (F1) | 44.18 | 0.4554 |

be a continuous range in progeny from resistant to highly susceptible (Table 3.13). These results suggest that the aphid resistance trait found in herbaceum A1-52 and herbaceum A1-124 may be more complex, possibly resulting from multiple genes. This would make the process of identifying and locating the genes responsible for aphid resistance much more difficult. It was noted, however, that the resistance trait in herbaceum A1-124 was greatly masked by the susceptible parent in comparison to herbaceum A1-52. In herbaceum A1-52, more progeny performed better than or equal to the resistant parent than in herbaceum A1-124. Therefore, herbaceum A1-52 may be more valuable as a source of aphid resistance than herbaceum A1-124.

Table 3.13. Summary of F2 aphid infestation, Test four.

| | # Aphids/Plant | |
|---|---|---|
| F2 Progeny | Mean | Range |
| A1-52XA2-128 | 92 | 9 - 275 |
| A1-52XA2-61 | 124 | 3 - 500 |
| A1-52XA2-19 | 49 | 8 - 260 |
| A1-124XA2-128 | 201 | 50 - 500 |
| A1-124XA2-61 | 182 | 39 - 450 |
| A1-124XA2-19 | 153 | 19 - 450 |

# CHAPTER IV

# CONCLUSIONS

Varying levels of aphid-resistance and susceptibility existed among cotton genotypes throughout this study. Herbaceum lines A1-52 and A1-124 clearly and repeatedly demonstrated host plant resistance to the cotton aphid. These two genotypes consistently out-performed all other genotypes in each of the infestation trials. Other tested cotton genotypes developed high aphid populations in one or more of the infestation tests, and are therefore considered susceptible to the cotton aphid.

Attempts to determine the exact mechanism of resistance in these genotypes were unsuccessful. Although differences in leaf sugar levels did exist among genotypes, a high level of variation between tests also existed. Variations could be due to environmental factors such as daylength, temperature, and soil fertility, or other unknown factors. A significant correlation did exist between maltose levels and aphid numbers. However, further research is necessary, and should be considered, to determine the exact effects of maltose on aphid populations.

Trichome density results were opposite of what was expected. Smooth leaves have been found to retard aphid population development, and it was expected that the same would be true in this study. However, smooth leaf genotypes developed larger aphid populations than hairy genotypes. The two most aphid-resistant genotypes, herbaceum A1-52 and A1-124, had higher trichome densities than all genotypes except arboreum A2-19. The aphid-resistance trait found in A1-52 and A1-124 appears to over-ride any effect of plant pubescence on aphid population development. Nevertheless, it is clear that high aphid populations are capable of developing on either hairy or smooth leaf cotton genotypes.

Results of the inheritance study indicate that the resistance trait found in herbaceum A1-52 and A1-124 may be complex. It was anticipated that F2 progeny would segregate into distinct groups, ranging from resistant to susceptible. Instead, however, progeny followed a continuous range from resistant to susceptible, failing to establish groups. These results suggest that further research with significantly larger plant populations may be necessary to determine the inheritance of this aphid resistance trait.

While the mechanism and inheritance of the aphid-resistance trait found in herbaceum A1-52 and A1-124 may not be understood, attention

should be given to the fact that high aphid populations failed to develop on these two genotypes throughout this study. Herbaceum A1-52 and A1-124 could serve as valuable sources in the development of future aphid-resistant cotton varieties.

# REFERENCES CITED


Akey, D. H., and G. D. Butler, Jr. 1989. Developmental rates and fecundity of apterous *Aphis gossypii* on seedlings of *Gossypium hirsutum*. Southwest. Entomol. 14: 295-299.

Allen, C. T., W. L. Multer, and V. Lucero. 1990. Seasonal changes in cotton aphid susceptibility to insecticides in west Texas. pp. 287-289. In Proc. Beltwide Cotton Conf. Nat. Cotton Council of Amer. Memphis, TN.

Armstrong, J. S. 1999. Personal Communication. Texas Tech University, Lubbock, TX.

Auclair, J. L. 1963. Aphid feeding and nutrition. Ann. Rev. Entomol. 8: 439-440.

Chapman, R. F. 1982. The insects: structure and function. 3rd. Ed. Harvard University Press, Cambridge, MA. pp. 919.

Drees, B. M. Aphid management. http://extension-horticulture.tamu.edu/greenhouse/pest/bdaphid.html.

Ewing, K. P. 1943. Cotton aphid damage and control in Texas. J. Econ. Entomol. 36: 598-601.

Freeman, B. L., and R. H. Smith. Cotton aphids. http://www.acesag.auburn.edu/department/ipm/aphid.htm.

Fuchs, T. W., and R. Minzenmayer. 1995. Effect of *Aphis gossypii* on cotton development and yield in West Texas. Southwest. Entomol. 20: 341-349.

Godfrey, L. D. and J. F. Leser. 1999. Cotton aphid management- status and needs. pp. 37-42. In Proc. Beltwide Cotton Conf. Nat. Cotton Council of Amer. Memphis, TN.

Hales, D. F. and T. E. Mittler. 1986. Chromosomal sex determination in aphids controlled by juvenile hormone. Genome. 29: 107-109.

Hardee, D. D., and G. A. Herzog. 1992. 45th annual conference report on cotton insect research and control. pp. 626-644. In Proc. Beltwide Cotton Conf. Nat. Cotton Council of Amer. Memphis, TN.

Hardee, D. D., M. T. Smith, and A. A. Weathersbee III. 1993. Sampling of the cotton aphid, *Aphis gossypii* Glover, in cotton. pp. 738-740. In Proc. Beltwide Cotton Conf. Nat. Cotton Council of Amer. Memphis, TN.

Hardee, D. D., M. T. Smith, A. A. Weathersbee III, and G. L. Snodgrass. 1994. Sampling of the cotton aphid (Homoptera: Aphididae) in cotton. Southwest. Entomol. 19: 33-44.

Isely, D. 1946. The cotton aphid. Ark. Ag. Exp. Sta. Bull. No. 462.

Kennedy, J. S., and H. L. G. Stroyan. 1959. Biology of aphids. Ann. Rev. Entomol. 4: 139-160.

Kidd, P. W. 1995. Impact of insect predators and a pyrethroid insecticide on field populations of the cotton aphid, *Aphis gossypii* Glover, in Texas High Plains cotton. M.S. Thesis, Entomology, Texas Tech University.

Kidd, P. W, and D. R. Rummel. 1997. Effect of insect predators and a pyrethroid insecticide on cotton aphid, *Aphis gossypii* Glover, population density. Southwest. Entomol. 22: 381-393.

Kidd, P. W., D. R. Rummel, and H. G. Thorvilson. 1996. Effect of cyhalothrin on field populations of the cotton aphid, *Aphis gossypii* Glover, in the Texas High Plains. Southwest. Entomol. 21: 293-301.

Kring, J. B. 1959. The life cycle of the melon aphid, *Aphis gossypii* Glover, an example of facultative migration. Ann. Entomol. Soc. Amer. 52: 284-286.

Leser, J. F., C. T. Allen, and T. W. Fuchs. 1992. Cotton aphid infestations in west Texas: A growing management problem. pp. 823-827. In Vol. II. Proc. Beltwide Cotton Conf. Nat. Cotton Council of Amer. Memphis, TN.

Metcalf, R. L., and W. H. Luckman. 1994. Introduction to Insect Pest Management, 3rd. Ed. John Wiley & Sons, Inc., New York.

O'Brien, P. J., M. B. Stoetzel, and D. D. Hardee. 1990. Verification of male and oviparous morphs of the cotton aphid in mid-south cotton (*Gossypium hirsutum* L.). J. Entomol. Sci. 25: 73-74.

O'Brien, P. J., M. B. Stoetzel, B. R. Leonard, and J. B. Graves. 1992. Taxonomy and biology of *Aphis gossypii* Glover in the mid-south. pp. 646-648. In Proc. Beltwide Cotton Conf. Nat. Cotton Council of Amer. Memphis, TN.

O'Brien, P. J., M. B. Stoetzel, R. C. Navasero, and J. B. Graves. 1993. Field biology studies of the cotton aphid, *Aphis gossypii* Glover. Southwest. Entomol. 18(1): 25-35.

Painter, R. H. 1951. Insect Resistance in Crop Plants. The Macmillan Co., New York.

Palmer, M. A. 1952. Aphids of the Rocky Mountain Region. A. B. Hirschfeld Press, Denver, CO. Thomas Say Foundation, Vol. V. 452pp.

Puterka, G. J. and J. E. Slosser. 1983. Inducing oviparae and males of biotype C greenbugs, *Schizphid graminum* (Rond). Southwest. Entomol. 8: 268-272.

Reed, B., J. Gannaway, D. R. Rummel, and H. G. Thorvilson. 1999. Screening for resistance in cotton genotypes to *Aphis gossypii* Glover, the cotton aphid. pp. 1002-1007. In Proc. Beltwide Cotton Conf. Nat. Cotton Council of Amer. Memphis, TN.

Rosenheim, J. A., K. J. Fuson, and L. D. Godfrey. 1995. Cotton aphid biology, pesticide resistance, and management in the San Joaquin Valley. In Proc. Beltwide Cotton Conf. Nat. Cotton Council of Amer. Memphis, TN.

Rummel, D. R. 1975. Focus on entomology. Texas Agri. Ext. Ser. Newsletter. 14: 7 pp.

Rummel, D. R., M. D. Arnold, J. E. Slosser, K. C. Neece, and W. E. Pinchak. 1995. Cultural factors influencing the abundance of *Aphis gossypii* Glover in Texas High Plains cotton. Southwest. Entomol. 20: 395-406.

Russell, G. E. 1978. Plant Breeding for Pest and Disease Resistance. Butterworth and Co., Boston, MA.

Sanchez-Pena, S. R. 1993. Entomogenous fungi associated with the cotton aphid in the Texas High Plains. Southwest. Entomol. 18: 69-71.

Slosser, J. E., W. E. Pinchak, and D. R. Rummel. 1989. A review of known and potential factors affecting the population dynamics of the cotton aphid. Southwest. Entomol. 14(3): 302-313.

Stoetzel, M. B., G. L. Miller, P. J. O'Brien, and J. B. Graves. 1996. Aphids (Homoptera: Aphididae) colonizing cotton in the United States. Florida Entomol. 79: 193-205.

Wall, R. E. 1933. A study of color and color-variation in *Aphis gossypii* Glover. Ann. Entomol. Soc. Amer. 26: 426-464.

Weathersbee III, A. A., D. D. Hardee, and W. R. Meredith, Jr. 1994. Effects of cotton genotypes on seasonal abundance of cotton aphid (Homoptera: Aphididae). J. Agric. Entomol. 11: 29-37.

Young, M. T., and G. L. Garrison. 1949. Aphid collections at Tallulah, Louisiana, from 1941 to 1947. J. Econ. Entomol. 42: 993-994.

PERMISSION TO COPY

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Texas Tech University or Texas Tech University Health Sciences Center, I agree that the Library and my major department shall make it freely available for research purposes. Permission to copy this thesis for scholarly purposes may be granted by the Director of the Library or my major professor. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my further written permission and that any user may be liable for copyright infringement.

Agree (Permission is granted.)

Disagree (Permission is not granted.)

_____ _____

Student Signature Date