IDENTIFYING THE POTENTIAL ROLE OF SQUARE SIZE IN RESISTANCE TO COTTON FLEAHOPPER (*PSEUDATOMOSCELIS SERIATUS*) IN UPLAND COTTON

A Thesis

by

BARRET BAXTER PAGE

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Steven Hague
Committee Members,	C. Wayne Smith
	Greg Sword
Head of Department,	David Baltensperger

August 2018

Major Subject: Plant Breeding

ABSTRACT

Cotton fleahopper (CFH) (*Pseudatomoscelis seriatus*) is an early season pest of upland cotton (*Gossypium hirsutum*, L). Feeding damage results in abscission of young floral buds (squares), and consequently a delay in maturation and potential yield losses. Traditional efforts to breed for CFH have focused on the role that pubescence has played in preferential feeding by CFH. The physical square morphology was investigated as a characteristic of resistance. Fourteen lines derived from the fleahopper breeding efforts and six elite lines from the Texas A&M AgriLife Research Cotton Improvement Lab were grown in a split-block spray non spray design to ascertain fleahopper resistance. Squares were measured throughout the growing season to obtain growth patterns for the 20 lines. Differences in square sizes were observed across the lines. Some relationships between square size, days of susceptibility to CFH feeding, CFH damage and square retention were observed but the data were not conclusive enough in determining whether square size impacts CFH resistance.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supported by a thesis committee consisting of Professor Steve Hague (advisor) and C. Wayne Smith of the Department of Soil and Crop Sciences and Professor Gregory Sword of the Department of Entomology.

The data analyzed for 2014 field trials and ovary depth was provided by Dr. Laura Ann McCloud.

All other work conducted for the thesis was completed by the student independently.

Funding Sources

Graduate study was supported by a fellowship from Texas A&M University and a research fellowship from Cotton Incorporated.

NOMENCLATURE

CFH	Cotton Fleahopper
CS	College Station
Den df	Denominator degrees of freedom
HR	Hypersensitive response
Num df	Numerator degrees of freedom
6d	Six days before flowering
12d	Twelve days before flowering
20d	Twenty days before flowering

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

TABLE Page

1. INTRODUCTION

Upland cotton is a culturally and economically important crop in the southern United States. The National Cotton Council of America estimates that upland cotton is planted on 10 million acres of land and contributes $75 billion in U.S. economic activity. As with any crop species there are a multitude of insect pests that feed on or damage cotton and have negative economic impacts on the crop. In the United States, control efforts of the most damaging insect pest species has made great strides over the last few decades. Transgenic *Bt* (*Bacillus thuringiensis)* cotton has been genetically engineered to produce *Cry* proteins, that when ingested, kill Lepidopteran pests such as the cotton bollworm (*Helicoverpa zea).* Across most of the U.S. Cotton-belt, an integrated pest management approach has been successful at eliminating the boll weevil (*Anthonomus grandis).* Successful management of primary cotton pests has allowed cotton breeders to focus on host plant resistance for secondary insect pests, such as CFH.

CFH is an early season pest of cotton, migrating to cotton fields as early season weedy host species senesce (Almand et al., 1976). Cotton fleahopper damage is most severe in the southern cotton belt, particularly dryland regions of Texas (Ring et al., 1993). Over the last fifteen years, estimated yield loss in Texas due to CFH has been as high as 1.11% (2007) and averaged 0.4% (Williams, 2000-2016), but many individual fields can experience much higher rates of damage. CFH feed on the developing cotton flower buds (squares). Damage from cotton fleahopper feeding results in death and abscission of young squares causing yield loss, abnormal plant growth and delay in maturity of the crop (Almand et al., 1976, Ring et al., 1993).

Control of CFH is entirely chemical, with no commercially available cotton varieties possessing resistance. Studies evaluating preferential CFH feeding on cotton cultivars have identified the cultigen ‘Pilose’ as experiencing lower levels of injury (Walker et al., 1974, Knutson et al., 2013). Further work by McCloud et al. (2015) confirmed that the pilose trait contains natural resistance to CFH feeding damage. By crossing ‘Pilose’ to elite, high yielding lines and evaluating the progeny of those crosses and backcrosses, McCloud et al. (2015) concluded that the resistance of ‘Pilose’ to CFH is heritable and potentially independent from the pilose trait. The exact mechanism of the resistance is not known.

McCloud et al. (2016) showed that the ovary depth was correlated to the physical size of the square, and that once a square reached a certain size, the ovary was deeper than the maximum CFH feeding stylet penetration. Square size has been shown to impact the herbivory and oviposition of other cotton insect pests (Showler, 2005). With larger squares having more tissue between the sensitive ovary and the outside of the square, perhaps this physical trait contributes in some way to host plant resistance to CFH.

Objectives of this project were:

1) Estimate differences in square sizes among cotton genotypes.
2) Determine if square size contributes to resistance to CFH

2. LITERATURE REVIEW

Cotton fleahoppers (CFH) are a primarily herbivorous insect and but will feed on eggs of other insects if the opportunity arises (Pfannenstiel, 2005). Adult CFH are around 3.175 mm long. CFH eggs are laid under the bark of a host plant, with eggs hatching around eleven days after they deposit (Breene et al., 1969, Bohmfalk et al., 2005). After hatching, young nymphs molt a total of five times in the 14 to 15 days required to mature to sexual adulthood (Bohmfalk et al., 2005). Six to eight generations of CFH occur annually, with one to three generations occurring in the cotton field (Bohmfalk et al., 2005). CFH overwinters in the egg state on a variety of wild host plants including woolly croton (*Croton capitatus*), cutleaf evening primrose (*Oenothera laciniata*), showy sundrops (*Oenothera speciosa*), wooly tidestromia (*Tidestromia lanaginosa*), and silverleaf nightshade (*Solanum elaeagnifolium*) (Bohmfalk et al., 2005). The genus *Oenothera* are the preferred spring food source for CFH (Esquivel and Esquivel, 2009). Around the onset of squaring in cotton fields, many wild host species begin to senesce and CFH migrate to cotton fields (Almand et al., 1976).

Cotton fleahopper damage is most severe in the southern cotton belt, particularly dryland regions of Texas (Ring et al., 1993). Damage to cotton crops caused by CFH have been estimated at 91kg of lint per acre in central and south Texas (Parker, 2009). Over the last fifteen years, estimated yield loss in Texas due to CFH has been as high as 1.11% (2007) and averaged 0.4% (Williams, 2000-2016), but many individuals experience much higher rates of damage. In 1999, the cotton fleahopper was considered the most economically damaging insect pest of U.S. cotton, with farmers incurring an

estimated $196 million cost in control efforts and losses (Williams, 2000). Economic importance of CFH have fluctuated over the past decade, but they have consistently ranked among the most destructive insect pests in Texas cotton. CFH is an early season pest in upland cotton, migrating from weedy host species to cotton around the onset of cotton's early fruiting stage (Almand et al., 1976). CFH is reported to feed on ovary walls, developing anthers and leaves of cotton plants (Pack and Tugwell, 1976; Bell et al., 2007). Damage from cotton fleahopper feeding results in death and abscission of the young floral buds (squares). CFH feeding damage can also result in abnormal plant growth and delay of maturity of the crop (Almand et al., 1976, Ring et al., 1993). Destruction of cell walls by polygalacturonases (pectinases) present in fleahopper saliva causes damage to the squares that are ultimately dropped because of the production of stress ethylene (Martin et al., 1988). It is also believed that CFH vector bacterial pathogens into the sensitive squares and the pathogens, in turn, cause squares to be shed (Bell et al., 2007). Hypersensitive response is a type of defense against the spread of a bacterial infection in plants characterized by localized, programed cell death and is often associated with disease resistance (Hofius et al., 2011). Pathogens vectored by CFH may result in a hypersensitive response by the resulting host cotton and subsequent square drop.

Pathogens of the genera *Pseudomonas, Xanthomonas, Pantoea,* and *Serratia* have been isolated in the saliva of cotton fleahoppers (Duffey and Powell, 1979; Martin et al., 1987; Bell et al., 2007). Both *Fusarium sp.* and *Xanthomonas sp.* have been cultured from CFH infected plant tissue. Cotton squares infested with CFH produce

ethylene at five times the rate of pre-infestation conditions (Duffy and Powell, 1979). When *Fusarium sp.* and *Xanthomonas sp.* are applied to cotton squares in the absence of CFH, ethylene production rates increase, approximating those of infested squares (Duffy and Powell, 1979). This suggests that infection of the plants with these bacterial species during CFH feeding induces ethylene synthesis in the plants and may cause abscission of infected squares. Bell et al., (2007) reported that sterile water used to wash CFH and subsequently injected into cotton squares caused seed rot and boll rot indicating that bacterial species associated with CFH are pathogenic. The average depth of ovaries is well within the CFH ability to penetrate, allowing vectoring of the pathogenic bacteria into sensitive tissues of the plant and subsequently causing abscission of the squares (Bell et al., 2007). Bacterial species present in CFH salivary glands vary depending on the population and the diets of the CFH. The majority of CFH collected from lemon horsemint, *Monarda citriodora* in College Station, TX, had sterile saliva whereas CFH collected from woolly croton harbored *Penicillium sp.* and *Pantoea sp.* (Martin et al., 1978).

Current control of CFH is mainly through the use of chemical insecticides. Presently, cotton producers utilize insecticide treatments for CFH control, making one or more applications during the initial three to four weeks of squaring when the cotton is most susceptible to CFH damage (Parker et al., 2008). Most insecticides used for CFH control are broad spectrum and may adversely impact beneficial insects that assist in suppression of outbreaks of other cotton pest species such as aphids, bollworms and budworms (Martin et al., 1988). An integrated approach to CFH management is

necessary to reduce the negative impacts these insects have on the U.S. and especially Texas cotton industry. Dent, (1995), defines integrated pest management (IPM) to mean "A pest management system that in a socioeconomic context of farming systems, the associated environment and the population dynamics of the pest species, utilizes all suitable techniques in as compatible a manner as possible and maintains the pest population levels below those causing economic injury." An IPM system has four general guidelines: (1) Analyze the pest status to establish economic thresholds for pests. (2) Devise strategies to increase biotic factors to control pest reproduction. (3) Use the best combination of natural enemies, resistant plant varieties and environmental modification to control pest outbreak with minimal ecological disruption. And (4) systematically and regularly check for insects, diseases and weeds (Bottrell, 1979).

Many IPM approaches have been successful in controlling different insect pests in cotton. Transgenic *Bt* cotton has been engineered to produce a protein natively expressed in the soil born bacteria *Bacillus thuringiensis.* Once ingested by caterpillar pests, these toxins bind to receptors in the midgut of the insect and create pores that result in lysis of midgut epithelium cells and ultimately death of the insect (Bravo et al., 2007). An example of successful large area implementation of IPM is the eradication of the boll weevil. Through a combination of cultural control practices to create unfavorable overwintering environments, chemical control to eliminate already established populations and extensive monitoring utilizing pheromone traps, the boll weevil has been eradicated from the vast majority of cotton hectares in the United States. Although an expensive undertaking for the cotton industry, these eradication programs

have resulted in great economic and environmental benefits in eradicated areas (Smith, 1998).

Identification of economic injury levels is the cornerstone of successful IPM, but paradoxically, few have been quantitatively established (Poston et al., 1983). A basic component of the economic injury level is the relationship quantifying crop yield response with the level of pest density or injury (Ring, 1993). Current treatment economic thresholds recommended by Texas A&M Extension range from 10-25 CFH per 100 plants in the southern, eastern and Blackland areas of Texas (Knutson et al., 1993) to 25-30 CFH per 100 plants in the High Plains, Rolling Plains and Trans-Pecos areas of Texas (Fuchs et al., 1993). Accurate sampling of CFH numbers is important to determine if control actions should be taken. Parajulee et al., (2006) determined that visual sampling detected the highest number of fleahoppers, followed by beat bucket, drop cloth, vacuum and sweep net sampling; however, the most cost effective sampling method based on fixed precision cost reliability was sweep net sampling. Plant breeding fits within the tenants of IPM by providing cotton cultivars capable of compensating for the loss of fruit, or reducing the impact of pests with some other strategy, in turn raising the economic injury level and providing an additional tool for insect pest control.

The wide host range of CFH covers 35 families of plant species (Esquivel and Esquivel, 2009) and suggests that CFH have evolved adaptations to overcome a wide range of host plant defenses. CFH colonizes cotton after senescence of its favored weedy host species, suggesting that cotton is not a preferred food source over these weedy species and that it may have physical or chemical attributes that discourage colonization

by CFH (Knutson et al., 2014). Naturally occurring host plant resistance to CFH has been identified and further exploration of host plant resistance to CFH is necessary to add additional management tools. Painter (1958) defines host plant resistance as the phenomena by which plants under the same environmental conditions experience differing levels of injury due to diseases or insect herbivory. Three types of host plant resistance are described by Painter (1958), tolerance, antibiosis and non-preference, now commonly referred to as antixenosis (Kogan and Ortman, 1978).

Tolerance is the plants ability to survive and recover from feeding damage caused by an insect infestation and continue to produce economic product (Painter 1958). Plant species, as well as different individuals within that species differ for their level of tolerance to insect herbivory (Strauss and Agrawal, 1999). Some species suffer reduced fitness with low levels of herbivory, in contrast, other species like wild radish can experience up to a 25% loss in total leaf area without a significant drop in seed set (Lehtilä and Strauss, 1999). It should be noted that the degree of tolerance to herbivory may not directly relate to plant fitness, a plant with complete compensation for herbivory may still suffer a loss in fitness compared to a less tolerant plant with higher fitness in a damaged state (Strauss and Agrawal, 1999). Both the abiotic and biotic environment that a plant experiences can affect the tolerance to herbivory (Strauss and Agrawal, 1999). Different modes of action of herbivores my trigger different compensation tolerance responses in the host plants. Upland cotton (*Gossypium hirsutum*) plants that had floral buds mechanically removed to simulate damage from sucking and Lepidopteran pests, were able to completely compensate for the loss of early floral buds in a favorable

environment with high availability of resources whereas those in a more limited and stressful environment were unable to compensate for the damage (Sadras, 1996). In response to the removal of floral buds, the plants showed increased branching to compensate (Sadras, 1996). In contrast, cotton that was heavily damaged by aphids (*Aphis gossypii*) with leaf area reduced by up to 58% responded to the herbivory by significantly decreasing the number of branches (Rosenheim et al., 1997). For two distinct damage types, the cotton was able to fully compensate through differing tolerance responses.

Plants show variation in their tolerance response to herbivory, and heritable genetic variability in tolerance has been observed (Simms and Triplett, 1994). Heritable variation for tolerance to herbivory exists, but selection for tolerance may be constrained by tradeoffs with other fitness related traits (Strauss and Agrawal, 1999). Van der Meijden (1988) proposes that tolerance and resistance are alternative strategies that plants employ to cope with herbivore damage. Plants that are resistant to feeding do not experience selection for compensatory tolerance because they sustain less damage in the first place. Other evidence points to tolerance strategies and resistance strategies not being mutually exclusive. Mauricio et al., (1997) shows that selection for resistance traits or tolerance in *Arabidopsis thaliana* does not negatively impact the fitness levels, rather selection favored the retention of both tolerance and resistance. Although negative correlations that may generate mutual exclusivity of tolerance and resistance have been reported (Fineblum and Rausher, 1995), these may not be the case in all instances (Mauricio et al., 1997).

Antibiosis host plant resistance is the term for when an insect pest incurs a loss of fitness after herbivory of the host plant. Examples of antibiosis are commonplace with many chemical compounds utilized by plants for defense against insects. Effects of secondary metabolites on insects range from death in severe cases, limitation of developmental time in immature insects, to reduced fecundity and weight of adult insects (Awmack and Leather, 2002). Gossypol is a terpenoid secondary metabolite of cotton that is known to play a role in antibiosis against a variety of species. Boll weevils (*Anthonomus grandis*) show non preference for strains of cotton containing high gossypol levels, as well as a size reduction of weevils emerging from punctured squares of high gossypol cotton compared to those emerging from normal or glandless cotton (Singh and Weaver, 1972). Cotton bollworm (*Heliothis zea*) and tobacco budworm (*Heliothis virescens*) larvae showed significant adverse effects on pupal weight, days to pupation and days to adult when reared on gossypol containing media (Shaver and Parrott, 1970). Nymph survival of *Lygus hesperus* was significantly reduced on cotton with normal glands when compared to glandless cotton (Tingey et al., 1975).

Painter's (1958) third class of host plant resistance mechanisms was termed 'nonpreference'. He defines preference or nonpreference as 'Denotes the group of plant characteristics and insect responses that lead to or away from the use of a particular plant or variety, for oviposition, food, shelter or a combination of the three'. In 1978 the scientific community shifted to the use of antixenosis to describe nonpreference (Kogan and Ortman, 1978). Modes of antixenosis commonly studied include physical characteristics like trichromes and sizes of physical structures as well as chemical

deterrents produced by plants. Upon herbivory, plants can release a variety of chemicals that modify herbivore and natural enemy behavior (Hegde et al., 2012). When cotton is attacked by cotton aphid, *Aphis gossypii*, it emits a blend of volatile organic compounds, and given a choice, aphids prefer to feed on plants that are not infected already. Feeding damage induces a chemical response that in turn repels further insect attack (Hegde et al., 2012). Similar response by aphids to herbivory induced volatile organic compounds have been observed in wormwood, *Artemisia annua*, with aphids avoiding already infected plants (Sun et al., 2015). This study identified a large increase in production of (E)-β-farnesene (a volatile organic compound) in the aphid effected plants. Utilizing an olfactometer bioassay to determine aphid's preference for chemicals, they concluded that (E)-β-farnesene may play a role in potential aphid resistance through antixenosis (Sun et al., 2015).

Plant trichome presence or absence as well as density has been investigated relating to all manner of insects and the herbivory they exhibit on a wide range of plants. Trichomes originate from epidermal tissue and proceed to develop through growth to produce hair like projections from epidermal surfaces (Johnson, 1975). Trichome density and type varies from organ to organ and tissue to tissue. Pubescence can affect insect activity by mechanical or chemical means (Johnson, 1975). The mechanical effect of trichomes varies depending on a variety of characteristics including density, erectness, length and shape (Johnson, 1975). Pubescence can affect locomotion, attachment, ingestion, digestion and oviposition of insects (Kogan, 1978).

Plant trichomes do not have the same antixenotic effects on all insects. A highly pubescent structure might contribute to resistance or lack thereof to a certain stage in an insect life cycle. The tobacco budworm, *Heliothis virescens*, prefers to oviposit on cotton varieties that have smooth leaves (Robinson et al., 1980). Significantly fewer eggs were laid on smooth leaf cotton than on pubescent cottons whether or not the tobacco budworms had a choice of hosts (Robinson et al., 1980). In the same study, a laboratory test featuring different textured fabrics and papers showed that the tobacco budworm preferred the materials that had the highest number of loose ends. Glabrous cotton is antixenotic for oviposition by the tobacco budworm (Robinson et al., 1980). Wiklund (1974), postulated that adult oviposition and larval food suitability are determined by different genetic mechanisms, therefore the pubescence of a plant could play different roles regarding the different life stages of an insect. Although tobacco budworms prefer pubescent cottons for oviposition, the trichomes play a role in inhibition of the movement of the larvae (Ramalho et al., 1984). Trichome density and erectness both were negatively correlated with larval movement. Pubescence and trichome erectness may be useful traits to incorporate for resistance to tobacco budworm larval herbivory (Ramalho et al., 1984).

Trichome density correlates to oviposition preference by tobacco budworm, but whitefly, *Bemisia tabaci*, show no preferential oviposition in relation to pubescence levels of different cotton varieties. Boica et al., (2007), Jindal and Dhaliwal (2011), and Meagher et al., (1997) all found no correlation between leaf hairiness and fecundity of whitefly. A significant correlation between compactness of the vascular bundles and

number of eggs laid by whitefly was observed (Jindal and Dhaliwal, 2011). This result is similar to a finding where whitefly preferred to lay eggs on cantaloupe varieties that had more compact vascular bundles (Chu et al., 1995). In addition to vascular bundle compactness, leaf lamina thickness was correlated to number of eggs laid (Jindal and Dhaliwal, 2011. Butter and Vir, 1989). In both cases, the greater the leaf lamina thickness, the more preferred for oviposition by whitefly the variety.

Trichomes have farther reaching effects than just the immediate insect pests. Trichome density of cotton plants has been show to impact predatory species of cotton pests as well as the pests themselves (Treacy et al., 1985). No choice greenhouse and field studies showed an inverse relationship between plant trichrome density and the level of successful attacks on bollworm, *Heliothis zea*, eggs by both the parasitic *Trichogramma pretiosum* and the predatory *Chrysopa rufilabris* (Treacy et al., 1985). This suggests that plant damage from bollworm may be reduced on glabrous cotton varieties due to antixenosis and increased predatory effectiveness in comparison to hirsute and pilose phenotypes (Treacy et al., 1985). Overall, plant pubescence antixenotic effects vary from species to species and combine with a myriad of other parameters to form the final antixenosis phenotype.

Historically plant pubescence has been investigated as playing a role in the preference for CFH feeding. Initial studies (Lukefahr et al., 1966, 1968, 1970), focused on the role of leaf pubescence. The conclusion was reached that glabrousness was a trait that conferred resistance. This initial impression was based on counts of fleahoppers present on two different cultivars with different levels of pubescence, hirsute plants had

higher incidence of CFH in comparison to cotton plants that had no hair (Lukefahr et al., 1966). Lukefahr also postulated that the 'resistance' observed could have been due to the "open type terminal bud" architecture that the glabrous experimental variety exhibited. A follow up study again confirmed that CFH counts were significantly lower on glabrous genotypes than hirsute genotypes (Lukefahr et al., 1968). In the 1968 study, Lukefahr et al., also examined the presence or absence of pigment glands in different cotton varieties and found that this had no effect on CFH counts. A third study (1970) investigated the pilose trait and CFH counts. Pilose is a trait that confers dense pubescence across the entire plant, stem, leaves and squares. Once again Lukefahr concluded that there is a strong positive correlation between CFH counts and leaf pubescence and deemed the varieties with low CFH counts as resistant. He postulated that the pubescent material trapped volatile compounds that would otherwise antixenotically influence the CFH, thereby making the pubescent leaves a more favorable microenvironment for CFH.

These early studies showed a preference by CFH for pubescent plants, but did not assess damage or losses caused by CFH feeding. Walker et al., (1974) conducted another study investigating CFH and levels of plant pubescence. In addition to counts of fleahoppers, they also assessed number of squares, number of flowers and yield. They reported that higher numbers of CFH were found on hirsute genotypes and lower numbers on glabrous genotypes, consistent with the findings of Lukefahr (1966, 1968, 1970). The use of chemical control for CFH did not increase blooming in the hirsute or pilose genotypes, but did produce a marked increase in blooming in glabrous lines

(Walker et al., 1974). The findings showed clear damage to the glabrous cottons, even though these genotypes were infested with small populations of CFH (Walker et al., 1974). Although the glabrous genotypes did not always show greater damage than the commercial hairy standard the authors concluded that the probability of greater damage was present in the smooth lines. These studies showed a progression of tolerance to CFH feeding as trichome density increased culminating in a tolerant and extremely hairy cultigen, 'Pilose' (Walker et al., 1974). While following a consistent pattern of higher fleahopper counts on hirsute and pilose varieties, these trends do not carry over when assessing actual damage caused by CFH, and show an opposing trend of resistance in extremely hairy genotypes. Ring et al., (1993), came to a similar conclusion, where in caged field trials with varying densities of CFH, the most densely pubescent genotype exhibited the largest tolerance to high densities of CFH, where the glabrous varieties were determined to be highly susceptible.

In addition to pubescence, the nectariless trait also has been investigated in relation to CFH resistance. The nectariless trait, conditioned by recessive genes *ne1* and *ne2*, removes the extrafloral nectaries present on the leaves and involucral bracts of cotton (Meyer and Meyer 1961). Some studies have shown nectariless genotypes harboring fewer CFH (Schuster et al., 1976) and tarnished plant bugs, *Lygus Hesperus* (a plant bug related to CFH) (Meredith and Schuster, 1979). Other studies reported that nectaried and nectariless varieties did not differ in the number of CFH per plant or square damage (Mekala, 2004) or that nectariless genotypes experienced high levels of square damage from CFH (Lidell et al., 1986).

Knutson et al., (2013) directly measured CFH feeding damage by dissecting and observing squares in no-choice cage studies. They determined that 80 percent of squares damage by CFH were smaller than 1mm in size and 99 percent of damaged squares were less than 2mm in diameter. Consistent with the other studies conducted prior, in a choice field trial they reported a significant positive correlation between trichome density and CFH numbers. They also reported no significant correlation between trichome density and damage to cotton squares, suggesting that trichome density does not play a role in the preferential feeding by CFH on one cultivar over another. Knutson et al., (2013) identified a cultigen named 'Pilose' that was the most tolerant of CFH feeding in both choice and no-choice feeding studies. Further wok by McCloud et al., (2015) confirmed that the pilose cultigen contains natural resistance to CFH feeding damage. By crossing Pilose to elite yielding lines and evaluating the progeny of those crosses and backcrosses, McCloud et al., (2015) concluded that the resistance of Pilose to CFH is heritable and potentially independent from the pilose trait. Pilose pubescence is either causative of the resistance or the resistance trait is tightly linked to genes controlling pubescence (McCloud et al., 2015).

There is a complex interaction between plant pubescence and resistance or preferential feeding by CFH, but other factors may be at play in determining resistance to CFH. Analysis of CFH feeding behavior showed significant differences in feeding behavior among different cotton lines (McCloud et al., 2015). Lines deemed resistant had a 100 fold decrease in the time that adult CFH were observed feeding on squares compared to susceptible lines. The resistant genotype may lack the chemical or physical

characters that signal a feeding response, or there may be compounds repellant to feeding (McCloud et al., 2015). Differences in feeding times suggest antixenosis as a resistance factor. In another study, McCloud et al., (2016) analyzed the depth of the developing ovary in matchhead cotton squares. Significant differences were found in the depth of the developing ovary among different cotton lines. These findings have two important implications. The first being that deeper ovaries may be protected from direct infection by the pathogens vectored by CFH feeding and from digestive enzymes in the saliva of CFH, and that this may distinguish between more susceptible plants that shed squares, or more resistant plants that retain squares when fed upon by CFH. Secondly, the inability of fleahoppers to penetrate the ovary when feeding may play a role in preferential feeding and duration of feeding.

McCloud et al., (2016) showed that the ovary depth was correlated to the physical size of the square, and that once a square reached a certain size, the ovary was deeper than the maximum CFH feeding stylet penetration. They also showed that the size a square must reach for the ovary to be safe from CFH varies among different genotypes. Square size may play a potential role in the nonpreference feeding behavior observed in CFH, with smaller squares being targeted because of access to the ovary. Square size has been shown to play a role in boll weevil, *Anthonomus grandis,* preferential feeding and fecundity (Showler, 2005). Large squares were more commonly used for oviposition than the other size classes, having developed a critical volume for immature boll weevils and the nutrients that contribute toward increased fecundity (Showler, 2005). In a laboratory study, boll weevils laid more eggs in larger squares and

no immature weevils survived to adulthood in match-head (2-3mm) sized squares (Greenberg et al., 2003).

This line of inquiry leads to the purpose of this investigation. The potential role that the physical size of the square plays in conveying resistance to CFH. Square size has been shown to impact the herbivory and oviposition of other insect pests. With larger squares having more tissue between the sensitive ovary and the outside of the square, perhaps this physical trait contributes in some way to host plant resistance to CFH. Not only are larger squares potentially important, but also the growth of the squares. The less time that the squares spend with the ovaries at an exposing depth, potentially the less susceptible they will be to CFH feeding damage. This study aims to examine the relationship between square size and resistance to CFH in upland cotton.

Objectives of this project were:

3) Estimate differences in square sizes among cotton genotypes.
4) Determine if square size contributes to resistance to CFH

3. MATERIALS AND METHODS

3.1 Square Size Material

A total of twenty genotypes were included in these tests. Fourteen of these lines are BC_1F_3 lines that were created previously to attempt to incorporate CFH resistance into high yielding lines. TAM07V-45 (96WD-22/02Q-42), a line with glabrous leaves and stems, and TAM06WE-14 (DPL491/96WD-22//AP9257/96WD-22), a line with relatively hairy stems and leaves, were used as recurrent parents for the backcrossing, the other side of the pedigrees consist of seven F_3 plants derived from the cross Pilose/'Deltapine50' (DP 50; PVP 8400154) (Table 4.0). The original cross that the BC_1F_3 were derived from was made in 2011. F_1 plants were crossed with the recurrent parents, TAM06WE-1 and TAM07V-45, in 2012. These BC_1F_1 plants were self-pollinated for two generations to create the BC_1F_3 lines. The other six lines are elite lines derived by the Texas A&M AgriLife Research Cotton Improvement Lab breeding program.

3.2 Evaluation of Cotton Fleahopper Resistance

In 2016, four row plots of the twenty genotypes were grown at College Station, TX in a single replication under irrigated conditions. At the end of the growing season, before defoliation, 18 plants were randomly identified from each plot and plant mapping data were collected. The presence or absence of a boll was recorded for the first and second position fruiting node on the first five fruiting branches of each plant. Data were collected on the earlier fruiting positions under the assumption that these were the fruit

that were squaring when the cotton is most affected by CFH feeding damage in the early stages of squaring.

In 2017, in College Station, a split-block design was used with main plots being insecticide treated and untreated, subplots of the original twenty genotypes, with six replications. Insecticide treated plots were sprayed once a week for three consecutive weeks beginning at square initiation with Acephate® (O,S-Dimethyl acetylphosphoramidothioate) at a rate of 140.31 L ha^{-1}. Data were collected on stands of the plots. Any plots with low or no stand, as well as the neighboring plots were discarded from the lint yield analysis as the low stand would impact the yield of the plot itself and give less competition to the neighboring plot, thus artificially inflating its plot weight. Plots were machine harvested at the end of the growing season and lint yield and fiber quality traits were measured.

3.3 Square Measurement

In 2016, square measurements were taken on nineteen genotypes, (Tamcot 73 was excluded due to a planting error) using an electric Performance Tools® dial calipers. Bracts were carefully opened and the largest width of the internal flower bud structure was measured. To insure that the same age squares were being measured, a first sympodial position open flower was located and using the growth patterns of cotton plants, two squares were measured, one two mainstem nodes above the white flower and one for mainstem nodes above the whote flower. The squares were estimated to bloom in six and twelve days respectively. Both of these squares were first position sympodial

squares. Square size data were collected over two weeks in July across all plots, with plants that fit the measurement criteria being measured, then marked so as not to be measured a second time.

In the first week of July 2017, five plants from each genotype from two replications of the split block yield trial, (for a total of twenty plants per genotype) were selected. The youngest main stem first position sympodial square available from those selected plants was tagged and measured. These squares were measured every Monday, Wednesday, and Friday until the square was absized or flowered. Using these data, growth patterns for squares were determined. Using the date of flowering as a constant point, the same 6D and 12D size of the squares was determined from the growth curves. From the growth curves of individual squares, 6d and 12d square sizes as well as the rate of growth per day for the squares and the total number of days before flowering for a square were determined.

3.4 Data Analysis

All data were analyzed using SAS (SAS v.9.4, SAS Institute, 2013). Data from the plant mapping in 2016, the square size for 2016and 2017, and split block yield trial 2017 were analyzed using a PROC GLM procedure. The combined 2016 and 2017 square size ANNOVA was conducted using a PROC MIXED procedure with year treated as a random effect and genotype as a fixed effect. CFH resistance trial data, and ovary depth data from McCloud, (2015) were used. CFH resistance data from 2014 (McCloud, 2015) were used and correlated to the combined square size. Additionally,

ovary depth regression equations identified by McCloud et al. (2015) were used to calculate the size a square must attain to be safe from the feeding proboscis of a CFH. Using the square growth curves from the 2017 experiment, the number of days that the ovaries are within the feeding distance of a CFH. Number of susceptible days and square size were correlated, as well as the combined square size and square growth rates. All correlations were determined using the PROC CORR procedure.

4. RESULTS AND DISCUSSION

In 2016, fourteen backcross genotypes, and six elite germplasm genotypes from the Texas A&M Agrilife Research Cotton Improvement Lab breeding program (Table 4.0) were grown at College Station, Texas and data on the size of the squares of these plants were collected. These backcross lines were derived from a previous effort to introduce resistance to CFH into more elite germplasm, utilizing Pilose as a source of resistance. Differences were observed in both the 6d and 12d square sizes (Table 4.1, 4.2) and a strong correlation between 6d and 12d squares was observed (Figure 4.1). (Square size data were not collected for Tamcot 73 in 2016 due to a planting error). Plant mapping data from the plots in 2016 were taken. A subsample of seven plants randomly selected from each row was plant mapped. This involved noting if a boll was present at a fruiting position. The first and second fruiting position was observed for the first five fruiting branches of the plant. Data were collected on the lower fruiting branches only, because these were the fruiting positions that would be most susceptible to CFH early in the growing season. Data were collected prior to defoliation at the end of the growing season. Significant differences in the number of bolls retained were found among the 20 lines (Table 4.4, 4.5).

Table 4.0. Line designations and pedigrees for parental, backcross and elite lines. Pubescence information provided for non-segregating lines.

Genotype ID	Pedigree	Pubescence
TAM07V-45	96WD-22/02Q-42	Smooth
TAM06WE-14	DPL491/96WD-22//AP9257/96WD-22	Normal/Hairy
GH-02	Pilose/TAM96 WD-69s	
GH-04	Pilose/Deltapine50	
GH-07	Pilose/All-Tex Atlas	
GH13-6	Pilose/Deltapine50	Pilose
GH15-2	Pilose/Deltapine50	Pilose
GH18-1	Pilose/Deltapine50	Pilose
GH18-3	Pilose/Deltapine50	Smooth
GH20-1	Pilose/Deltapine50	Normal/Hairy
GH20-2	Pilose/Deltapine50	Normal/Hairy
LA-01	TAM06WE-14 //TAM06WE-14 /GH15-2	
LA-02	TAM06WE-14 //TAM06WE-14 /GH13-6	
LA-03	TAM06WE-14 //TAM06WE-14 /GH18-3	
LA-04	TAM07V-45//TAM07V-45/GH18-3	
LA-05	TAM06WE-14 //TAM06WE-14 /GH20-1	
LA-06	TAM07V-45//TAM07V-45/GH15-2	Pilose
LA-07	TAM07V-45//TAM07V-45/GH15-2	Smooth
LA-08	TAM07V-45//TAM07V-45/GH18-1	Pilose
LA-09	TAM07V-45//TAM07V-45/GH18-1	Smooth
LA-10	TAM07V-45//TAM07V-45/GH13-6	Pilose
LA-11	TAM07V-45//TAM07V-45/GH13-6	Smooth
LA-12	TAM07V-45//TAM07V-45/GH20-2	Normal/Hairy
LA-13	TAM07V-45//TAM07V-45/GH20-2	Smooth
LA-14	TAM06WE-14 //TAM06WE-14 /GH20-1	Normal/Hairy
15 EE-40	06 B-69/Garant	
15 EE-48	DP90/Tamcot73	
15 EE-52	BRS 269/08 WZ-51	
15 FF-21	Carter Long Staple/06 C-79	
13 Q-18	TAM 96WD-18/03WZ-37	
Tamcot 73		

Table 4.1. Analysis of variance of 6d square sizes, College Station, TX (2016), * P<.05, ** P<.01

Effect	Num df	Den df	F Value
Genotype	18	1090	12.30**
Row	3	17	3.17
Genotype*row	17	1096	1.25
Error	1090		

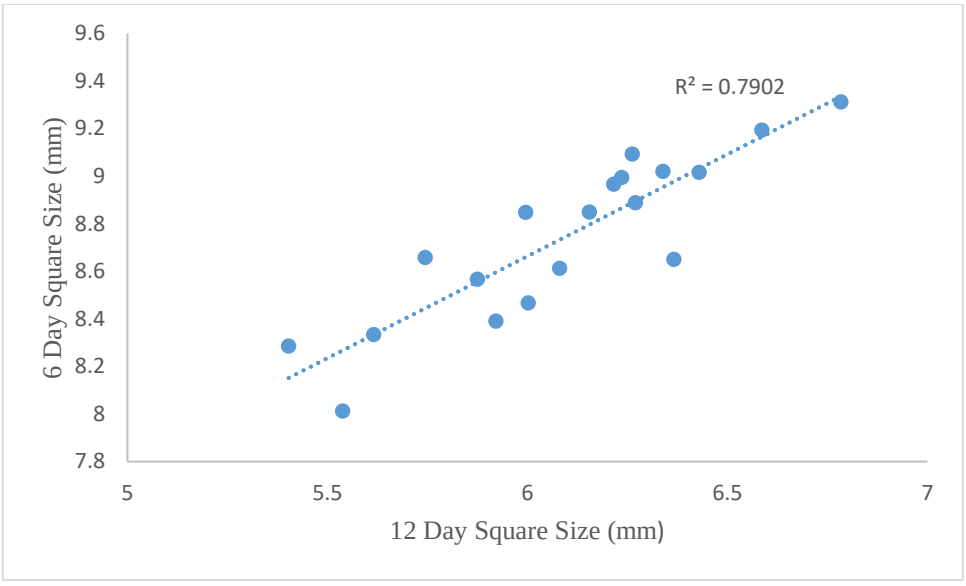
Table 4.2 Analysis of variance of 12d square sizes, College Station, TX (2016), * P<.05, ** P<.01

Effect	Num df,	Den df	F Value
Genotype	18	1090	14.63**
Row	3	17	0.52
Genotype*row	17	1090	0.95
Error	1090		

Table 4.3. Mean separation of 12d square size, College Station, TX (2016)

Genotype	Mean (mm)
15 EE-52	6.78 a[†]
13 Q-18	6.59 ab
LA-08	6.43 bc
15 FF-21	6.37 bcd
LA-10	6.34 bcd
LA-13	6.27 cde
LA-09	6.26 cde
LA-12	6.24 cde
LA-14	6.22 cde
15 EE-40	6.15 cdef
LA-02	6.08 def
LA-01	6.00 efg
15 EE-48	5.99 efg
LA-05	5.92 fg
LA-11	5.87 fgh
LA-04	5.74 ghi
LA-06	5.62 hij
LA-03	5.54 ij
LA-07	5.40 j

[†]Means connected by the same letter are not different at α=0.05, Duncan MRT



p<0.001

Figure 4.1. Regression of 12d square size means and 6d square size means (2016)

Table 4.4 Analysis of variance of plant mapping data, number of bolls left per plant, College Station, TX (2016), * P<.05, ** P<.01

Effect	Num df,	Den df	F Value
Genotype	19	38	4.43**
Genotype*Row	38	296	1.02
Row	3	296	2.36
Error	296		

Table 4.5. Mean separation of plant mapping data, number of bolls left per plant, College Station, TX (2016)

Genotype	Mean (# Bolls)
Tamcot 73	7.12 a[†]
15 FF-21	6.41 ab
15 EE-48	5.78 bc
LA-12	5.56 bcd
LA-03	5.44 bcde
13 Q-18	5.33 bcdef
LA-10	5.33 bcdef
15 EE-52	5.28 bcdef
15 EE-40	5.22 bcdef
LA-14	5.17 bcdef
LA-07	5.06 bcdefg
LA-05	4.63 cdefgh
LA-11	4.61 cdefgh
LA-04	4.44 cdefgh
LA-02	4.17 defgh
LA-01	4.00 efgh
LA-13	3.89 fgh
LA-09	3.89 fgh
LA-06	3.67 gh
LA-08	3.61 h

[†]Means connected by the same letter are not different at α=0.05, Duncan MRT

Plant mapping data were collected at the end of the growing season. Boll retention can be attributed to a large variety of factors, but CFH damage, particularly on early-setting fruit, can be substantial in the testing region if plants are left untreated. Lines LA-08 and LA-06 were pilose strains and had the lowest number of retained squares (Table 4.5) where the opposite would be expected if the dropped bolls at the beginning fruiting positions were primarily caused by CFH feeding damage. Overall, the bottom fifty percent of lines that retained the least amount of bolls were all LA strains (Table 4.5) that were derived from crosses meant to introgress CFH resistance into more elite germplasm. Generally, the elite material and lines retained more bolls than the fleahopper resistant germplasm (Table 4.5). Ultimately, this type of plant mapping may not be effective as a measure of CFH feeding damage because the data can be affected by too many other factors. Physiological stress, including cloudy weather and drought, can result also in square abscission (Mauney and Henneberry 1979). No significant correlation was found between 12d square size and number of bolls left from the plant mapping data in 2016 (Figure 4.2).

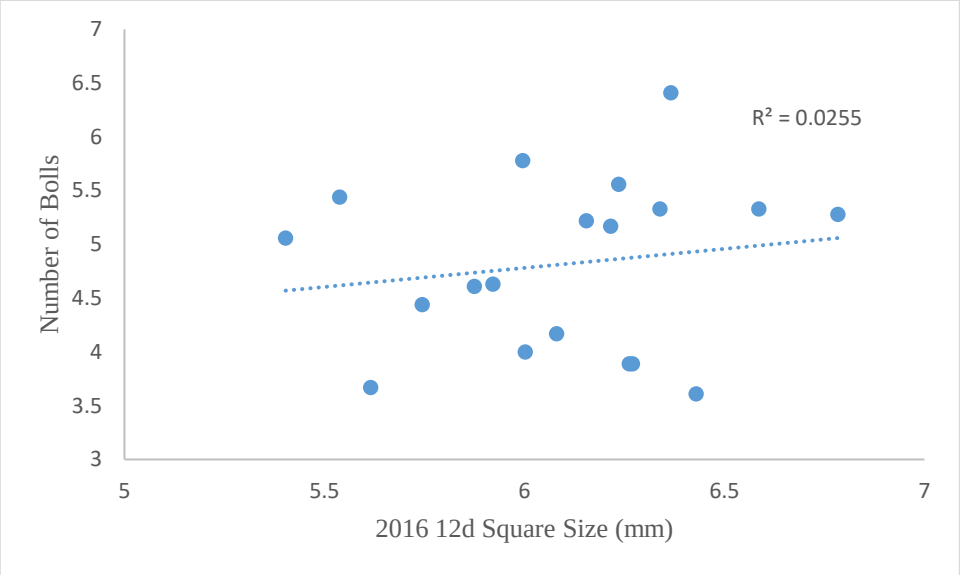


Figure 4.2. Regression of 12d square size means mean number of bolls per early fruiting branches (2016)

In 2017, the same twenty genotype's squares were measured. This year, the same square was measured over a period of time from pinhead size until flowering. Growth curves for individual squares were constructed, and all on the 2017 square data were extrapolated from these individual square growth curves (Figure 4.3). For 2017 at the location these plants were grown, using days as a measure of time was sufficient in obtaining linear curves. For less stable environments or years, some measurement of temperature included with time such as growing degree days, may be necessary to obtain linear growth curves. Flowering dates for each square that was measured were also collected. From these growth curves, we can tell that squares grow linearly throughout their existence, from a new pinhead square through flowering. The lowest Pearson's correlation coefficient value for an individual square's growth curve was 0.975, with the average being 0.999 across all squares that were measured. From these growth curves,

values for individual square size were calculated at the same twelve days before flowering as was measured in 2016. Significant differences among the lines were observed in the 12d square sized in 2017 (Table 4.6, Table 4.7). Significant differences among the lines in growth rate of squares were also observed (Table 4.8, Table 4.9). Although growth rates are significantly different, numerically they are extremely similar across the lines. It is therefore doubtful the difference in square size is different enough to affect CFH feeding damage. 15EE-40 has the fastest growing squares, growing 0.485 mm/day, and 15FF-21 had the slowest growing squares at 0.443 mm/day. The difference between the fastest and slowest growing squares was only 0.042 mm/day. From the growth curves total time for each square to flower was calculated and significant differences between the lines tested were observed (Table 4.10, 4.11).

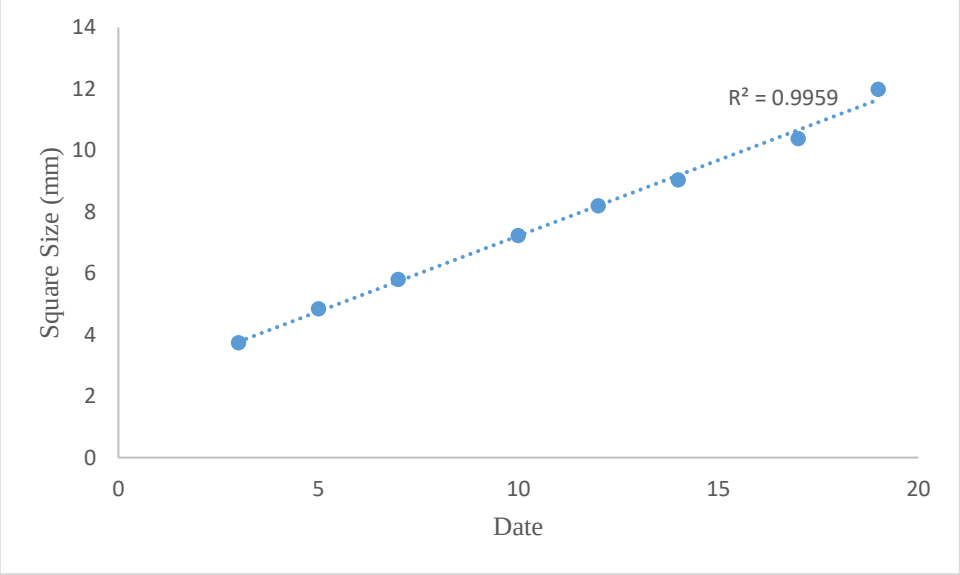


Figure 4.3. Sample growth curve for an individual square. Measurements were taken every 2-3 days throughout the life of the square.

Table 4.6. Analysis of variance of 12d square sizes, split block, College Station, TX (2017), * P<.05, ** P<.01

Effect	Num df,	Den df	F Value
Genotype	19	19	5.23**
Rep	1	293	0.04
Insecticide	1	1	14.22
Genotype*Insecticide	19	293	0.46
Rep*Insecticide	1	293	0.45
Error	293		

Table 4.7. Mean separation of 12d square size, College Station, TX (2017)

Genotype	Mean (mm)
15 EE-52	6.60 a[†]
LA-02	6.44 ab
LA-12	6.42 abc
Tamcot73	6.30 bcd
LA-03	6.26 bcde
LA-04	6.22 cde
LA-01	6.21 de
LA-09	6.18 def
13 Q-18	6.15 def
LA-08	6.14 def
LA-07	6.13 def
LA-06	6.11 defg
15 EE-40	6.10 defg
LA-14	6.09 defg
LA-13	6.07 efg
15 EE-48	6.06 efg
15FF-21	6.04 efg
LA-05	5.97 fg
LA-10	5.90 g
LA-11	5.70 h

[†]Means connected by the same letter are not different at α=0.05, Duncan MRT

Table 4.8. Analysis of variance of square growth rates, College Station, TX (2017), * P<.05, ** P<.01

Effect	Num df,	Den df	F Value
Genotype	19	19	3.27**
Rep	1	293	7.79**
Insecticide	1	1	0.15
Genotype*Insecticide	19	293	0.22
Rep*Insecticide	1	293	0.42
Error	293		

Table 4.9. Mean separation of square growth rate, College Station, TX (2017)

Genotype	Mean (mm/day)
15 FF-21	0.443 a[†]
LA-13	0.443 a
13 Q-18	0.453 ab
LA-11	0.461 abc
LA-07	0.462 abc
15 EE-48	0.463 abc
LA-06	0.464 abc
LA-05	0.468 abc
LA-02	0.469 abc
LA-03	0.469 abc
LA-10	0.470 abc
LA-04	0.474 bc
Tamcot73	0.478 bc
15 EE-52	0.478 bc
LA-01	0.482 c
LA-08	0.482 c
LA-12	0.484 c
LA-14	0.484 c
LA-09	0.484 c
15 EE-40	0.485 c

[†]Means connected by the same letter are not different at α=0.05, Duncan MRT

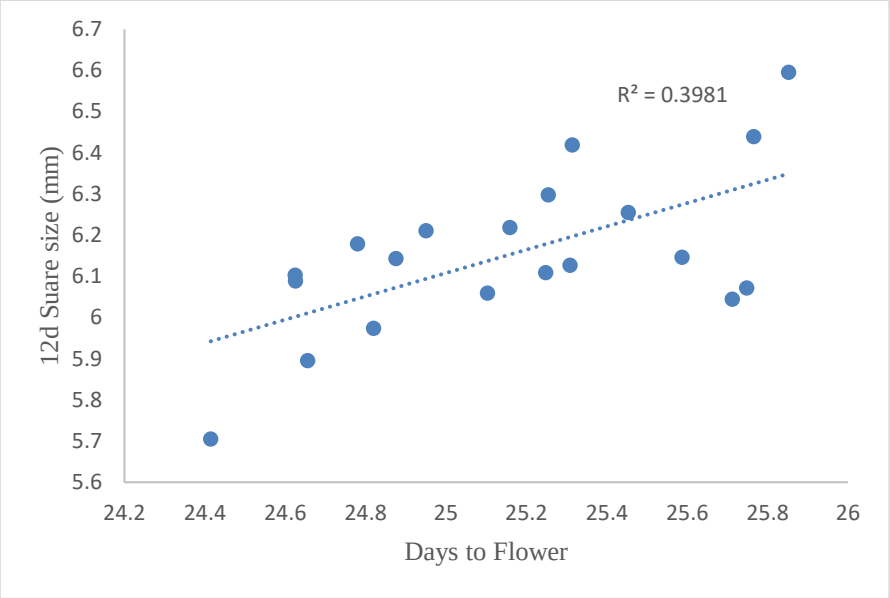
Table 4.10. Analysis of variance of total days to flower, College Station, TX (2017), * P<.05, ** P<.01

Effect	Num df,	Den df	F Value
Genotype	19	57	2.46**
Rep	3	274	4.89**
Genotype*Rep	57	274	1.56*
Error	274		

Table 4.11. Mean separation of total days to flower, College Station, TX (2017)

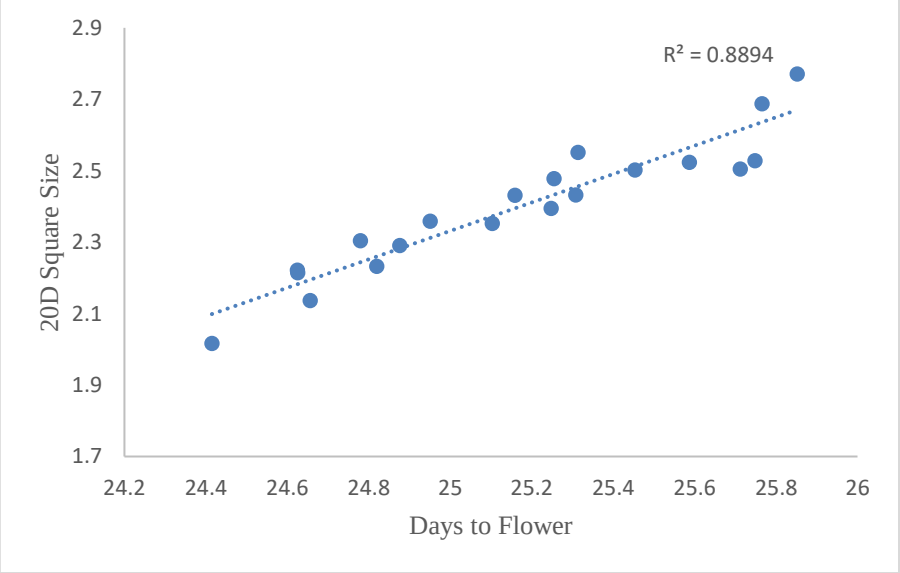
Genotype	Mean (mm)
15 EE-52	25.9 a[†]
LA-02	25.8 ab
LA-13	25.7 ab
15 FF-21	25.7 ab
13 Q-18	25.6 abc
LA-03	25.5 abcd
LA-12	25.3 abcde
LA-07	25.3 abcde
Tamcot73	25.3 abcde
LA-06	25.2 abcde
LA-04	25.2 abcde
15 EE-48	25.1 bcdef
LA-01	25.0 cdef
LA-08	24.9 cdef
LA-05	24.8 def
LA-09	24.8 def
LA-10	24.7 ef
LA-14	24.6 ef
15 EE-40	24.6 ef
LA-11	24.4 f

[†]Means connected by the same letter are not different at α=0.05, Duncan MRT



p=0.008

Figure 4.4. Regression of Days to Flower and 12d Square Size College Station TX, (2017)



p=.000

Figure 4.5. Regression of Days to Flower and 20d Square Size College Station TX, (2017)

A significant correlation between day to flower and 12d square size in 2017 was observed (Figure 4.4). This is important to note as it shows that the measurements of 12d square sizes are confounded by the fact that, while those squares have the same time left before they flower, they are not the same age. This can be illustrated by observing the correlation of much younger squares (20d) and days to flower (Figure 4.5). The Pearson's correlation coefficient is much higher for the younger 20d squares than it is for the 12d squares (Figures 4.4, 4.5) at 0.943 and 0.631 respectively. When the squares are younger and time is measured backwards from the date of flowering, more of the differences are due to the different ages of the squares, whereas when the squares age, more of the differenced in square size can be attributed to the different square growth rates between lines (Table 4.8).

A combined analysis of the 2016 and 2017 12d square sizes shows significant differences among the lines tested, and a significant Line*Year interaction effect. The year effect was not significant however (Table 4.12). From this combined analysis we calculated least squared means estimate for the 12d square sizes (Table 4.13). Based on rank performance in 2016 and 2017 (Table 4.14), seven lines showed consistent square sizes (a rank shift within 25 percentile points or less) and the other 12 lines had notable rank shifts when comparing 2016 and 2017 12d square sizes. The largest rank shift belonged to line LA-03, having the second smallest squares in 2016, and the fourth largest squares in 2017. The different lines exhibited different levels of GXE interaction across years, with some having no rank shift, and other lines moving as much as 14 places (Table 4.14). Line 15EE52 had the largest squares in both 2016 and 2017 (Table

4.14). When comparing the estimated ls means square size, the difference between 15EE-52 (largest squares) and 13Q-18 (second largest), is larger than the difference between LA-07 (smallest squares) and 13Q-18. 15EE-52 has largest squares in both years of testing.

Table 4.12. Combined analysis of variance of 12d square sizes, College Station, TX (2016 and 2107), * P<.05, ** P<.01

Effect	Num df,	Den df	F Value
Genotype	19	1421	6.47**
Year	1	6	1.64
Genotype*Year	18	1421	4.26**
Error	1421		

Table 4.13. Least Squared Means estimate from combined analysis of 12d square size, College Station, TX (2016 and 2017)

Genotype	Estimate (mm)
LA-07	5.77
LA-11	5.79
LA-06	5.86
LA-03	5.89
LA-05	5.95
LA-04	5.98
15 EE-48	6.03
LA-01	6.11
LA-10	6.12
15 EE-40	6.13
LA-14	6.15
LA-13	6.17
15 FF-21	6.21
LA-09	6.22
LA-02	6.26
LA-08	6.29
LA-12	6.33
13 Q-18	6.37
15 EE-52	6.69

Table 4.14. Line performance ranked by 12d square size in College Station (2016) and College Station (2017). Rank shift is the change in performance rank from 2016 to 2017.

Genotype	Rank		
	2016	2017	Rank Shift
15 EE-52	1	1	0
13 Q-18	2	8	-6
LA-08	3	9	-6
15 FF-21	4	16	-12
LA-10	5	18	-13
LA-13	6	14	-8
LA-09	7	7	0
LA-12	8	3	5
LA-14	9	13	-4
15 EE-40	10	12	-2
LA-02	11	2	9
LA-01	12	6	6
15 EE-48	13	15	-2
LA-05	14	17	-3
LA-11	15	19	-4
LA-04	16	5	11
LA-06	17	11	6
LA-03	18	4	14
LA-07	19	10	9

The 2017 split block spray/non spray yield trial showed significant differences in lint yield for the twenty lines (Table 4.15, Table 4.16) but no differences in insecticide or the insecticide*genotype interaction effect (Table 4.15). These results could be caused by different reasons. There may not have been a heavy infestation of CFH that year, although CFH were observed in the plots. If there were not enough CFH present to cause significant yield loss, the insecticide treatment would be insignificant, although even the presence of large CFH populations might still not yield a significant insecticide treatment on lint yield. This exact result was reported by McCloud 2015, where a split block trial in 2014 showed no significant treatment effect of insecticide despite recording high levels of damage and large populations of CFH. An additional factor that may explain the insignificant insecticide effect is Hurricane Harvey. Harvey hit at the end of August where it rained approximately 550 mm College Station, within four days. The timing of this large rainfall event coincides with the opening of the earliest bolls, the same bolls most vulnerable to the early season feeding habits of the CFH. The destruction of the early bolls across the trial may have eliminated any effect the insecticide treatment had on protecting these early flowers from CFH damage.

Table 4.15. Analysis of variance of yield (kg ha^{-1}) of experimental fleahopper strains and elite material checks College Station, TX (2017), * P<.05, ** P<.01

Effect	Num df,	Den df	F Value
Genotype	19	126	7.86 **
Insecticide	1	4	0.18
Genotype*Insecticide	19	126	0.8
Rep	4	126	5.12**
Rep*Insecticide	4	126	3.3*
Error	126		

Table 4.16. Means separation of lint yield (kg ha^{-1}) of lines in split block yield trial in College Station, TX (2017)

Genotype	Mean (kg ha^{-1})
15 FF-21	1270 a[†]
13 Q-18	1253 ab
LA-05	1228 abc
LA-14	1188 abc
15 EE-48	1144 abcd
LA-13	1129 bcd
LA-03	1128 bcd
15 EE-52	1117 bcd
Tamcot73	1108 cde
LA-01	1104 cde
LA-11	1088 cdef
LA-04	1087 cdef
15 EE-40	1001 defg
LA-12	971 efgh
LA-07	965 fgh
LA-10	944 gh
LA-09	941 gh
LA-02	929 gh
LA-08	904 gh
LA-06	842 h

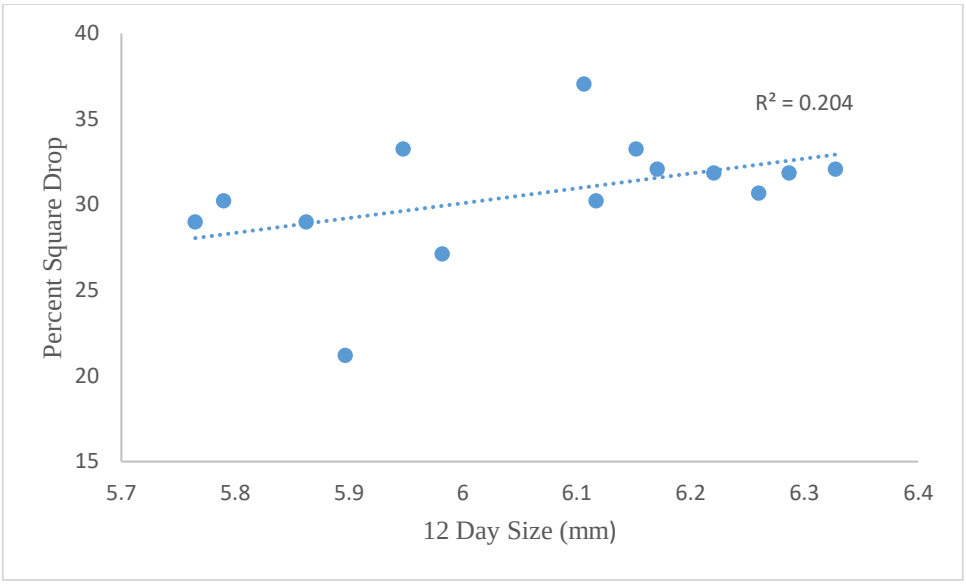
[†]Means connected by the same letter are not different at α=0.05, Duncan MRT

Both the post season plant mapping in 2016 and the split block spray/non spray yield trial in 2017 were unsuccessful in ascertaining useful information about the levels of injury due to CFH or potential resistance. While not containing the same 20 lines used in these studies, McCloud 2015, collected percent square loss data on the 14 BC_1F_3 strains that were tested (Table 4.17). Historical CFH feeding damage from 2014 was utilized and correlated to square size. CFH at College Station and Corpus Christi represent two distinct genotypes, based on amplified fragment length polymorphism (AFLP) analysis of populations collected from differing host sources (Barmen et al., 2012), so only data from College Station were used. Utilizing the ls means estimated for 12d square size and the percent square loss from College Station, 2014, a small but significant (α=0.1) correlation exists with a Pearson's correlation coefficient of 0.452 (Figure 4.6). This correlation suggests that among the backcross progeny, the plants with larger squares have a larger percentage square loss.

Table 4.17*. Means separation of percent square loss of parental and backcross progeny lines in untreated plots in College Station TX during the third week of data collection (2014)

Untreated		
College Station		
Genotype	**Pct Sq Loss**	
GH15-2	17.48	a†
GH18-1	18.73	a
LA-03	21.20	ab
GH13-6	21.84	ab
GH18-3	23.75	ab
LA-04	27.12	bc
TAM07V-45	27.65	bc
GH20-2	28.62	bc
LA-07	29.00	cd
LA-10	30.22	cd
LA-02	30.67	cd
GH20-1	31.58	cd
LA-08	31.85	cd
LA-12	32.08	cd
LA-14	33.25	cd
LA-01	37.04	d
TAM06WE-14	38.52	d

†Means sharing the same letter are not different at α=0.05, t-grouping
*Source: McCloud 2015



p=0.10

Figure 4.6. Regression of 12d square size ls means estimations with percent square drop of 2014 College Station trial.

McCloud 2015, also calculated regressions for ovary depth and square width (Figure 4.7). Using images taken during the McCloud study and CFH proboscis penetration depth equations published be Esquivel (2011) McCloud et al. calculated that the maximum proboscis penetration depth for adult CFH was 0.549 mm (Figure 4.7). Using the regressions of ovary depth and square size, we were able to determine a size that a square must attain to be large enough to escape ovary penetration by a CFH. Estimates for each strain were determined, then using the individual growth curves for the 2017 squares, the number of days required for the square to reach the size where the ovaries were protected was calculated (Table 4.18, 4.19). The total time that the squares spend susceptible to CFH varies from 2.93 days for LA-12 to 3.74 days for LA-04. Correlating 12d square size and the time it takes for a square to become large enough for the ovaries to be protected, a significant positive correlation is observed (Figure 4.8).

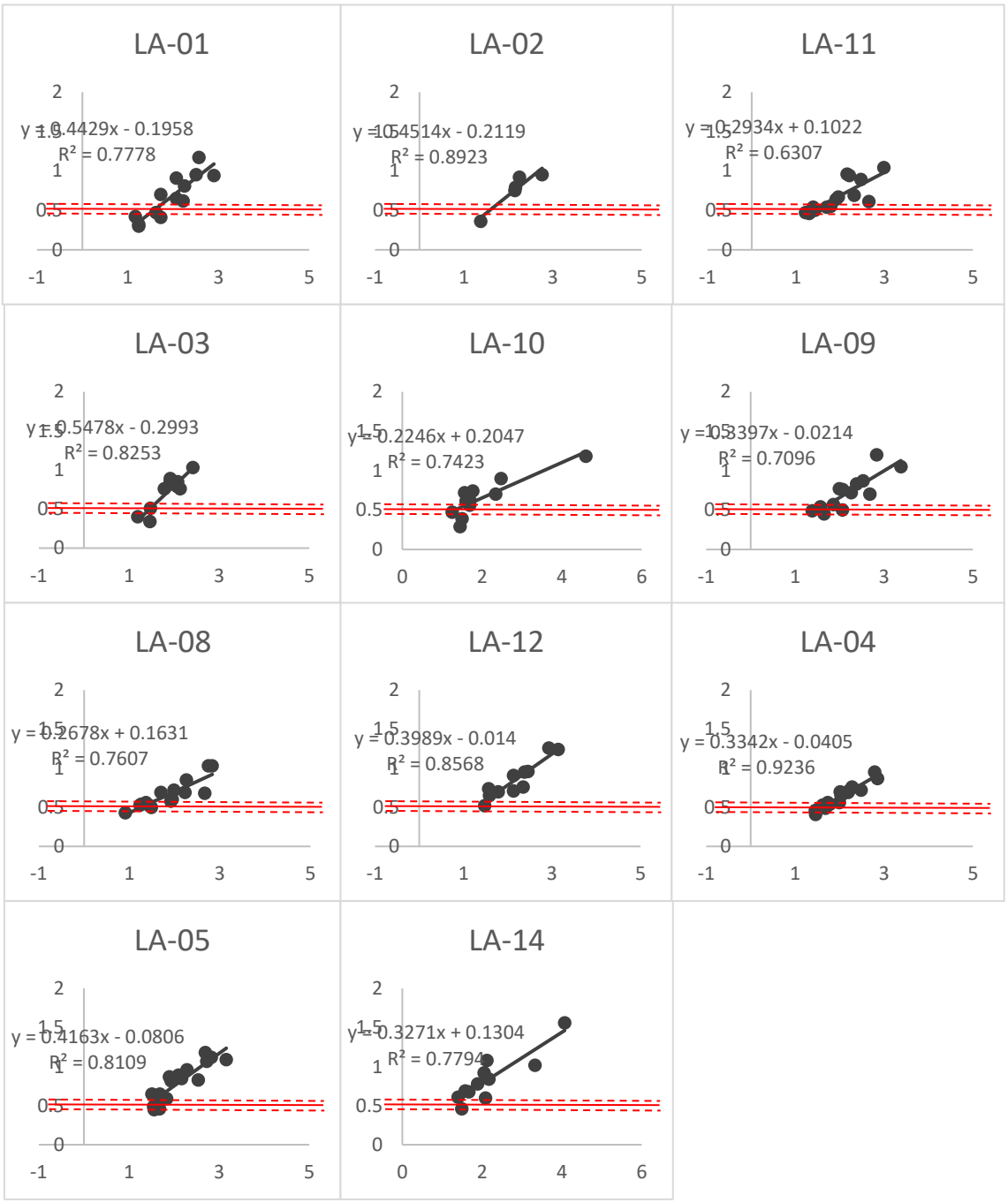


Figure 4.7*. Regression of ovary depth (y) on square width (x) for backcross progeny strains. The solid red line indicates a threshold of susceptibility of 0.549±0.05 mm, determined by estimating the maximum proboscis penetration depth of adult cotton fleahoppers during feeding
*Source: McCloud 2015

Table 4.18. Analysis of variance days of square susceptibility to CFH College Station, TX (2017 and 2106), * P<.05, ** P<.01

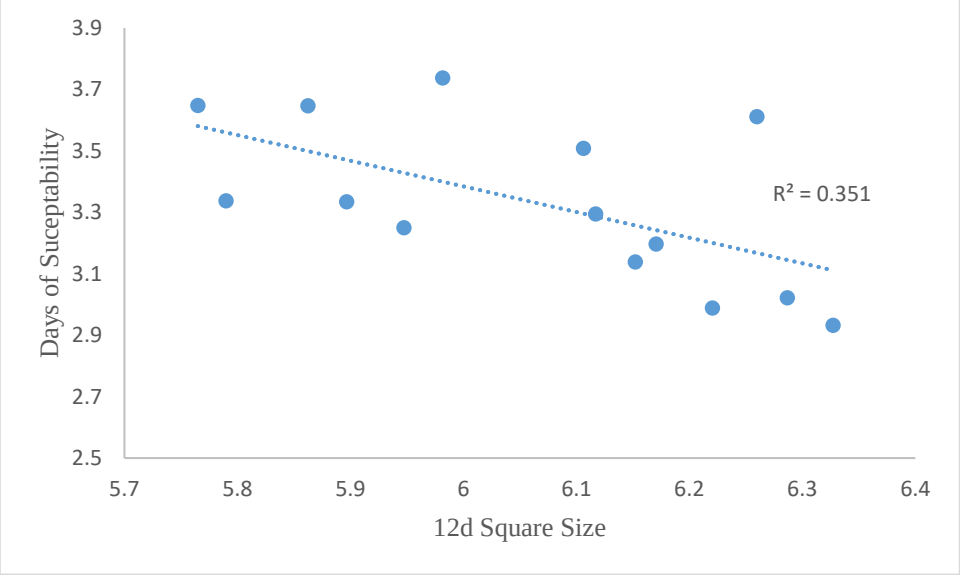
Effect	Num df, Den df	Den df	F Value
Rep	3	222	3.56*
Genotype	13	222	18.44**
Error	222		

Table 4.19. Mean separation of days of susceptibility for backcross strains College Station, TX (2017)

Genotype	Mean (days)
LA-04	3.74 a[†]
LA-07	3.65 ab
LA-06	3.65 ab
LA-02	3.61 ab
LA-01	3.51 bc
LA-11	3.34 cd
LA-03	3.33 cd
LA-10	3.29 de
LA-05	3.24 de
LA-13	3.20 def
LA-14	3.14 efg
LA-08	3.02 fgh
LA-09	2.99 gh
LA-12	2.93 h

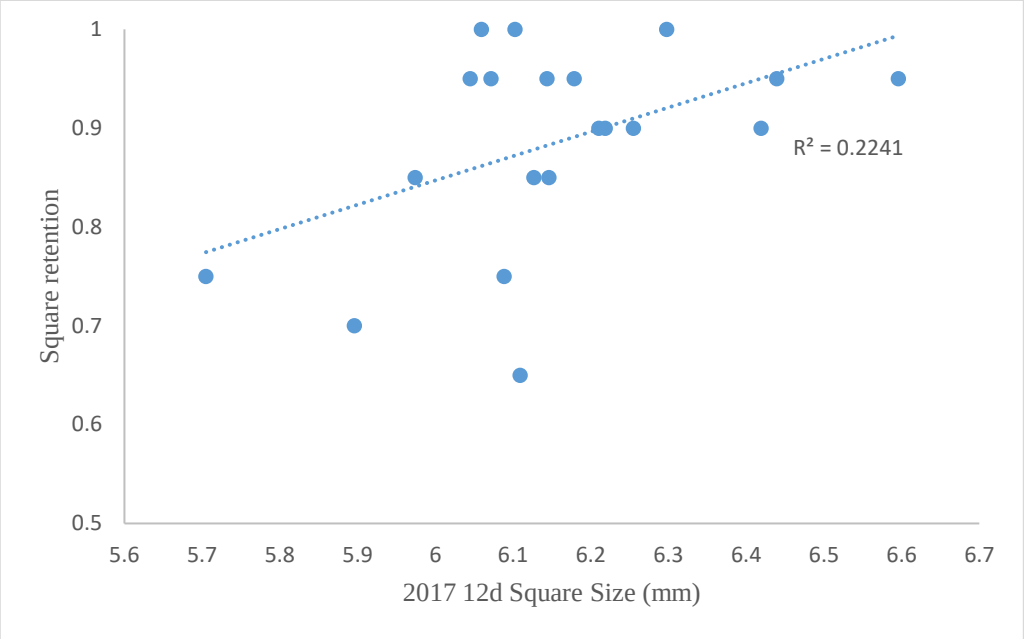
[†]Means connected by the same letter are not different at α=0.05, Duncan MRT

Knutson et al., (2013) reported that 99% of CFH feeding damage was observed on squares that were less than 2.0 mm in diameter. All of these lines' squares attain a size of less than 2.0 mm before the ovaries are deep enough to be protected from CFH feeding damage. The trend of larger squares being vulnerable for a lesser amount of time (Figure 4.8) would indicate that perhaps these lines show a greater degree of resistance to CFH. However, Figure 4.6 indicates a weak correlation suggesting, the opposite is true, i.e. the lines with larger squares experienced a greater loss of squares than smaller squared lines.



p=0.026

Figure 4.8. Regression of ls means square size estimates and calculated days of susceptibility for 14 backcross strains.



p=0.035

Figure 4.9. Regression of 2017 12d square size and proportion of retained squares throughout measurement.

As squares were being measured throughout their growth, not all initially selected were retained through flowering. A significant positive correlation between square size and square retention throughout the life of the growing square was observed (Figure 4.9). It is important to note that the square retention measured during this study was not affected by CFH. The first size measurements for the squares were made around match-head size (2-3mm). Squares of this size are already larger than the size range preferred by CFH according to Knutson et al. (2013). Showler (2005), showed that other insect pests in cotton have preferred square sizes for feeding and oviposition. This slight correlation shows that larger squares are more likely to reach flowering after they have survived past CFH feeding susceptibility. Additional data are necessary to determine if the correlation between square size and retention is repeatable. If it is, selecting for

larger squares would potentially increase square retention, an important trait where plants in a limiting environment are not as capable of compensating for loss of squares (Sadras, 1996).

Table 4.20. Analysis of variance of High Volume Information (HVI) fiber properties—length (mm), strength (kN m kg^{-1}), micronaire, uniformity (%) and elongation—of lines in College Station, TX (2017), * P<.05, ** P<.01

		Length	Strength	Micronaire	Uniformity	Elongation
Effect	**Num df, Den df**	**F Value**				
Genotype	19, 76	22.3**	12.59**	43.8**	16.86**	9.29**
Rep	2, 76	6.3**	4.96*	19.98**	1.14	0.35
Insecticide	1, 2	0.16	2.28	0.22	0.34	1.54
Rep*Insecticide	2, 76	6.33**	1.34	4.77*	2.7	3.4*
Genotype*Insecticide	19, 76	1.86*	0.98	1.53	1.13	1.44

Table 4.21. Means separation of high volume instrument (HVI) fiber properties—length (mm), strength (kN m kg^{-1}), micronaire, uniformity (%) and elongation—of parental lines in untreated plots in College Station and Corpus Christi, TX (2014)

Genotype	Length (mm)		Strength (kN m kg^{-1})		Micronaire		Uniformity (%)		Elongation	
13 Q-18	30.6	bc[†]	333	a	4.6	ijk	85.1	abc	5.12	de
15 EE-40	28.8	fgh	307	bcd	5.1	d	84.6	bcd	5.00	e
15 EE-48	31.0	b	341	a	5.0	de	85.6	abc	5.05	e
15 EE-52	28.7	gh	312	bc	5.5	c	84.1	cd	5.22	cde
15 FF-21	28.9	efgh	314	bc	5.4	c	85.3	abc	6.27	ab
Tamcot 73	30.4	bcd	339	a	4.9	def	86.0	ab	5.38	cde
LA-01	29.5	cdefg	306	bcd	4.7	ghij	84.0	dc	5.45	cde
LA-02	33.0	a	317	b	4.3	l	86.3	a	5.43	cde
LA-03	30.5	bc	304	bced	4.7	fghi	84.7	bcd	4.40	f
LA-04	29.4	defg	309	bc	4.8	efghi	84.6	bcd	5.60	cde
LA-05	29.8	cdef	291	defg	4.9	efg	84.4	cd	5.42	cde
LA-06	25.8	i	280	g	6.1	a	78.4	g	6.40	a
LA-07	29.9	bcde	312	bc	4.6	hijk	84.7	bcd	5.62	cde
LA-08	27.9	h	285	fg	5.5	c	82.0	e	6.70	a
LA-09	29.8	cdef	290	efg	4.5	jk	83.3	de	5.68	cd
LA-10	25.9	i	283	g	5.8	b	80.0	f	6.42	a
LA-11	28.8	fgh	301	bced	4.8	efgh	84.2	cd	5.75	bc
LA-12	30.1	bcd	304	bced	4.4	kl	85.4	abc	5.10	de
LA-13	29.9	bcde	299	cdef	4.7	ghij	84.9	abc	5.43	cde
LA-14	29.7	cdefg	300	cde	4.9	efg	84.7	bcd	5.23	cde

[†]Means connected by the same letter are not different at α=0.05, Duncan MRT

Among the backcross strains, LA-02 had excellent fiber properties with a length of 33.0mm, a strength 317 kNm kg^{-1}, and a micronaire of 4.3 (Table 4.21). With length and micronaire values that are superior to any of the elite breeding lines LA-02 has potential use in a breeding program with its high-quality fiber. It did not perform well in lint yield being not statistically different from the poorest yielding line and numerically 17^{th} out of the 20 lines tested (Table 4.16). The elite material line 13Q-18 showed good fiber quality with a length of 30.6mm and the highest strength in the test at 333.44 kNm kg^{-1} (Table 4.21), high yield potential, being statistically similar to the top yielding line and second numerically as well as having extremely consistent performance across replications.

5. CONCLUSION

This project had two objectives: (1) determine if differences in square sizes between genotypes exist and (2) establish if there is a relationship between square size and cotton fleahopper resistance. With regards to the first objective, it was established that differences in square size exist. Across both years there were highly significant differences in square size, however, from one year to another the size of a genotype can change so much so that there is no correlation between square sizes in 2016 and 2017. Some lines such as 15EE-52 showed consistent performance across both years, but other lines such as LA-03, LA-04 LA-10 and 15FF-21 shifted more than 50 percentile points from 2016 to 2017. In the combined analysis, year was not a significant source of variation, but the interaction of year and genotype was. The differences between the two years show a large range in genotype by environment interaction effects. Some of the genotype by environment interactions may be explained by the fact that the measurements of square size are confounded by the squares being different ages. The only consistent way to measure squares of the "same" age is to count backwards from the date of flowering, however differences in the time it takes each genotype to flower causes these backwards age calculations to be different ages in actuality. The growth pattern of a cotton plant is a much more complex trait than the size of a square, so it is plausible that this added complexity of the measurement helps to explain the large environmental interaction. Square size is a highly quantitative trait, and although no direct estimate for heritability can be made from the data collected, because the

repeatability for year to year is low, this leads us to believe that the heritability would be relatively low as well.

The second objective of the study was to determine if square size played a role in the host plant resistance mechanisms against CFH. From the individual square growth curves and ovary depth data, see a significant correlation between larger squares and less time spent susceptible to CFH feeding damage is observed. This correlation supports the theory of larger squares conferring greater resistance against CFH, but the best available CFH resistance data do not support this theory. During a heavy CFH year in 2014, a slight, lowly significant correlation shows that among the backcross strains, the lines with larger squares sustained a larger degree of damage. McCloud (2015) identified LA-03 as exhibiting lower injury levels than either of its parents and the lowest among the backcross progeny lines in 2014 when CFH populations were the highest. This line is in the middle of the range of days of susceptibility at 3.33 days (Table 4.19). It has the fourth smallest ls mean 12d square size (Table 4.13) of all the lines and the largest rank shift from 2016 to 2017 (Table 4.14).

Due to the growth patterns of cotton, there are squares of varying sizes on any one plant at a given time. CFH preferentially feed on smaller squares where they can reach the ovaries inside. Even though the larger squares escape the threshold of CFH damage faster than the smaller squares, the larger squared varieties still spend multiple days with susceptible squares. The inconsistency between the square retention and days of susceptibility with regards to square size suggests that square size does not play a role

in the complex matrix of host plant resistance mechanisms that ultimately determine the level of resistance a cotton cultivar exhibits against cotton fleahoppers.

REFERENCES

Almand, L.K., W.L. Sterling, C.L. Green. 1976. Seasonal abundance and dispersal of the cotton fleahopper as related to host plant phenology. Texas Agric. Exp. Stn. Bull. 1170.

Awmack, C.S. and S.R. Leather. 2002. Host plant quality and fecundity in herbivorous insects. Ann. Rev. Entomol. 47: 817-844.

Barman, A.K., M.N. Parajulee, C.G. Sansone, C.P.C. Suh, R.F. Medina. 2012. Geographic pattern of host-associated differentiation in the cotton fleahopper, *Psudatomoscelis seriatus*. Entomol. Exp. App. 143:31-41.

Bell, A. A., E.G. Medrano, J.D. Lopez, R.K. Luff. 2007. Transmission and importance of *Pantoea ananatis* during feeding on cotton buds (*Gossypium hirsutum* L.) by cotton fleahoppers (*Pseudatomoscelis seriatus* Reuter). World Cotton Research Confernce, Lubbock, TX.

Bell, A.A., J.D. Lopez, and E.G. Medrano. 2006. Frequency and identification of cotton-rotting bacteria from cotton fleahoppers. *In* Proc. Beltwide Cotton Conferences. National Cotton Council of America: pp 97-104.

Bohmfalk, G.T., R.E. Frisbie, W.L. Sterling, R.B. Metzer, A.E. Knutson. Identification, biology and sampling of cotton insects. Texas Cooperative Ext. Texas A&M University <http://www.soilcropandmore.info/crops/CottonInformation/insect/B-933/b- 933.htm>.

Boica, A. L., Jr., Campos, Z. R., Lourencao, A. L., & Campos, A. R. 2007. Adult attractiveness and oviposition preference of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) B-biotypes in cotton genotypes. Scientia Agricola, *64*, 147–151.

Bottrell, Dale R. 1979. Integrated Pest Management. Washington, DC: Council on Environmental Quality.

Bravo, A., S.S. Gill, M. Soberón. 2007. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon 49(4): 423-435.

Breene, R.G., W.R. Martin Jr., D.A. Dean, W.L. Sterling. 1989. Rearing methods for the cotton fleahopper. Southwest. Entomol. 14(3): 249-253.

Butter, N. S., & Vir, B. K. (1989). Morphological basis of resistance in cotton to the whitefly, *Bemisia tabaci*. *Phytoparasitica, 17*, 251–261.

Chu, C. C., Henneberry, T. J., & Cohen, A. C. (1995). *Bemisia argentifolii* (Homoptera: Aleyrodidae): host preference and factors affecting oviposition and feeding site preference. Environmental Entomology, *24*, 354–360.

Dent D (1995) Integrated pest management. Chapman and Hall, London

Duffey, J.E. and R.D. Powell. 1979. Microbial induced ethylene synthesis as a possible factor of square abscission and stunting in cotton infested by cotton fleahopper. Ann. Entomol. Soc. Am. 72(5): 599-601.

Esquivel, J. F. 2011. Estimating potential stylet penetration of southern green sting bug--a mathematical modeling approach. Entomol. Exp. App. 140: 163-170.

Esquivel, J. and S. Esquivel. 2009. Identification of cotton fleahopper (Hemiptera: Miridae) host plants in Central Texas and compendium of reported hosts in the United States. Environ. Entomol. 38(3): 766-780.

Fineblum, W. L. , and M. D. Rausher . 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature **377**: 517–520.

Fuchs, T. W., C. T. Allen, E. P. Boring III & J. F. Leser. 1993. Management of cotton insects in the High Plains, Rolling Plains, and Trans-Pecos areas of Texas. Texas Agric. Ext. Servo B-1209.

Greenberg, S. M, T. W. Sappington, M. Sétamou, R. J. Coleman. 2003. Influence of Different Cotton Fruit Sizes on Boll Weevil (Coleoptera: Curculionidae) Oviposition and Survival to Adulthood, Environmental Entomology. 33(2): 443–449

Hegde, Mahabaleshwar, Janser N. Oliveira, Joao G. da Costa, Elisa Loza-Reyes, Ervino Bleicher, Antonio E.G. Santana, John C. Caulfield, Patrick Mayon, Sarah Y. Dewhirst, Toby J.A. Bruce, John A. Pickett, Michael A. Birkett. 2012. Aphid antixenosis in cotton is activated by the natural plant defence elicitor cis-jasmone. Phytochemistry. 78: 81-88.

Hofius, D., D. Munch, S. Bressendorff, J. Mundy, M. Petersen. 2011. Role of autophagy in disease resistance and hypersensitive response-associated cell death. Cell Death Differ. 18: 1257-1262.

Jindal, V. & Dhaliwal, G.S. 2011. Mechanisms of resistance in cotton to whitefly (*Bemisia tabaci)*: antixenosis. Phytoparasitica. 39: 129

Johnson, H. B. 1975. Plant pubescence: an ecological perspective. Bot. Rev. 41: 233-258.

Knutson, A. E., R. D. Parker, R. L. Huffman, C. G. Sansone & J. S. Swart. 1993. Management of cotton insects in the southern, eastern, and Blackland areas of Texas. Texas Agric. Ext. Servo B-1204.

Knutson, A.E., K.D. Mekala, C.W. Smith, C. Campos. 2013. Tolerance to feeding damage by cotton fleahopper (Hemiptera: Miridae) among genotypes representing adapted germplasm pools of United States upland cotton. J. Econ. Entomol. 106(2): 1045-1052.

Kogan, M. and E.F. Ortman. 1978. Antixenosis--a new term proposed to define Painter's 'Nonpreference' modality of resistance. Bull. Entomol. Soc. Am. 24(2): 175-176.

Kogan, M. 1978. Pubescence: role in breeding for plant resistance, pp. 13-23. In Proceedings of the Third Biennial Research Workshop, Gainesville, Fla.

Lehtilä and Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, Raphanus raphanistrum. Ecology, 80: 116-124

Lidell, M. C., G. A. Niles, and J. K. Walker. 1986. Response of nectariless cotton genotypes to cotton fleahopper (Heteroptera: Miridae) infestation. J. Econ. Entomol. 79: 1372–1376.

Lukefahr, M.J., C.B. Cowan, R.R. Pfrimmer, L.W. Noble. 1966. Resistance of experimental cotton strain 1514 to the bollworm and cotton fleahopper. J. Econ. Entomol. 59(2): 393-395.

Lukefahr, M.J., C.B. Cowan, L.A. Bariola, J.E. Houghtaling. 1968. Cotton strains resistant to the cotton fleahopper. J. Econ. Entomol. 61(3): 661-664.

Lukefahr, M.J., C.B. Cowan, J.E. Houghtaling. 1970. Field evaluations of improved cotton strains resistant to the cotton fleahopper. J. Econ. Entomol. 63(4): 1102-1103.

Martin, W. R., M.P. Grisham, C.M. Kenerly, W.L. Sterling, P.W. Morgan. 1987. Microorganisms associated with cotton fleahopper, *Pseudatomoscelis seriatus* (Heteroptera: Miridae). Ann. Entomol. Soc. Am. 80(2): 251-255.

Martin, W. R., Jr., P. W. Morgan, W. L. Sterling, and R. W. Meola. 1988. Stimulation of ethylene production in cotton by salivary enzymes of the cotton fleahopper (Heteroptera: Miridae). Environ. Entomol. 17: 930–935.

Mauricio, R., Rausher, M. D. and Burdick, D. S. (1997), Variation in the defense strategies of plants: Are resistance and tolerance mutually exclusive?. Ecology, 78: 1301–1311

Mauney, J. R., and T. J. Henneberry. 1979. Identification of damage symptoms and patterns of feeding of plant bugs in cotton. J. Econ. Entomol. 72: 496–501

McLoud, Laura Ann, Allen Knutson, Manuel Campos-Figueroa, C. Wayne Smith, Steven Hague (2015). Evaluating Pilose, a Cultigen of *Gossypium hirsutum* , as a Source of Resistance to Cotton Fleahopper (Hemiptera: Miridae) , *Journal of Economic Entomology* 108(4): 2048–2054

McLoud, Laura Ann, Steven Hague, Allen Knutson, C. Wayne Smith, Michael Brewer; Cotton Square Morphology Offers New Insights into Host Plant Resistance to Cotton Fleahopper (Hemiptera: Miridae) in Upland Cotton, *Journal of Economic Entomology*. 109(1): 392–398

Meagher, R. L., Jr., Smith, C. W., & Smith, W. J. (1997). Preference of *Gossypium* genotypes to *Bemisia argentifolii*(Homoptera: Aleyrodidae). *Journal of Economic Entomology, 90,* 1046–1052.

Mekala, K. D. 2004. Screening upland cotton for resistance to cotton fleahopper (Heteroptera: Miridae). M.S. thesis, Texas A&M University, College Station, TX.

Meredith, W. R., and M. F. Schuster. 1979. Tolerance of glabrous and pubescent cottons to tarnished plant bug. Crop Sci. 19: 484–488.

Meyer, J. R., and V. G. Meyer. 1961. Origin and inheritance of nectariless cotton. Crop Sci. 1: 167–169.

Pack, T.M. and P. Tugwell. 1976. Clouded and tarnished plant bugs on cotton: a comparison of injury symptoms and damage on fruit parts. Arkansas Agri. Exp. Stn. Report Series 226.

Painter, R.H. 1958. Resistance of plants to insects. Ann. Rev. Entomol. 3:267-290.

Parajulee, M. N., R. B. Shrestha, J. F. Leser. 2006. Sampling Methods, Dispersion Patterns, and Fixed Precision Sequential Sampling Plans for Western Flower Thrips (Thysanoptera: Thripidae) and Cotton Fleahoppers (Hemiptera: Miridae) in Cotton, Journal of Economic Entomology, 99(2): 568–577

Parker, R. D., M. J. Jungman, S. P. Biles and D. L. Kerns. 2008. Managing cotton insects in the Southern, Eastern, and Blackland areas of Texas. Texas Agrilife Ext. Serv. Bull., E-5, 17 pp.

Parker, R.D. Response of cotton to fleahopper control based on growing condtions: using the fleahopper to adjust fruit load. Proc. of the Beltwide Cotton Conferences. January 2009.

Pfannenstiel, R. S. Nocturnal predation of Lepidopteran eggs in south Texas cotton. Proc. of the Beltwide Cotton Conferences, San Antonio, TX, January 2004.

Poston, F. L., L. P. Pedigo & S. M. Welch. 1983. Economic injury levels: reality and practicality. Bull. Entomol. Soc. Am. 29: 49-53.

Ramalho, F. S. , W. L. Parrott, J. N. Jenkins, J. C. McCarty. 1984. Effects of Cotton Leaf Trichomes on the Mobility of Newly Hatched Tobacco Budworms (Lepidoptera: Noctuidae), Journal of Economic Entomology. 77(3): 619-621

Ring, D.R, J.H Benedict, M.L. Walmsley, M.F. Treacy. 1993. Cotton yield response to cotton fleahopper (Hemiptera: Miridae) infestations on the lower Gulf Coast of Texas. J. Econ. Entomol. 86(6): 1811-1819.

Robinson, S. H., D. A. Wolfenbarger, and R. H. Dilday. 1980. Antixenosis of Smooth Leaf Cotton to the Ovipositional Response of Tobacco Budworm1. Crop Sci. 20:646-649

Rosenheim, J. A., Wilhoit, L. R., Goodell, P. B., Grafton-Cardwell, E. E. and Leigh, T. F. (1997), Plant compensation, natural biological control, and herbivory by *Aphis gossypii* on pre-reproductive cotton: the anatomy of a non-pest. Entomologia Experimentalis et Applicata, 85: 45–63.

Sadras, V.O. 1996. Cotton compensatory growth after loss of reproductive organs as affected by availability of resources and duration of recovery period. Oecologia, 106: 432-439

Schuster, M. F., M. J. Lukefahr, and F. G. Maxwell. 1976. Impact of nectariless cotton on plant bugs and natural enemies. J. Econ. Entomol. 69: 400–402.

Shaver, T.N. and W.L. Parrott. 1970. Relationship of larval age to toxicity of gossypol to bollworms, tobacco budworms, and pink bollworms. J. Econ. Entomol. 63(6): 1802-1804.

Showler, Alan T. 2005. Relationships of different cotton square sized to boll weevil (Coleoptera: Curculionidae) feeding and oviposition in field conditions. Journal of Economic Entomology. 98(5): 1572-1579.

Simms, E. L. and Triplett, J. (1994), Costs and benefit of plant responses to disease: Resistance and tolerance. Evolution, 48: 1973–1985.

Singh I. D., J. B. Weaver. 1972. Growth and Infestation of Boll Weevils on Normal-Glanded, Glandless, and High-Gossypol Strains of Cotton, Journal of Economic Entomology. 65(3): 821–824

Smith, James W. 1998. Boll Weevil Eradication: Area-Wide Pest Management, Annals of the Entomological Society of America. 91(3): 239–247

Stewart, S.D. and W.L. Sterling. 1989. Causes and temporal patterns of cotton fruit abscission. J. Econ. Entomol. 954-959.

Strauss, S.Y. and A.A. Agrawal., 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14(5): 179-185

Sun, Hainan, Fei Zhang,Sumei Chen,Zhiyong Guan,Jiafu Jiang,Weimin Fang,Fadi Chen. 2015. Effects of aphid herbivory on volatile organic compounds of Artemisia annua and Chrysanthemum morifolium. Biochemical Systematics and Ecology 60: 225-233

Tingey, W. M., T. F. Leigh, and A. H. Hyer. 1975. Glandless Cotton: Susceptibility to *Lygus hesperus* Knight1. Crop Sci. 15:251-253

Treacy, M.F., Zummo, G.R. and Benedict, J.H., 1985. Interactions of host-plant resistance in cotton with predators and parasites. Agric. Ecosystems Environ. 13: 151-- 157.

Van der Meijden, E., Wijn, M., & Verkaar, H. (1988). Defence and Regrowth, Alternative Plant Strategies in the Struggle against Herbivores. *Oikos, 51*(3), 355-363.

Walker, J.K., G.A. Niles, J.R. Gannaway, J.V. Robinson, C.B. Cowan, M.J. Lukefahr. 1974. Cotton fleahopper damage to cotton genotypes. J. of Econ. Entomol. 67(4): 537-542.

Wiklund, C. 1974. Oviposition preferences in Papilio machaon in relation to the host plants of the larvae. Entomol. Exp. Appl. 17: 189-198

Williams, M. 2000-2014. Texas Summary. Cotton Crop Loss Data. Mississippi State University. http://www.entomology.msstate.edu/resources/cottoncrop.asp