AN INTEGRATED APPROACH TOWARD IDENTIFYING RESISTANCE TO COTTON FLEAHOPPER (*PSEUDATOMOSCELIS SERIATUS*) IN UPLAND COTTON

A Dissertation

by

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ABSTRACT

Cotton fleahoppers (*Pseudatomoscelis seriatus*) are an early season pest of upland cotton. Feeding damage is characterized by death and abscission of developing cotton floral buds, called squares, and is thought to result from infection of the plant tissue with the bacterial pathogen, *Pantoea ananatis*, which is vectored by the insect during feeding. Heavy infestations cause delayed maturity and can result in substantial yield loss. Cotton fleahoppers are primarily controlled by chemical insecticides, and thus there exists a strong need to identify resistance in the available upland germplasm for resistance breeding purposes. To that end, three integrated projects were designed to identify and characterize host plant resistance in the available upland germplasm: (1) field evaluation of candidate germplasm to identify resistance and introgression of the resistance trait through backcross breeding, (2) characterization of resistance identified in the first objective through assays of feeding behavior and morphological analysis of the plants and cotton fleahoppers, and (3) RNA-seq transcriptome analysis of plant response to herbivory in one susceptible and three resistant genotypes identified in the first objective.

Germplasm obtained from a previous cotton fleahopper breeding effort at Texas A&M and from the Texas A&M AgriLife Research Cotton Improvement Lab at College Station was screened for resistance by estimating percent square loss in three years of field tests in College Station and Corpus Christi, TX and included two high-yielding breeding lines and 18 lines derived from crosses of Pilose (a densely pubescent cultigen resistant to cotton fleahopper) with ‘Deltapine50,’ ‘All-Tex Atlas,’ and ‘TAM 96 WD-

69s'. Field evaluations identified resistance to cotton fleahoppers in lines derived from crosses with Pilose. Field evaluations of backcross progeny lines identified one line, 12525, with high resistance to cotton fleahoppers in both College Station and Corpus Christi and good yield and fiber traits. Behavioral assays examined the interactions of adult cotton fleahoppers with excised cotton squares. Behavior was categorized as walking, resting, probing, feeding or cleaning. Analysis revealed significant differences among parental and backcross progeny lines in time cotton fleahoppers spent feeding, indicating non-preference as a mechanism of resistance. Morphological analysis of square structure, in which square width and length and depth of the developing ovary were measured, indicated variation in depth of the developing ovary may contribute to resistance to cotton fleahoppers; squares with greater ovary depth may escape direct penetration by the proboscis of a feeding cotton fleahopper. RNA-seq transcriptome profilining examined the effects of cotton fleahopper herbivory on gene expression. Analysis revealed differential expression of transcripts associated with three regulators of the hypersensitive response (HR)—*myb* transcription factor, alternative oxidase (AOX), and BAX inhibitor-1— and indicated the difference between susceptible types (plants that shed squares) and resistant types (plants that retain squares) may lie in regulation of HR-associated lesion formation. Together, the projects presented in this dissertation indicate that the relationship between cotton fleahopper and upland cotton is complex and involves several host plant resistance mechanisms that can be exploited in future efforts to breed for resistance to this insect in cotton.

DEDICATION

This work is dedicated to my family, who supported me through this process.

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NOMENCLATURE

CFH	Cotton fleahopper(s)
ET	Economic threshold
GO	Gene ontology
HR	Hypersenstive response
AOX	Alternative oxidase

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

LIST OF TABLES

TABLE Page

TABLE Page

TABLE Page

TABLE Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Upland cotton (*Gossypium hirsutum*) is the primary fiber crop in the southern United States. Annually, upland cotton contributes an estimated $100 billion in economic value, according to a recent estimate by the National Cotton Council of America. In 1996, *Bt* cotton was introduced to the market to control Lepidoptera pests of the crop, and, according to estimates in 2014 by the USDA Economic Research Service, 96% of all cotton grown in the US is genetically modified (GM). *Bt* cotton is engineered to produce *cry* proteins, toxins natively expressed in the soil-borne bacteria, *Bacillus thuringiensis*. Briefly, once ingested by caterpillar pests of cotton, these toxins bind to receptors in the midgut membrane and create pores, which results in lysis of the midgut epithelium cells (Bravo et al., 2007). While GM cotton has been very successful in controlling Lepidopteran pests, piercing-sucking insects, like the cotton fleahopper (CFH) (*Pseudatomoscelis seriatus*), are not susceptible to GM cotton.

Cotton fleahoppers are an early season pest of upland cotton, and damage is generally most severe in the central and southern portions the U.S. Cotton Belt, particularly in the dryland regions of Texas (Ring et al., 1993), where cotton is bred for early maturity and begins squaring around the time of senescence of the CFHs' weedy spring hosts. Damage resulting from CFH feeding has been estimated to be responsible for the loss of 91 kg of lint per acre in South and Central Texas (Parker, 2009). In the last decade (2000-2010), estimated losses in Texas due to CFH have been as high as 108,057 bales (in 2007), a 1.11% yield reduction. In perspective, in 2007, estimated

yield reductions in Texas due to bollworm/budworm (*Helicoverpa armigera*/*Heliothis virescens*) and *Lygus* spp. were 0.81% and 0.11%, respectively. On average, CFH, over the past decade, have resulted in an estimated yearly yield reduction of 0.4% (Williams, 2000-2014).

Cotton fleahopper is primarily herbivorous, feeding on over 160 wild hosts (Esquivel and Esquivel, 2009), and facultatively insectivorous, feeding on the eggs of other cotton pests, like the bollworm and tobacco budworm (Pfannenstiel, 2005; Gravena and Sterling, 1983; McDaniel and Sterling, 1979). The life cycle of the CFH spans 47-50 days. An adult female lays eggs just under the bark of the host plant by inserting her ovipositor into the plant tissue, and eggs hatch in one to two weeks (Breene et al., 1969; Bohmfalk et al., 2005). Immatures, called nymphs, undergo five molts over a period of about two weeks, the last of which represents the transition from nymph to adult (Bohmfalk et al., 2005). In East Texas, CFH overwinter as eggs in the stems of woolly croton, *Croton capitatus* (Breene et al., 1989), and feed on a variety of wild hosts in the spring, with plants of the genus *Oenothera* generally thought of as the preferred spring hosts (Esquivel and Esquivel, 2009). As wild host plants begin to senesce, CFH migrate to cotton fields (Almand et al., 1976).

Cotton fleahoppers are reported to feed on the leaves, ovary wall, and developing anthers of the cotton plant (Reinhard 1926; Pack and Tugwell, 1976; Bell et al., 2007). Cotton fleahopper feeding damage results in blasting, or death and abscission, of the cotton squares. Like other Mirids, the saliva of CFH contains a pectinase, called polygalacturonase, which aids in digestion of pectins in the middle lamella (Miles, 1972;

Martin et al., 1988). Martin et al. (1988) conjectured that polygalacturonase in the saliva may be responsible for the plant tissue lesion characterisitic of CFH feeding. An early study by Painter (1930) of CFH morphology revealed the presence of "vacuolated droplets…[that had] the general appearance of yeast cells" in the anterior portion of the insect's salivary glands, which suggested the presence of bacteria in the salivary glands. Studies have since identified a cocktail of bacteria that can be isolated from the salivary glands of CFH, including known plant pathogens of the genera *Pantoea*, *Serratia*, *Xanthomonas*, and *Pseudomonas* (Duffey and Powell, 1979; Martin et al., 1987; Bell et al., 2007). *Pantoea ananatis* (Enterobacteriaceae), isolated from CFH salivary glands by Bell et al. (2007), is often regarded as an epiphyte but is also well-known for its pathogenicity. It is a broadly adapted species but was first described as the cause of fruit rot in pineapples in the Phillippines (Coutinho and Venter, 2009). Duffey and Powell (1979) reported that cotton squares infested with CFH produced ethylene at rates 5x that of pre-infestation conditions. Both *Fusarium sp.* and *Xanthomonas sp.* were cultured from CFH-infested plant tissue. When applied to cotton squares, in the absence of CFH, *Fusarium sp*. and *Xanthomonas sp*. triggered ethylene production rates that approximated ethylene production rates in squares infested with CFH; *Fusarium sp*. produces ethylene in culture, but *Xanthomonas sp.* produced negligible amounts of ethylene in culture. Duffey and Powell (1979) suggested that infection of the plant with *Xanthomonas* sp. during CFH feeding induced ethylene synthesis in infested plants, which may have caused abscission of infested squares. Bell et al. (2007) more definitively reported that bacteria vectored during CFH feeding is the cause of ovary rot

in fed upon squares and may be the main cause of square shed after CFH feeding. Sterile water used to wash CFH and subsequently injected into cotton squares caused seed rot and boll rot, indicating that the CFH-associated bacteria was pathogenic. According to Bell et al. (2007), the average proboscis length of the CFH is 1.5 mm, and the average depth of the ovary is approximately 1.0 mm, so a CFH feeding on a developing square can easily penetrate the ovary wall and introduce pathogens (Bell et al., 2007). However, Bell et al. (2007) did not account for the fact that the entire length of the proboscis does not enter the plant tissue during feeding; penetration depth is limited by the length of the first and second labial segements, which hinge during insertion of the feeding stylets (Wheeler 2001; Esquivel 2011).

Microflora diversity in the salivary glands of CFH has been reported to vary with the host plant from which the insects were collected. The majority of CFH collected from lemon horsemint (*Monarda citriodora*) in College Station, TX had sterile saliva (Martin et al., 1987). However, when CFH were collected from woolly croton in College Station they were found to harbor *Penicillium* sp. and *Pantoea* spp. (Martin et al., 1987; Bell et al., 2006). Martin et al. (1988) reported that CFH saliva, after being filtered to remove most microorganisms, induced ethylene production when it was injected into cotton squares. Saliva from male CFH resulted in higher concentrations of ethylene than saliva from female CFH. Pectinases in the saliva, which attack the cell wall and middle lamellae (Bateman and Millar, 1966), were attributed with the ethylene burst observed after injection of the squares with CFH saliva. The cotton squares tested were excised

from the plant prior to injection, so whether or not ethylene production resulting from saliva injections alone can cause square abscission is unknown.

Control of CFH in cotton remains primarily chemical in nature (Parker et al, 2007). The need to exploit naturally occurring host plant resistance remains, as CFH continues to rank among the top pests in Texas cotton. Host plant resistance is defined as the phenomenon by which plants under the same environmental conditions experience different levels of injury due to insect herbivory (Painter, 1958); plants with comparatively little damage are often termed resistant, and those with comparatively more damage are often termed susceptible. Painter (1958) described three types of host plant resistance: tolerance, non-preference (now commonly referred to as antixenosis (Kogan and Ortman, 1978)), and antibiosis. Tolerance describes the effect of the insect on the plant. Tolerance is a plant's ability to survive and sufficiently recover from insect infestation and feeding damage to be able to produce biomass and reproduce (Painter, 1958; Reese et al., 1994; Strauss and Agrawal, 1999). For example, tolerance may manifest as regrowth of tissues damaged by insect herbivores (Strauss and Agrawal, 1999). Cotton is able to compensate for damage to its reproductive structures by CFH feeding but not without a delay in maturity of the crop (Stewart et al., 1989).

Antixenosis and antibiosis describe a plant's effect on the insect. Antixenosis is the aversion of the insect to feeding or even selecting the plant as a potential host. Plants may produce antifeedant compounds that limit or deter feeding or may fail to elicit chemical signals that attract insects during the host finding process. When an insect incurs a fitness cost from feeding on a plant it is called antibiosis. For example,

consumption of secondary metabolites during feeding on a host can lengthen developmental time for immature insects and affect weight and fecundity in adults (Awmack and Leather, 2002). Gossypol, a terpenoid secondary metabolite of cotton, is known to retard larval growth and reduce pupal weight in caterpillars of the *Heliothis*/*Helicoverpa* complex and to negatively impact nymph survival in *Lygus hesperus* (Miridae) (Shaver and Parrott, 1970; Tingey, 1975).

The most common strategy in identifying host plant resistance for CFH is evaluation of the role that plant pubescence plays in the insects' preference for feeding and/or capacity to feed. Lukefahr and colleagues (1966, 1968, 1970) published a series of papers reporting the role of cotton leaf pubescence in resistance to CFH. Lukefahr and colleagues (1966) initially alluded to glabrousness as the quality that confers resistance, but also noted that the plants' "open-type terminal buds" may also play a role. In 1968, Lukefahr and colleagues published a follow-up study in which cage, small plot, and field tests were conducted to evaluate hirsute and glabrous cotton cultivars under CFH stress. Again, CFH counts on glabrous cultivars were significantly lower than on hirsute cultivars. A third study (1970) investigated the role of the pilose cotton for potential resistance to CFH. The pilose trait, controlled by the H_2 gene, confers dense pubescence on the leaves, stems, and squares of the plant (Simpson 1947, Ramey 1962, Benedict et al. 1983). Lukefahr (1970) found a strong, positive correlation between pubescence and number of CFH per plant. He conjectured that trichomes play a role in trapping the plant's volatiles close to the surface of the leaf; on hirsute plants, he hypothesized, CFH are, in effect, protected from defensive compounds that volatilize from the plant,

whereas on glabrous plants, due to low trichome density, CFH are exposed to these defensive compounds, which confers resistance to the plant (Lukefahr et al., 1970). Despite these findings, Lukefahr et al. (1966, 1968, 1970) did not assess the damage due to CFH feeding, only the insects' preference for congregating on some cultivars over others.

Walker and colleagues (1974) offered a more complete view of the relationship between cotton plant trichome density and CFH preference and feeding. Much like studies previously conducted by Lukefahr and colleagues (1966, 1968, 1970), Walker et al. evaluated glabrous and hairy plants, including a genotype termed ‘Pilose’. In addition to CFH per 100 plants, squares and flowers were also counted, as well as lint production. Consistent with Lukefahr et al. (1966, 1968, 1970), Walker and colleagues reported that CFH numbers were lower on glabrous varieties, compared to hairy varieties. Walker et al. (1974) also reported that, despite lower CFH numbers on glabrous cultivars, these cultivars were more susceptible to CFH feeding damage than more hirsute cultivars, which harbored higher numbers of CFH.

While earlier studies used indirect measures (number of blooms, yield) of CFH injury, Knutson et al. (2013) directly measured CFH injury by observing injury in dissected squares in no-choice cage studies. In their study, examining CFH feeding tolerance in breeding lines and adapted germplasm, they reported, similarly, a significant, positive correlation between trichome density and CFH density. However, they reported no significant correlation between trichome density and square damage when square damage was determined by visual examination of squares for CFH feeding

injury. Knutson et al. (2013), citing Chu et al. (2001) and Johnson (1975), hypothesized that hairy leaf cotton may be more attractive to CFH because the dense pubescence of hairy leaf cotton creates a microclimate in which temperature and humidity are regulated. Knutson et al. (2013) also found that the Pilose cultigen was the most tolerant to CFH feeding in both choice and no-choice feeding studies. Knutson and colleagues (unpublished), evaluating a broader range of genotypes, assessed the role of the pilose trait in conferring resistance to CFH. Pilose was crossed with several commercial and breeding lines and progeny were screened for resistance to CFH feeding. Cotton fleahopper behavior was also assessed, and results suggested that the type of resistance conferred by pilose is non-preference and is a heritable trait.

Trichome density evidently is important in the dynamic between CFH and the plants upon which they feed. The following studies focus on the role of the pilose trait as a resistance factor and evaluate its potential in breeding for increased resistance to CFH feeding. Transcriptome profiling, using RNA sequencing, was also conducted to elucidate differences in gene expression between tolerant and susceptible lines exposed to CFH feeding in an effort to identify underlying mechanisms of host plant resistance in lines derived from crosses with Pilose.

CHAPTER II

IDENTIFICATION OF RESISTANCE TO COTTON FLEAHOPPER: GERMPLASM SCREENING AND INTROGRESSION BREEDING

Introduction

Insect control in cotton is dominated widely by genetically modified (GM) cultivars. However, piercing-sucking insects, like the cotton fleahopper (CFH) (*Pseudatomoscelis seriatus*), are not managed by GM cotton. Cotton fleahoppers are an early season pest of upland cotton, and damage generally is most severe in the central and southern portions of the U.S. Cotton Belt, particularly in the dryland production systems of Texas (Ring et al., 1993), where cotton cultivars have an early maturity habit and begin squaring around the time of senescence of the CFHs' weedy spring hosts. Cotton fleahoppers feed on leaves, ovary walls, and developing anthers of the cotton plant (Reinhard 1926, Pack and Tugwell; Bell et al., 2007). Feeding damage is characterized by death and abscission of the cotton squares, termed blasting, and can result in delayed maturity of the crop. In 2012 and 2013, CFH was the leading insect pest in cotton, in terms of bales lost (Williams, 2000-2014). Eradication of the boll weevil and widespread adoption of *Bt* cotton for control of Lepidopteran pests has dramatically decreased pesticide applications for these insects, which has led to the emergence of CFH as a major pest in Texas cotton (Knutson et al., 2013). Breeding for CFH resistance has not, to date, produced any marketed cultivars, but the need for naturally occurring resistance remains, as CFH continues to rank among the top insect pests in Texas cotton.

The history of evaluating upland cotton germplasm for resistance to CFH generally has focused on the role of plant pubescence in insect preference. Briefly, cotton with dense pubescence is reported to be more attractive to CFH, but shows comparatively lower feeding injury (Lukefahr 1970; Walker et al., 1974). While earlier studies used indirect measures (number of blooms, yield) of CFH injury, Knutson et al. (2013) directly measured CFH injury by observing injury in dissected squares in no-choice cage studies. In their study, examining CFH feeding tolerance in breeding lines and adapted germplasm, they reported, similarly, a significant, positive correlation between trichome density and CFH density. However, they reported no significant correlation between trichome density and square damage when square damage was determined by visual examination of squares for CFH feeding injury. Knutson et al (2013) also found that the Pilose cultigen was the most tolerant to CFH feeding in both choice and no-choice feeding studies, but did not find a significant relationship between trichome density and square damage. Knutson and colleagues (unpublished) assessed the role of Pilose, a densely pubescent cultigen, in conferring resistance to CFH. Pilose was crossed with several commercial and breeding lines and then progeny were screened for resistance to CFH feeding. Cotton fleahopper behavior was also assessed, and results suggested that the type of resistance conferred by Pilose is non-preference and that the resistance trait is heritable. This study utilizes the germplasm produced by Knutson and Smith (unpublished) for continued evaluation in the field and introgression of resistance traits into two, high-yielding breeding lines from the Texas A&M AgriLife Research

Cotton Improvement Lab at College Station, while continuing to examine the role of pubescence as a resistance factor.

Materials and Methods

Breeding Material

Two high-yielding lines were selected as female parents: 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. Male parents were selected from three families of F_3 progeny resulting from a CFH resistance breeding effort by Knutson and Smith (unpublished). The three families will be referred to a GH-02, GH-04, and GH-07. Respectively, the pedigrees of these families are Pilose (PI 528521)/'TAM 96 WD-69s' (PI 635878; Thaxton and Smith 2005), Pilose/'Deltapine50' (DP 50; PVP 8400154), and Pilose/'All-Tex Atlas' (PVP 9200188). Six lines were selected from each family for screening on the basis of leaf trichome density (smooth, normal/hairy, or pilose) and fiber quality (length, and micronaire), for a total of 18 lines. In 2011, crosses were made in the greenhouse using the 18 lines from GH-02, GH-04, and GH-07 as males, and TAM06WE-14 and TAM07V-45 as females. During summer 2012, F_1 plants were backcrossed to recurrent parents, TAM06WE-14 and TAM07V-45. In 2013, BC_1F_1 were self-pollinated and increased at a winter nursery in Mexico. In the summer of 2013, the BC_2F_1 generation was created. In 2014, BC_2F_1 and BC_1F_3 lines were planted for open pollination increase at College Station.

Field Screening for Resistance

In 2012, GH-02, GH-04, GH-07, TAM06WE-14, and TAM07V-45 were evaluated in the field for resistance to CFH feeding in College Station and Corpus Christi. Due to low seed quantity, an equal number of seed from each of the six lines in GH-02, GH-04, and GH-07 were bulked by family for planting, and these lines were evaluated on a family level. A split-plot design was used, with the main plots as insecticide-treated and untreated and subplots as genotype, with four replications per location. Insecticide-treated plots were sprayed once a week, beginning at square initiation and ending when 50% of plants had a first flower, with Acephate® (O,S-Dimethyl acetylphosphoramidothioate) at a rate of 140.31 L ha^{-1}. Plant mapping was used as the primary tool for monitoring square loss due to CFH feeding. Additionally, adult and nymph fleahopper counts were taken within each plot. Data were collected once a week, beginning at square initiation, for four weeks. During the data collection period, both fields were monitored for *Lygus* spp. and Lepidopteran pests. *Lygus* were not detected at either location. Tobacco budworms (*Heliothis virescens*) and square borers (*Strymon melinus*) were detected in some plots during the fourth week of data collection, so that week was excluded from analysis. After the last week of data collection, insect pests were controlled in all plots as needed. Plots were machine harvested at the end of the growing season.

Based on the data from 2012, family GH-04 was selected for further testing in 2013 and 2014, along with the backcross progeny generated from the lines in this family. Backcross progeny were evaluated in 2014 for the purpose of selecting lines with high

resistance to CFH. In 2013, the six lines from GH-04 (GH13-6, GH15-2, GH18-1, GH18-3, GH20-1, GH20-2) and TAM06WE-14 and TAM07V-45 were field-evaluated at College Station only, due to drought conditions in Corpus Christi; in 2014 all eight parental lines and 11 BC_1F_3 lines were evaluated at College Station and Corpus Christi. A split-plot design was used for each location, with main plots as insecticide-treated and untreated, subplots as genotype, and four replications per location. Insecticide-treated plots were sprayed once a week, beginning at square initiation and ending at 50% first flower, with Warhawk® (chlorpyrifos) in 2013 and Centric® (thiamethoxam) in 2014 at the labeled rate. The insecticide used varied due to availability. In 2013, data were collected once per week, beginning at square initiation. Five random plants were sampled from each plot, and the number of green squares, dead squares, and scars were counted to estimate percent square set (Knutson et al., 2013). Additionally, adult and nymph fleahopper counts were taken on each sampled plant. Plots were hand-harvested at the end of the growing season and lint yield and fiber quality determined. In 2014, parents and BC_1F_3 progeny were screened for resistance at College Station and Corpus Christi. Data collection was identical to methods used in 2013, except CFH were counted on 25 consecutive plants per plot. Plots were machine harvested at College Station, but hand harvested at Corpus Christi.

Data Analysis

Data for 2012 were analyzed in SAS (SAS v.9.4, SAS Institute, 2013), using PROC MIXED, after log transformation of the percent square loss data. Information gathered in 2012 was used to select a family for further testing in 2013 and 2014. Data

from 2013 and 2014 were analyzed as continuous proportion data, using PROC GLIMMIX and fit to a beta distribution (Stroup, 2015; SAS/STAT® 9.3 User's Guide: The GLIMMIX Procedure). Data for CFH preference (fleahopper count per plot) were analyzed using PROC GLIMMIX and fit to a Poisson distribution. For data that could not be fit to a Poisson distribution, a square root transformation was applied to approximate fit to a normal distribution. Goodness of fit for each model was determined by generalized Chi-square df^{-1}, which, when approximately equal to 1, indicates that variability in the data is adequately modeled (Schabenberger, 2005). Correlation of fleahopper counts and square loss was determined using PROC CORR.

Results and Discussion

In 2012, three families (comprised of six lines each), derived from crosses of Pilose x commercial cultivar (unpublished), and TAM06WE-14 and TAM07V-45 were evaluated for resistance to CFH at College Station and Corpus Christi (Table 2.1). Data from the 2012 study indicated line performance was consistent across locations (Table 2.2) and that each of the three families (GH-02, GH-04, GH-07) significantly outperformed TAM06WE-14 and TAM07V-45, in terms of resistance to CFH feeding, as measured by percent square loss (Table 2.3). Cotton fleahopper counts taken each week during the data collection period, indicated there was no difference between treated and untreated plots in College Station and that GH-02, GH-04, and GH-07 harbored more CFH, compared with TAM06WE-14 and TAM07V-45 (Tables 2.4 and 2.5), despite incurring less feeding damage. This finding was consistent with previous reports in the literature that CFH tend to congregate on hirsute plant types, in particular, pilose

types of plants, which were present in each of the three families (Lukefahr 1966, 1968, 1970; Knutson et al., 2013).

Table 2.1. Line designations and pedigrees of parental lines and backcross progeny. Pubescence information is provided for non-segregating lines

Line 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
12511	TAM06WE-14 //TAM06WE-14 /GH15-2	
12522	TAM06WE-14 //TAM06WE-14 /GH13-6	
12524	TAM06WE-14 //TAM06WE-14 /GH18-1	
12525	TAM06WE-14 //TAM06WE-14 /GH18-3	
12547	TAM07V-45//TAM07V-45/GH13-6	
12548	TAM07V-45//TAM07V-45/GH15-2	
12550	TAM07V-45//TAM07V-45/GH18-1	
12552	TAM07V-45//TAM07V-45/GH20-2	
12553	TAM07V-45//TAM07V-45/GH18-3	
12554	TAM06WE-14 //TAM06WE-14 /GH20-1	
12555	TAM06WE-14 //TAM06WE-14 /GH20-2	

Table 2.2. Analysis of variance of percent square damage in parental lines, combined across College Station and Corpus Christi, TX (2012)

Effect	Num df, Den df	F Value
Line	4, 340	33.88 **
Trt	1, 3	3.62
Line*Trt	4, 340	3.16 *
Loc	1, 340	2.10
Line*Loc	4, 340	0.86

Table 2.3. Percent square loss in parental lines in insecticide treated and untreated plots in College Station and Corpus Christi, TX (2012)

Untreated		Treated	
Genotype	Pct Square Loss	Genotype	Pct Square Loss
GH-07	15.09 a[†]	GH-02	13.65 a
GH-04	19.83 ab	GH-04	18.62 ab
GH-02	21.57 ab	GH-07	19.07 ab
TAM06WE-14	31.01 b	TAM06WE-14	30.36 bc
TAM07V-45	55.71 c	TAM07V-45	39.24 c

[†]Means sharing the same letter are not significantly different (α=0.05, Tukey-Kramer adjustment)

Table 2.4. Cotton fleahoppers per plant in College Station, TX in 2012

Line	CFH/plant	
TAM07V-45	0.21	a[†]
TAM06WE-14	0.37	b
GH02	0.73	c
GH04	0.86	c
GH07	0.89	c

[†]Means sharing the same letter are not significantly different (α=0.05, Tukey-Kramer adjustment)

Table 2.5. Cotton fleahoppers per plant in Corpus Christi, TX in 2012

Treated			Untreated		
Line	CFH/plant		Line	CFH/plant	
TAM07V-45	0.10	a[†]	TAM07V-45	0.33	a
GH02	0.22	ab	TAM06WE-14	0.42	ab
TAM06WE-14	0.24	ab	GH07	0.59	ab
GH07	0.29	b	GH04	0.65	b
GH04	0.36	b	GH02	0.69	b

[†]Means sharing the same letter are not significantly different (α=0.05, Tukey-Kramer adjustment)

Based on the performance of GH-02, -04, and -07 in 2012, and the performance of these families in a previous study conducted by Knutson (unpublished), GH-04 was selected for further screening in 2013 and 2014. In 2014, BC_1F_3 progeny derived from crosses between the six lines in GH-04 and TAM06WE-14 and TAM07V-45 (Table 2.1) were evaluated concurrently with parental lines.

Data from 2013 and 2014 were analyzed separately and by week of data collection due to differences in data distribution from week to week. In 2013, lines were evaluated only at College Station due to drought conditions at Corpus Christi that year. Data were collected once a week for four weeks. Data from week one in 2013 were excluded from analysis due to the large number of plants that had not initiated squaring; data from week four in 2014 were excluded because of square damage due to Lepidopteran pests. Genotypes differed significantly (in percent square loss) across all three weeks in 2013 and 2014 (Tables 2.6 and 2.7). A significant difference in the effect of treatment (insecticide treated or untreated) was only noted in the first week in 2013, and only in the second and third weeks for 2014 (Tables 2.6 and 2.7). In 2013, CFH numbers were consistently below economic threshold (ET) (25 insects per 100 plant terminals) across all three weeks of data collection for both treatments (Table 2.8). In 2014, in the untreated plots at College Station, CFH increased to ET levels from week one to week two and remained high through week three (Tables 2.9 and 2.10); at Corpus Christi, CFH populations in the untreated plots did not approach economic threshold levels until the third week of sampling (Tables 2.11 and 2.12). These fluctuations in CFH populations corresponded to significant differences between treatments for square

loss. For both years, during the weeks a treatment difference was noted, square loss was lower in treated plots, compared to untreated plots, and was lower than 20%. In order to more make more meaningful interpretations of these data, an ET in terms of CFH damage, was established at 20% square loss. This damage-based economic threshold was based on treatment recommendations for plant bugs (Hemiptera) that suggest applying insecticides when square retention falls below 80% (Stewart and McClure, 2014). Cotton fleahopper numbers in treated plots remained below the ET of 25 insects per 100 plant terminals in treated plots across 2013 and 2014 in CS and CC (Tables 2.4, 2.5, 2.8, 2.9, and 2.11), with the exception of plots of GH13-6 in week 3 of 2014. In 2012, CFH numbers were above ET in College Station and Corpus Christi. These data indicate that the insecticide treatment effectively controlled CFH only when Warhawk® or Centric® was used.

Table 2.6. Analysis of variance of percent square damage in parental lines in College Station, TX (2013), by week of data collection

	Week 1		Week 2		Week 3	
Effect	**Num df, Den df**	**F Value**	**Num df, Den df**	**F Value**	**Num df, Den df**	**F Value**
Line	7, 290.4	7.90 **	7, 289.7	7.87 **	7, 288	2.83 *
Trt	1, 3.37	1.54	1, 3.43	0.73	1, 6.74	4.13
Line*Trt	18, 290.50	2.33 *	7, 289.7	1.27	18, 288	0.93

Table 2.7. Analysis of variance of percent square damage in parental lines in College Station and Corpus Christi, TX (2014), by week of data collection

	Week 1		Week 2		Week 3	
Effect	**Num DF**	**F Value**	**Num DF**	**F Value**	**Num DF**	**F Value**
Line	18, 1123	1.79 *	18, 1259	2.13 *	18, 1256	2.94 **
Trt	1, 6.247	1.18	1, 2.74	37.75 *	1, 3.06	12.94 *
Line*Trt	18, 1123	1.25	18, 1259	1.49	18, 1256	2.21 *
Line*Loc	18, 1124	0.95	1, 1255	28.87	1, 1249	832.70 *
Loc	1, 1066	22.51 **	18, 1259	1.42 **	18, 1256	1.69 **

Table 2.8. Cotton fleahoppers per plant in College Station, TX (2013)

Week 1			
Untreated		**Treated**	
Line	**CFH/plant**	**Line**	**CFH/plant**
GH20-2	0.0000 a[†]	GH20-2	0.0000 a
TAM07V-45	0.0000 a	GH15-2	0.0000 a
TAM06WE-14	0.0000 a	GH13-6	0.0000 a
GH13-6	0.0005 ab	GH18-1	0.0000 a
GH18-3	0.0005 ab	TAM07V-45	0.0000 a
GH20-1	0.0005 ab	GH20-1	0.0005 a
GH15-2	0.0020 ab	TAM06WE-14	0.0005 a
GH18-1	0.0100 b	GH18-3	0.0038 a
Week 2		**Week 3**	
Combined Treatments		**Combined Treatments**	
Line	**CFH/plant**	**Line**	**CFH/plant**
GH20-2	0.0000 a	GH20-2	0.0001 a
TAM07V-45	0.0001 a	TAM07V-45	0.0001 a
TAM06WE-14	0.0002 a	GH20-1	0.0001 a
GH20-1	0.0005 a	TAM06WE-14	0.0027 ab
GH18-3	0.0040 ab	GH13-6	0.0031 ab
GH15-2	0.0163 b	GH18-3	0.0035 ab
GH13-6	0.0184 b	GH18-1	0.0107 ab
GH18-1	0.0256 b	GH15-2	0.0180 b

[†]Means sharing the same letter are not significantly different (α=0.05, Tukey-Kramer adjustment)

Table 2.9. Cotton fleahoppers per plant in insecticide treated plots in College Station, TX (2014)

College Station, Treated								
Week 1			Week 2			Week 3		
Line	CFH/plant		Line	CFH/plant		Line	CFH/plant	
12554	0.00	a[†]	TAM06WE-14	0.01	a	12548	0.02	a
12552	0.00	a	TAM07V-45	0.01	a	GH18-3	0.02	a
12555	0.00	a	12550	0.01	a	12552	0.04	ab
12511	0.00	a	12525	0.03	ab	TAM06WE-14	0.06	abc
GH13-6	0.00	a	GH18-3	0.03	ab	12511	0.06	abc
12522	0.00	a	12522	0.04	abc	12525	0.06	abc
GH20-2	0.00	a	12548	0.04	abc	12553	0.06	abc
TAM06WE-14	0.01	ab	GH20-2	0.04	abc	12555	0.06	abc
TAM07V-45	0.01	ab	12553	0.05	abcd	GH20-1	0.06	abc
12548	0.01	ab	12554	0.05	abcd	12522	0.07	abc
12550	0.01	ab	GH20-1	0.05	abcd	GH20-2	0.07	abc
12525	0.03	ab	12511	0.06	abcd	TAM07V-45	0.09	abc
12553	0.03	ab	12555	0.06	abcd	12550	0.09	abc
GH18-3	0.03	ab	12524	0.08	bcd	12547	0.10	bc
GH20-1	0.03	ab	12552	0.08	bcd	12524	0.11	cd
12547	0.04	ab	GH13-6	0.08	bcd	12554	0.14	cde
GH18-1	0.04	ab	GH18-1	0.10	cd	GH15-2	0.21	de
12524	0.05	bc	12547	0.11	d	GH18-1	0.23	ef
GH15-2	0.09	c	GH15-2	0.11	d	GH13-6	0.36	f

[†]Means sharing the same letter are not significantly different (α=0.05, Tukey-Kramer adjustment)

Table 2.10. Cotton fleahoppers per plant in untreated plots in College Station, TX (2014)

College Station, Untreated								
Week 1			**Week 2**			**Week 3**		
Line	**CFH/plant**		**Line**	**CFH/plant**		**Line**	**CFH/plant**	
12552	0.00	a[†]	TAM07V-45	0.01	a	12547	0.04	a
12554	0.00	a	12552	0.01	a	GH18-3	0.04	a
12555	0.00	a	12553	0.03	ab	12552	0.05	ab
12553	0.00	a	12547	0.06	abc	12554	0.05	ab
TAM07V-45	0.00	a	12525	0.08	bcd	12555	0.07	ab
GH20-1	0.00	a	GH20-2	0.08	bcd	GH20-2	0.07	ab
12550	0.00	a	GH20-1	0.10	cde	TAM07V-45	0.09	abc
TAM06WE-14	0.01	a	12522	0.11	cde	12550	0.09	abc
12525	0.01	a	TAM06WE-14	0.12	cdef	12553	0.10	abcd
GH18-3	0.01	a	12554	0.12	cdef	12511	0.13	bcde
GH20-2	0.01	a	12555	0.14	cdef	12548	0.16	cdef
12522	0.03	ab	12524	0.14	def	12525	0.17	cdef
12547	0.03	ab	12550	0.16	def	12522	0.18	cdef
12548	0.03	ab	GH18-3	0.18	efg	12524	0.20	def
GH13-6	0.03	ab	12548	0.21	fg	TAM06WE-14	0.21	ef
12524	0.04	abc	GH13-6	0.21	fg	GH15-2	0.23	ef
GH18-1	0.04	abc	GH15-2	0.21	fg	GH13-6	0.26	f
GH15-2	0.07	bc	GH18-1	0.21	fg	GH18-1	0.27	f
12511	0.08	c	12511	0.27	g	GH20-1	0.27	f

[†]Means sharing the same letter are not significantly different (α=0.05, Tukey-Kramer adjustment)

Table 2.11. Cotton fleahoppers per plant in insecticide treated plots in Corpus Christi, TX (2014)

Corpus Christi, Treated								
Week 1			Week 2			Week 3		
Line	CFH/plant		Line	CFH/plant		Line	CFH/plant	
12511	0.00	a[†]	12554	0.00	a	12548	0.02	a
12548	0.00	a	12511	0.00	a	TAM07V-45	0.03	a
12552	0.00	a	12548	0.00	a	12552	0.03	a
12553	0.00	a	12547	0.01	ab	12550	0.03	ab
12554	0.00	a	12550	0.01	ab	12525	0.05	abc
GH15-2	0.00	a	12552	0.01	ab	12547	0.05	abc
GH18-3	0.00	a	TAM06WE-14	0.01	abc	12554	0.05	abc
12550	0.00	a	12522	0.01	abc	TAM06WE-14	0.08	bcd
12555	0.00	a	GH18-3	0.01	abc	12553	0.08	cd
GH13-6	0.00	a	TAM07V-45	0.02	abc	GH20-1	0.08	cd
12522	0.00	a	12553	0.02	abc	12511	0.08	cd
TAM06WE-14	0.00	a	GH20-2	0.02	abc	12524	0.08	cd
TAM07V-45	0.00	a	12555	0.02	abcd	GH13-6	0.09	cd
GH20-2	0.00	a	12524	0.02	bcde	GH18-3	0.09	cd
12524	0.01	a	GH20-1	0.02	bcde	GH20-2	0.10	cd
12547	0.01	a	GH15-2	0.03	cde	12522	0.12	d
GH18-1	0.01	a	GH18-1	0.04	de	12555	0.12	d
GH20-1	0.01	a	12525	0.04	ef	GH18-1	0.13	d
12525	0.01	a	GH13-6	0.06	f	GH15-2	0.14	d

[†]Means sharing the same letter are not significantly different (α=0.05, Tukey-Kramer adjustment)

Table 2.12. Cotton fleahoppers per plant in untreated plots in Corpus Christi, TX (2014)

Corpus Christi, Untreated								
Week 1			**Week 2**			**Week 3**		
Line	**CFH/plant**		**Line**	**CFH/plant**		**Line**	**CFH/plant**	
TAM06WE-14	0.00	a[†]	12524	0.00	a	TAM07V-45	0.03	a
12550	0.00	a	12553	0.00	a	12553	0.06	ab
GH18-3	0.00	a	12550	0.00	a	12550	0.07	abc
12553	0.00	a	GH20-2	0.00	a	TAM06WE-14	0.09	bc
GH20-2	0.00	a	12548	0.00	a	12511	0.09	bc
12554	0.00	a	TAM06WE-14	0.01	ab	12547	0.09	bc
TAM07V-45	0.00	a	12522	0.01	ab	12552	0.09	bc
12511	0.00	a	12525	0.01	ab	GH20-2	0.11	bcd
12548	0.00	a	12555	0.01	ab	12548	0.13	cde
12555	0.00	ab	GH13-6	0.01	ab	12554	0.15	cdef
12552	0.00	ab	GH18-3	0.01	ab	GH18-3	0.15	cdef
12525	0.01	abc	12511	0.01	ab	GH20-1	0.16	cdef
GH20-1	0.01	abc	12552	0.02	ab	12522	0.17	cdef
12547	0.02	bc	GH20-1	0.02	ab	GH15-2	0.18	cdefg
GH13-6	0.02	bc	TAM07V-45	0.02	bc	12555	0.18	defg
GH18-1	0.02	bc	12554	0.03	bc	12524	0.19	efg
12522	0.03	c	GH15-2	0.03	bc	GH18-1	0.19	efg
12524	0.05	d	GH18-1	0.03	bc	12525	0.23	fg
GH15-2	0.06	d	12547	0.04	c	GH13-6	0.28	g

[†]Means sharing the same letter are not significantly different (α=0.05, Tukey-Kramer adjustment)

Across all weeks in 2013, the lines from GH-04 showed less square loss than TAM06WE-14 and TAM07V-45, with the exception of GH18-3 in week 1 and GH20-2 in week 3, which was generally consistent with the performance of GH-04 in 2012 (Tables 2.3 and 2.13). Line performance was more variable in 2014. For the first two weeks of data collection, there was no significant line by location interaction (Tables 2.7 and 2.14). During the third week of data collection, square loss combined across lines was significantly higher at College Station, compared to Corpus Christi, where all lines had injury level below ET. Cotton fleahopper numbers were also higher at College Station than Corpus Christi during the third week. The same trend of higher CFH numbers at College Station was also noted in 2012 (Tables 2.4 and 2.5). Across both locations during the last week of data collection in 2014, among the parental lines in untreated plots, GH13-6, GH15-2, GH18-1, consistently had the lowest damage from CFH; not surprisingly the phenotype of these lines was pilose. However, when the CFH population approached or exceeded ET, as it did in untreated College Station plots in 2014, the pilose lines exhibited damage near or exceeding the ET of 20% used in this study (Tables 2.15).

Table 2.13. Means separation of percent square loss of parental lines in College Station, TX (2013)

Week 1					
Untreated			**Treated**		
Line	**Pct Sq Loss**		**Line**	**Pct Sq Loss**	
GH18-3	0.30	a[†]	GH20-1	1.33	a
GH13-6	0.76	ab	GH18-1	1.58	ab
GH20-2	1.82	ab	GH20-2	1.79	ab
GH20-1	2.47	ab	GH13-6	3.18	ab
GH15-2	2.85	b	GH15-2	3.40	ab
GH18-1	3.48	b	TAM07V-45	4.56	b
TAM07V-45	4.69	bc	GH18-3	6.02	bc
TAM06WE-14	8.87	c	TAM06WE-14	9.21	c
Week 2			**Week 3**		
Combined Treatments			**Combined Treatments**		
Line	**Pct Sq Loss**		**Line**	**Pct Sq Loss**	
GH18-3	0.65	a	GH20-1	0.19	a
GH15-2	1.21	ab	GH18-3	0.52	a
GH20-1	1.49	ab	GH18-1	0.36	ab
GH18-1	1.67	ab	GH13-6	0.41	abc
GH13-6	1.78	ab	GH15-2	0.52	abcd
GH20-2	2.08	b	TAM07V-45	0.78	bcd
TAM07V-45	3.63	c	TAM06WE-14	0.88	cd
TAM06WE-14	5.05	c	GH20-2	0.89	d

[†]Means sharing the same letter are not significantly different (α=0.05, t-grouping)

Table 2.14. Means separation of percent square loss of parental and backcross progeny lines in College Station and Corpus Christi, TX during the first and second weeks of data collection (2014)

Week 1			Week 2		
Line	Pct Sq Loss		Line	Pct Sq Loss	
GH18-1	0.00	a[†]	GH13-6	4.81	a
GH20-2	0.00	a	12522	4.90	ab
12547	0.00	a	GH18-3	4.92	ab
12555	0.00	a	TAM06WE14	5.41	abc
12548	0.70	ab	GH15-2	5.61	abcd
12554	0.71	ab	GH18-1	5.73	abcde
GH20-1	0.82	ab	12555	5.93	abcde
12552	1.04	ab	12525	6.20	abcde
GH13-6	1.44	ab	12550	6.23	abcde
12525	1.61	ab	TAM07V45	6.50	abcdef
GH18-3	1.74	ab	GH20-2	6.69	abcdef
12524	2.13	ab	12547	6.78	abcdef
12522	2.36	ab	12554	7.96	abcdefg
TAM06WE14	2.44	ab	12524	8.01	bcdefg
TAM07V45	2.54	ab	12552	8.55	cdefg
GH15-2	2.72	ab	12553	8.76	defg
12553	3.01	ab	12548	8.86	efg
12511	4.04	ab	GH20-1	9.71	fg
12550	6.01	b	12511	10.23	g

[†]Means sharing the same letter are not significantly different (α=0.05, t-grouping)

Table 2.15. Means separation of percent square loss of parental and backcross progeny lines in untreated plots in College Station and Corpus Christi, TX during the third week of data collection (2014)

Untreated					
College Station			**Corpus Christi**		
Line	**Pct Sq Loss**		**Line**	**Pct Sq Loss**	
GH15-2	17.48	a[†]	12511	2.97	a
GH18-1	18.73	a	GH15-2	3.38	ab
12525	21.20	ab	GH13-6	5.40	abc
GH13-6	21.84	ab	12525	5.47	abc
GH18-3	23.75	ab	GH18-1	5.98	abcd
12553	27.12	bc	12552	7.37	bcd
TAM07V-45	27.65	bc	TAM06WE-14	7.38	bcd
GH20-2	28.62	bc	12547	7.39	bcd
12548	29.00	cd	12524	7.47	bcd
12547	30.22	cd	12522	7.50	bcd
12522	30.67	cd	GH20-1	7.93	bcd
GH20-1	31.58	cd	GH18-3	7.98	bcd
12550	31.85	cd	GH20-2	8.02	bcd
12552	32.08	cd	12550	9.59	cd
12554	33.25	cd	12554	10.67	d
12511	37.04	d	TAM07V-45	11.60	de
TAM06WE-14	38.52	d	12555	11.89	de
12524	38.95	d	12553	14.69	de
12555	39.08	d	12548	15.76	e

[†]Means sharing the same letter are not significantly different (α=0.05, t-grouping)

Among the BC_1F_3 progeny during week three (CS, 2014), line 12525 (TAM06WE-14 //TAM06WE-14 /GH18-3) consistently exhibited low CFH feeding damage in untreated plots across both locations (Tables 2.15 and 2.16). At Corpus Christi alone, 12511 (TAM06WE-14 //TAM06WE-14 /GH15-2) had the lowest square damage in untreated plots, and all BC_1F_3, lines except 12555 (TAM06WE-14 //TAM06WE-14 /GH20-2), 12553 (TAM07V-45//TAM07V-45/GH18-3), and 12548 (TAM07V-45//TAM07V-45/GH15-2) exhibited less than 10% square loss. Based on performance rank (Table 2.16), backcross lines with TAM07V-45 as the recurrent parent generally performed better at College Station in comparison to backcross lines with TAM06WE-14 as the recurrent parent. The opposite was true for backcross line performance at Corpus Christi, where lines with TAM06WE-14 as the recurrent parent generally outperformed lines with TAM07V-45 as the recurrent parent (Table 2.16). Interestingly, TAM07V-45 outperformed TAM06WE-14 at College Station, but performance of these lines was reversed in the trials at Corpus Christi in 2014. It was also noted, that at College Station, backcross lines with the same donor parent showed similar levels of injury; at Corpus Christi, injury level seemed to be more dependent on the recurrent parent, with the exception of lines with GH13-6 as the donor parent (Table 2.16).

Table 2.16. Parental and backcross progeny line performance ranked by percent square loss in the third week of data collection in College Station (CS) and Corpus Christi (CC), TX in 2014. Rank shift is the change in performance rank from College Station to Corpus Christi.

Line	Rank		Rank Shift
	CS	CC	
12553	6	18	-12
12548	9	19	-10
TAM07V-45	7	16	-9
GH18-3	5	12	-7
GH20-2	8	13	-5
GH18-1	2	5	-3
GH15-2	1	2	-1
12525	3	4	-1
12550	13	14	-1
12554	15	15	0
GH13-6	4	3	1
GH20-1	12	11	1
12522	11	10	1
12547	10	8	2
12555	19	17	2
12552	14	6	8
12524	18	9	9
TAM06WE-14	17	7	10
12511	16	1	15

Based on rank of performance, ten of the lines had consistent performance across both locations in week three; the other nine lines showed notable rank shift when comparing square loss at College Station and Corpus Christi (Table 2.16). Lines 12553, 12548, TAM07V-45, GH18-3, and GH20-2 ranked in the top 10 for lowest square loss at College Station, but in the bottom 10 at Corpus Christi. Conversely, lines 12552, 12524, TAM06WE-14, and 12511 ranked in the bottom 10 at College Station, but in the top 10

at Corpus Christi (Table 2.16). Correlation analysis of percent square loss and CFH per 25 plants revealed a similar trend, in that the size of the correlation between number of CFH and square loss for a given line in a given location was often indicative of line performance in that location (Tables 2.17, 2.18, 2.19, 2.20). Barman et al. (2012) reported that 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. They postulated that host associated differentiation (HAD), or reproductive isolation due to availability of host plants, resulted in the emergence of unique genotypes in these locations. The genetic difference in CFH populations at College Station and Corpus Christi due to HAD could indicate differences in CFH host preference and thus account for the changes noted in line performance across these locations. However, there is some dispute over the validity of HAD in CFH, an insect that migrates from one host species to another as hosts senesce (Almand et al., 1976; Knutson and Brewer, personal communication). More testing in College Station and Corpus Christi, as well as in Weslaco, TX and the Texas high plains, areas purportedly inhabited by a third distinct CFH genotype (Barman et al., 2012), is needed to substantiate or refute the hypothesis that HAD in CFH is responsible for location-dependent preference differences in cotton.

Table 2.17. Pearson's correlation analysis of percent square loss and cotton fleahopper density in parental lines in untreated plots (2014). The correlation value and p-value are beneath each genotype, respectively.

CFH/25 plants	Percent Square Loss							
	Corpus Christi							
	TAM07V-45	**TAM06WE-14**	**GH13-6**	**GH15-2**	**GH18-1**	**GH18-3**	**GH20-1**	**GH20-2**
	0.2100	0.4202	0.4130	0.1677	0.4336	0.6523	0.0649	0.1127
	0.0809	*0.0017*	*0.001*	*0.2884*	*0.0009*	*<0.0001*	*0.6313*	*0.3952*
	College Station							
	TAM07V-45	**TAM06WE-14**	**GH13-6**	**GH15-2**	**GH18-1**	**GH18-3**	**GH20-1**	**GH20-2**
	0.4776	0.4254	0.1687	0.0980	0.3105	0.1137	0.5615	0.4016
	0.0009	*0.0036*	*0.268*	*0.522*	*0.0379*	*0.4572*	*0.0002*	*0.0062*

Table 2.18. Pearson's correlation analysis of percent square loss and cotton fleahopper density in BC_1F_3 lines in untreated plots (2014). The correlation value and p-value are beneath each genotype, respectively.

	Percent Square Loss					
	Corpus Christi					
CFH/25 plants	**12511**	**12522**	**12524**	**12525**	**12547**	**12548**
	-0.0212	0.1066	0.0544	0.1127	0.0892	0.2360
	0.8724	*0.4177*	*0.6796*	*0.4127*	*0.4979*	*0.0695*
	12550	**12552**	**12553**	**12554**	**12555**	
	-0.2285	0.2960	0.4683	0.2358	0.4752	
	0.1561	*0.0282*	*0.0002*	*0.0697*	*0.0003*	
	College Station					
	12511	**12522**	**12524**	**12525**	**12547**	**12548**
	0.2130	0.6339	0.3752	0.6789	0.0554	0.2150
	0.1601	*<0.0001*	*0.0111*	*<0.0001*	*0.7178*	*0.1561*
	12550	**12552**	**12553**	**12554**	**12555**	
	0.2327	0.2971	0.3567	0.1255	0.3843	
	0.1076	*0.0475*	*0.011*	*0.4115*	*0.0144*	

Table 2.19. Pearson's correlation analysis of percent square loss and cotton fleahopper density in parental lines in treated plots (2014). The correlation value and p-value are beneath each genotype, respectively.

	Percent Square Loss							
	Corpus Christi							
CFH/25 plants	**TAM07V-45**	**TAM06WE-14**	**GH13-6**	**GH15-2**	**GH18-1**	**GH18-3**	**GH20-1**	**GH20-2**
	-0.0021	-0.0685	0.0565	-0.0055	0.2679	-0.0729	-0.0689	0.6201
	0.9875	*0.6029*	*0.6765*	*0.968*	*0.0502*	*0.597*	*0.6174*	*<0.0001*
	College Station							
	TAM07V-45	**TAM06WE-14**	**GH13-6**	**GH15-2**	**GH18-1**	**GH18-3**	**GH20-1**	**GH20-2**
	0.5524	0.6429	0.1369	0.0873	0.3046	0.2154	0.6215	0.4886
	<0.0001	*<0.0001*	*0.3700*	*0.5686*	*0.0419*	*0.1553*	*<0.0001*	*0.0007*

Table 2.20. Pearson's correlation analysis of percent square loss and cotton fleahopper density in BC_1F_3 lines in treated plots (2014). The correlation value and p-value are beneath each genotype, respectively.

	Percent Square Loss					
	Corpus Christi					
CFH/25 plants	**12511**	**12522**	**12524**	**12525**	**12547**	**12548**
	0.0149	0.2079	0.2824	-0.1572	0.2108	-0.0541
	0.9141	*0.1109*	*0.0367*	*0.2562*	*0.1224*	*0.6948*
	12550	**12552**	**12553**	**12554**	**12555**	
	0.0303	0.0750	-0.0586	-0.0256	0.2684	
	0.828	*0.5861*	*0.6711*	*0.8627*	*0.0497*	
	College Station					
	12511	**12522**	**12524**	**12525**	**12547**	**12548**
	0.1316	0.4648	0.2072	0.2377	0.2694	0.1135
	0.3889	*0.0013*	*0.1721*	*0.1159*	*0.0735*	*0.4579*
	12550	**12552**	**12553**	**12554**	**12555**	
	0.4704	0.2296	0.5034	0.2708	0.6909	
	0.0013	*0.1292*	*0.0004*	*0.079*	*<0.0001*	

Consistent with previous reports in the literature (Lukefahr, 1970; Knuston et al., 2013), the pilose lines (GH13-6, GH15-2, GH18-1) had the highest populations of CFH in the untreated plots across all weeks and locations in 2013 and 2014. However, as previously discussed, these three lines exhibited the lowest percent square loss. Locating a suitable host may involve odor or visual cues, or a combination of both (Bernays and Chapman, 1994). While studies have not been conducted with CFH, the literature documents the use of visual cues by other Hemipterans, like aphids and Lygus, in orientation towards suitable hosts (Blackmer et al., 2005; Döring et al., 2009). Assuming that migrating CFH also rely to some extent on visual cues for locating a host, and taking into account the visual similarity between pilose cotton and other pubescent CFH hosts, like woolly croton, I hypothesize that, upon first encountering the field of cotton, CFH are initially attracted to pilose plants based on visual cues, even if the pilose plants are less suitable for feeding compared to plants in neighboring plots (data discussed in Chapter II). This phenomenon would explain higher CFH populations on pilose plants, despite recording less feeding damage on these plants. Correlation analysis of 2014 data supports this hypothesis for GH15-2, but is less clear for GH13-6 and GH18-1 (Table 2.17). Knutson et al. (2013), hypothesized that hairy leaf cotton may be more attractive to CFH because the dense pubescence of hairy leaf cotton creates a microclimate in which temperature and humidity are regulated.

In addition to square loss, the performance of each line was also measured in terms of yield (kg ha^{-1}) (Tables 2.21 and 2.22) and fiber quality (Tables 2.23 and 2.24). Fiber quality traits of interest were: length (mm), strength (kN m kg^{-1}), micronaire,

uniformity (%) and elongation (%). In 2013, there was no difference in the effect of treatment (insecticide application) on yield (Table 2.21); in 2014, there was a significant interaction between location and genotype, and a treatment effect on yield was present in the trial at Corpus Christi, but not at College Station (Tables 2.22, 2.25, 2.26). The lack of treatment effect on yield at College Station was surprising, given the high level of damage and high population of CFH at that location, particularly during the last week of data collection (Tables 2.15, 2.27, 2.9). Lint yield at College Station was higher in 2013 when fleahopper pressure was low, compared with 2014, when fleahopper pressure was high, suggesting that the infestation severity may affect lint yield (Tables 2.28, 2.29, 2.30). An analysis of variance and means separation of the differences in yield (Δ) between treated and untreated plots in Corpus Christi (2014) indicated that some cotton lines yielded more in the untreated plots and that the differences in yield (Δ) were significant between lines (Table 2.31).

Table 2.21. Analysis of variance of yield (kg ha^{-1}) of parental lines in College Station, TX (2013)

Effect	Num df, Den df	F Value
Line	7,41	11.41 **
Trt	1,3	0.73
Line*Trt	7,41	0.50

Table 2.22. Analysis of variance of yield (kg ha^{-1}) of parental and backcross progeny lines in College Station and Corpus Christi, TX (2014)

Effect	Num df, Den df	F Value
Line	18,229	11.46 **
Trt	1,3	2.31
Location	18,229	1180.39 **
Line*Loc	18,229	5.50 **
Trt*Line	18,229	0.80

Table 2.23. Analysis of variance of advanced fiber information system (AFIS) fiber properties—length (mm), strength (g/den), micronaire, uniformity (%) and elongation—of parental and backcross progeny lines in College Station, TX (2013)

		Length	Strength	Micronaire	Uniformity	Elongation
Effect	Num df, Den df	F Value				
Line	7,41	36.37 **	47.62 **	4.88 *	5.18 *	10.10 **
Trt	1,3	1.85	0.10	6.99	0.00	4.39
Line*Trt	7,41	0.61	0.46	0.49	0.27	0.69

Table 2.24. Analysis of variance of advanced fiber information system (AFIS) fiber properties—length (mm), strength (kN m kg^{-1}), micronaire, uniformity (%) and elongation—of parental and backcross progeny lines in College Station and Corpus Christi, TX (2014)

		Length	Strength	Micronaire	Uniformity	Elongation
Effect	**Num df, Den df**	**F Value**				
Line	18, 92	59.16 **	27.44 **	23.73 **	12.80 **	13.85 **
Trt	1, 1	1.66	1.48	0.50	2.30	1.57
Loc	1, 92	754.51 **	194.45 **	11.83 *	317.48 **	59.68 **
Line*Loc	18, 92	1.55	1.33	1.44	0.6	2.19 *
Line*Trt	18, 92	2.62 *	1.87 *	2.82 *	2.05 *	2.39 *

Table 2.25. Analysis of variance of yield (kg ha^{-1}) of parental and backcross progeny lines in College Station, TX (2014)

Effect	**Num df, Den df**	**F Value**
Line	18,107	10.34 **
Trt	1,3	0.47
Line*Trt	18,107	0.77

Table 2.26. Analysis of variance of yield (kg ha^{-1}) of parental and backcross progeny lines in Corpus Christi, TX (2014)

Effect	**Num df, Den df**	**F Value**
Line	18,97	4.07 **
Trt	1,3	0.69
Line*Trt	18,97	2.05 *

Table 2.27. Means separation of percent square loss of parental and backcross progeny lines in insecticide treated plots in College Station and Corpus Christi, TX during the third week of data collection (2014)

Treated			
College Station		**Corpus Christi**	
Line	**Pct Sq Loss**	**Line**	**Pct Sq Loss**
GH15-2	7.13 a[†]	12548	0.21 a
12552	8.53 ab	12525	0.96 ab
GH18-3	8.72 ab	12554	1.03 ab
12547	8.81 ab	GH20-1	1.38 ab
12548	9.35 ab	12555	1.51 ab
12511	10.24 ab	GH18-1	1.89 ab
12554	10.82 ab	12547	1.99 ab
GH20-2	11.42 b	GH15-2	2.19 ab
TAM06WE-14	11.63 b	GH13-6	2.27 ab
GH13-6	12.12 b	GH18-3	2.36 ab
12524	12.84 b	12522	2.78 ab
GH20-1	13.01 b	12553	3.10 ab
12553	13.03 b	12511	3.15 ab
12550	13.33 b	GH20-2	3.23 ab
GH18-1	13.35 b	TAM07V-45	3.50 ab
12555	13.56 b	TAM06WE-14	4.13 b
12525	13.91 b	12552	4.26 b
TAM07V-45	14.05 b	12524	4.29 b
12522	14.47 b	12550	4.55 b

[†]Means sharing the same letter are not significantly different (α=0.05, t-grouping)

Table 2.28. Means separation of yield (kg ha^{-1}) of parental lines combined across treatments in College Station, TX (2013)

Line	Mean (kg ha^{-1})	
TAM07V-45	1712.10	a†
GH20-2	1682.25	a
GH20-1	1474.64	ab
TAM06WE-14	1101.82	abc
GH18-3	934.82	bcd
GH15-2	831.26	cd
GH13-6	743.36	cd
GH18-1	306.31	d

†Means connected by the same letter are not significantly different (α=0.05, Tukey HSD)

Table 2.29. Means separation of yield (kg ha^{-1}) of parental and backcross progeny lines combined across treatments in College Station, TX (2014)

Line	Mean (kg ha^{-1})	
12511	968.19	a†
12525	963.63	a
TAM07V-45	916.11	a
12552	909.45	a
12555	900.66	a
TAM06WE-14	880.97	a
12524	880.50	ab
12553	878.65	ab
12554	871.35	ab
12522	858.40	ab
12547	857.75	ab
12548	808.37	ab
12550	696.77	abc
GH20-1	688.07	abc
GH20-2	669.29	abcd
GH18-3	574.60	bcde
GH18-1	493.14	cde
GH13-6	367.94	de
GH15-2	344.08	E

†Means connected by the same letter are not significantly different (α=0.05, Tukey HSD)

Table 2.30. Means separation of yield (kg ha^{-1}) of parental and backcross progeny lines in insecticide treated and untreated plots in Corpus Christi, TX (2014)

Untreated			Treated		
Line	**Mean (kg ha^{-1})**		**Line**	**Mean (kg ha^{-1})**	
GH20-2	299.78	a†	TAM07V-45	311.66	a
12525	292.89	a	12552	270.69	ab
12511	287.39	a	12555	257.04	ab
12554	284.02	a	12522	253.81	ab
TAM07V-45	272.71	ab	12553	245.61	ab
12548	266.59	ab	12550	240.78	ab
12522	257.95	ab	12524	232.04	ab
12553	256.24	ab	TAM06WE-14	228.06	ab
12552	252.08	ab	12548	224.52	ab
TAM06WE-14	238.46	ab	12547	222.98	ab
GH20-1	229.65	ab	GH20-1	220.05	ab
12547	228.61	ab	GH20-2	214.91	ab
12524	228.18	ab	GH18-3	164.70	ab
12555	211.55	ab	12511	160.97	ab
12550	187.48	ab	GH15-2	156.81	ab
GH18-3	146.81	ab	GH18-1	155.10	ab
GH13-6	138.34	ab	12554	149.90	ab
GH18-1	136.93	ab	12525	132.60	b
GH15-2	112.41	b	GH13-6	121.17	b

†Means connected by the same letter are not significantly different (α=0.05, Tukey HSD)

Table 2.31. Means separation of differences in yield (Δ) between treated and untreated plots in Corpus Christi (2014)

Line	Δ (kg ha^{-1})	
12550	113.17[†]	a
12555	40.60	ab
GH15-2	39.61	ab
12524	34.41	ab
TAM07V-45	23.94	ab
12552	17.61	ab
GH18-1	16.21	ab
12522	9.39	abc
GH18-3	6.33	abc
12553	-2.98	abcd
12547	-5.02	abcd
GH20-1	-8.57	abcd
GH13-6	-14.46	bcd
TAM06WE-14	-16.73	bcd
12548	-58.64	bcde
GH20-2	-73.11	bcde
12511	-112.78	cde
12554	-119.66	de
12525	-143.01	e

[†]Means connected by the same letter are not significantly different (α=0.05, Tukey HSD)

Among the parental lines, TAM07V-45, TAM06WE-14, GH20-1, and GH20-2 consistently had the highest yields (Tables 2.28, 2.29, 2.30). Lines GH20-1 and GH20-2 have normal pubescence, performed relatively well in terms of square loss, and have relatively good fiber properties. In terms of fiber length, GH20-1 and GH20-2 were comparable to TAM07V-45 and TAM06WE-14 (Tables 2.32, 2.33, and 2.34). Both of these lines, GH20-1 and GH20-2, have potential as a high yielding, moderately resistant lines with good fiber qualities.

Among the backcross progeny, line 12525 (TAM06WE-14 //TAM06WE-14 /GH18-3), had the highest lint yield in untreated plots at Corpus Christi and, at College Station, where treatment did not impact yield, had the second highest yield among all parent and backcross lines (Table 2.29 and 2.30). With a length of 26.92 mm and micronaire of 4.51 (Tables 2.33 and 2.34), and one of the best and most consistent performances in terms of square loss, 12525 has the greatest potential among the backcross lines for a high yielding, resistant line with good fiber quality.

Table 2.32. Means separation of advanced fiber information system (AFIS) fiber properties—length (mm), strength (kN m kg^{-1}), micronaire, uniformity (%) and elongation—of parental lines in College Station, TX (2013)

Line	Length (mm)		Strength (kN m kg^{-1})		Micronaire		Uniformity (%)		Elongation	
TAM07V-45	27.94	b[†]	300.73	ab	4.35	bcd	81.51	a	6.46	a
TAM06WE-14	29.21	ab	305.44	ab	4.37	cd	81.78	a	5.98	ab
GH20-2	29.72	a	312.513	a	3.89	ab	82.16	a	4.99	c
GH20-1	28.70	ab	303.18	ab	4.22	abcd	81.91	a	4.83	c
GH18-3	27.94	b	288.06	b	3.94	abc	80.53	ab	6.16	ab
GH18-1	23.11	d	238.09	c	4.01	abc	78.85	b	6.05	ab
GH15-2	25.91	c	269.31	c	4.44	d	80.31	ab	6.43	a
GH13-6	26.42	c	245.45	c	3.87	a	78.98	b	5.54	bc

[†]Means connected by the same letter are not significantly different (α=0.05, Tukey HSD)

Table 2.33. Means separation of advanced fiber information system (AFIS) 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)

Line	Length (mm)		Strength (kN m kg^{-1})		Micronaire		Uniformity (%)		Elongation	
TAM07V-45	27.18	ab[†]	307.30	a	4.59	abc	82.20	a	7.28	a
TAM06WE-14	28.19	ab	283.74	abc	4.76	abc	81.48	ab	6.30	abcd
GH20-2	28.19	ab	301.90	ab	4.31	a	82.50	a	5.35	d
GH20-1	27.43	ab	276.38	abc	4.45	ab	82.10	a	6.28	abcd
GH18-3	26.92	b	282.76	abc	4.20	a	82.65	a	7.30	a
GH18-1	22.86	cd	231.02	d	5.04	cd	79.13	bcd	6.98	ab
GH15-2	21.08	d	228.27	d	5.54	d	77.93	d	7.38	a
GH13-6	23.62	c	226.30	d	5.01	cd	78.78	cd	6.50	abcd
12555	27.18	ab	270.78	bc	4.56	abc	80.53	abc	6.23	abcd
12554	26.92	ab	256.25	cd	4.70	abc	81.35	abc	6.25	abcd
12553	26.67	b	273.73	abc	4.36	ab	81.13	abc	7.05	a
12552	27.43	ab	292.09	ab	4.85	bc	81.75	a	7.30	a
12550	27.43	ab	284.03	abc	4.49	abc	81.98	a	6.68	abc
12548	28.96	a	293.85	ab	4.33	ab	82.28	a	6.65	abc
12547	27.43	ab	292.58	ab	4.79	abc	82.03	a	6.40	abcd
12525	28.19	ab	287.96	abc	4.68	abc	82.15	a	5.60	cd
12524	27.69	ab	278.63	abc	4.21	a	81.30	abc	7.15	a
12522	27.94	ab	270.98	bc	4.30	a	81.18	abc	6.90	ab
12511	27.94	ab	272.25	abc	4.60	abc	81.95	a	5.83	bcd

[†]Means connected by the same letter are not significantly different (α=0.05, Tukey HSD)

Table 2.34. Means separation of advanced fiber information system (AFIS) fiber properties—length (mm), strength (kN m kg^{-1}), micronaire, uniformity (%) and elongation—of parental lines in insecticide treated plots in College Station and Corpus Christi, TX (2014)

Line	Length (mm)		Strength (kN m kg^{-1})		Micronaire		Uniformity (%)		Elongation	
TAM07V-45	27.94	abc[†]	292.58	ab	4.51	abc	81.70	ab	6.73	abc
TAM06WE-14	28.19	ab	292.38	ab	4.61	abcd	81.78	ab	6.40	abcd
GH20-2	28.70	a	306.81	a	4.21	a	83.55	a	5.30	d
GH20-1	27.18	abc	283.05	abc	4.44	ab	82.28	ab	6.70	abc
GH18-3	26.92	abc	266.85	bcd	4.31	ab	81.80	ab	6.93	ab
GH18-1	23.37	d	229.94	ef	5.05	d	80.09	bc	7.03	ab
GH15-2	21.59	d	237.89	def	5.63	e	78.60	c	7.38	a
GH13-6	23.11	d	213.84	f	5.09	d	78.78	c	5.95	bcd
12555	27.94	abc	278.63	abc	4.71	abcd	82.18	ab	6.63	abc
12554	26.42	bc	248.40	cdef	4.67	abcd	80.90	bc	5.68	cd
12553	26.92	abc	262.14	bcde	4.37	ab	80.58	bc	6.60	abc
12552	28.45	a	304.85	a	4.24	a	81.80	ab	6.28	abcd
12550	25.91	c	285.70	ab	5.01	cd	80.35	bc	7.35	a
12548	27.18	abc	274.90	abc	4.65	abcd	80.45	bc	6.78	abc
12547	26.42	bc	273.23	abc	4.82	bcd	80.30	bc	6.58	abc
12525	26.92	abc	259.69	bcde	4.51	abc	81.40	ab	5.23	d
12524	27.43	abc	279.81	abc	4.48	abc	80.65	bc	6.53	abc
12522	28.45	a	277.16	abc	4.30	a	81.28	abc	6.70	abc
12511	27.18	abc	276.67	abc	4.67	abcd	81.60	ab	6.40	abcd

[†]Means connected by the same letter are not significantly different (α=0.05, Tukey HSD)

Conclusions

This project was designed to accomplish two objectives: (1) evaluate resistance to CFH feeding in eight parental lines and (2) to introgress resistant traits into high-yielding lines through backcrossing. In regards to the first objective, field evaluations over three years at College Station and Corpus Christi indicated that pilose, or densely pubescent, lines have a high resistance to CFH feeding, compared to lines with smooth or normal phenotype. However, when the CFH population approached or exceeded economic threshold levels in these plots, the pilose lines exhibited damage near or exceeding economic threshold. These data indicate that resistance can be overwhelmed by high fleahopper populations, but at lower populations, fewer insecticide treatments may be needed to maintain yield. The smooth and normal lines also maintained injury levels below economic threshold in all but week three at College Station in 2014. Again, resistance to CFH feeding appeared to be overwhelmed by CFH numbers above economic threshold in that week.

Data from the final week of data collection in 2014 indicated a difference in preference between the College Station CFH and the Corpus Christi CFH, measured by feeding injury; lines that showed little feeding injury at College Station showed greater feeding injury at Corpus Christi, and vice versa. Barman et al, in 2012, reported that CFH at College Station and Corpus Christi are genetically distinct as a result of host associated differentiation. Our data showed a potential difference in cotton genotype preference between the two fleahopper genotypes. The existence of two fleahopper genotypes in Texas (and possibly in other states) could strongly affect cotton breeding

programs by necessitating development of cotton lines that are regionally specific. However, more extensive screening is needed to support the hypothesis of location-dependent preference difference among the cotton genotypes tested in this study.

The second objective of this study was to introgress resistant traits into TAM07V-45 and TAM06WE-14 (recurrent parents) using the GH-04 lines that showed tolerance in 2012 and 2013 as donor parents. Among the backcross progeny, 12525 exhibited lower injury levels than either of its parents (TAM06WE-14 and GH18-3), and resistance comparable to those of the other donor parent lines, when CFH populations were the highest in 2014. Line 12525 is a normal/hairy line, indicating that resistance is not linked to the pilose trait. This line also had superior fiber quality, with a length of 26.92 mm and micronaire of 4.51. Additional breeding may be able to increase fiber length to make this line more competitive.

Data also indicated parent-dependent resistance in the backcross progeny across locations, perhaps indicating difference in host plant preference between the distinct CFH genotypes in these locations, but more years of testing are needed to validate or refute this hypothesis. A similar difference in preference was noted in TAM07V-45 and TAM06WE-14, which reflected differences in injury level noted in backcross progeny originating from these recurrent parents. Evaluation of backcross progeny occurred in only one year, with segregating backcross populations. In 2014, BC_1F_3 lines were grown for increase and plants were hand-harvested, by pubescence phenotype. These lines should be evaluated by phenotype to assess the effects of pubescence on CFH

preference, resistance to CFH feeding, and the success of transferring a resistance trait not linked to the pilose trait.

In summary, these studies revealed three important findings: 1) resistance was identified in the available upland germplasm and was prominent in cotton lines derived from crosses with the densely pubescent cultigen, Pilose; 2) evaluation of backcross progeny indicated that the resistance trait was heritable and could be separated from the pilose trait; 3) resistance was influenced by location and future studies should focus on validating this interaction between genotype and location and identifying underlying causes.

CHAPTER III

CHARACTERIZATION OF RESISTANCE TO COTTON FLEAHOPPER: BEHAVIORAL ASSAYS AND ANALYSIS OF SQUARE STRUCTURE

Introduction

Host plant resistance is defined as the phenomenon by which plants under the same environmental conditions experience different levels of injury due to insect herbivory (Painter, 1958); plants with comparatively little damage are often termed resistant and those with comparatively more damage are often termed susceptible. Host plant resistance can be described using three terms: tolerance, antixenosis, and antibiosis. Briefly, tolerance is a plant's ability to survive and sufficiently recover from insect infestation to produce economic product; antixenosis is the aversion of the insect to feeding on or even selecting the plant as a potential host; and antibiosis describes a fitness cost for the insect feeding on the plant (Painter, 1958; Reese et al., 1994; Strauss and Agrawal, 1999).

Cotton fleahoppers feed primarily on developing cotton flower buds, or squares, early in development, when the squares are of pinhead (1-2 mm in diameter) or match-head size (2-3mm in diameter) (Showler, 2009; Knutson et al., 2013). Feeding injury is characterized by abscission of squares and thus delayed maturity of the crop. Bell et al. (2007) reported that CFH are capable of vectoring pathogens during feeding and these pathogens, if delivered into the developing ovary, may be responsible for necrosis of ovary tissue that is characteristic of squares shed after being fed on by CFH. The most

common and abundant bacteria isolated from CFH was *Pantoea ananatis*, which is known to cause fruit and ovary rot in other plant species (Bell et al., 2007; Coutinho and Venter, 2009). Previous studies indicate degrees of resistance to CFH among cultivars evaluated in field studies (Lukefahr 1970; Walker et al., 1974) and in field and cage studies (Knutson et al., 2013). Evaluations of potentially resistant cotton lines in this study, described in the previous chapter, also revealed significant differences in performance, in terms of square loss due to CFH feeding. This chapter examines underlying mechanisms that may confer greater resistance to some cotton lines over others.

Materials and Methods

Cotton Fleahopper Rearing Protocol

The cotton fleahopper rearing protocol was derived from the methods of Breene et al. (1989), Gaylor and Sterling (1975), and Allen Knutson (personal communication). Woolly croton (*Croton capitatus*) stems were collected in burlap sacks at College Station in January 2012-2014. Stems were stored long-term in a cold storage seed room (approximately 15° C, 50% RH). As needed, stems were removed from the sacks, broken into smaller pieces and placed in 4.73 L plastic buckets, the openings of which were covered with mesh and secured with rubber bands. The buckets were filled with water for 20 minutes, drained, and placed in an incubator at 27.0±1° C (12 hr light: 12 hr dark). After a week of soaking in this manner every other day, the buckets were checked for hatched nymphs by inverting and shaking over a black counter top. Nymphs that fell out of the buckets were collected with an aspirator and transferred to plastic

Tupperware® containers covered with organza and lined with a Kimwipe (Kimberly-Clark®) and placed in the incubator. Adults and nymphs were fed store-bought, certified organic green beans. Green beans were replaced every other day or as needed.

Behavioral Assays

Behavioral assays were conducted with parental and backcross lines described in the previous chapter: parental lines—TAM07V-45, TAM06WE-14,GH13-6, GH15-2, GH18-1, GH18-3, GH20-1, GH20-2; and backcross progeny (BC_1F_3)—12511, 12522, 12524, 12525, 12547, 12548, 12550, 12552, 12553, 12554, 12555—derived from crossing each of the six GH- lines (donor parents) with TAM07V-45 and TAM06WE-14 (recurrent parents). Plants were grown in a growth chamber in a completely randomized design. Assays were replicated five times for each genotype.

Beginning two weeks after square initiation, a match-head size square was excised from each plant. Immediately following excision, the square was placed in a petri dish, along with a single adult CFH. Prior to use in the experiment, CFH were held overnight with water but no food. The petri dish containing insect and square was positioned under a dissecting microscope, and the actions of the CFH were filmed for 30 minutes using a digital camera mounted on the microscope. Following a protocol similar to that designed by Knutson (unpublished), a CFH's behavior during a 30 minute session was categorized as feeding, probing, cleaning, walking, or resting. Probing was characterized by walking with the proboscis forward, tapping and quickly inserting the proboscis into the substrate; feeding was characterized as prolonged insertion of the proboscis into the plant tissue, accompanied by pumping action of the head; cleaning, as

preening of the proboscis or antennae; walking, as taxis with the proboscis held against the body; and resting was characterized by the insect staying in place and not cleaning, probing or feeding. Several instances were noted in which the CFH fed (proboscis inserted with pumping action of the head) and then sat still, with the proboscis still inserted but without the characteristic pumping of the head. These instances were characterized as resting. Data were analyzed as a CRD in SAS (SAS v.9.4, SAS Institute, 2013), using PROC GLM.

Ovary Depth and Proboscis Penetration

Lines used in this study (TAM07V-45, TAM06WE-14, GH13-6, GH15-2, GH18-1, GH18-3, GH20-1, GH20-2 and BC_1F_3 progeny) were grown in a growth chamber (12 hr light: 12 hr dark; RH 50%) with three plants per pot. Beginning a few days after square initiation, pinhead (1-2mm diameter) and match-head size squares (2-3mm diameter) were excised from the plants and collected for measurements. Using a scalpel, the squares were cut approximately in half. Measurements of square width or diameter, length, and ovary depth were recorded under a dissecting microscope mounted with a camera. Video of the square was streamed to a desktop computer and measurements were recorded using ToupView software (v.3.2), which was calibrated for measurement with a stage micrometer to extract real measurements from pixels. Ovary depth was measured as the shortest distance from the outer edge of the bract to the wall of the developing ovary. Data were analyzed in SAS with PROC GLM, using ovary depth as the response variable and square width and length as covariates.

Cotton fleahopper proboscis penetration depth was estimated with the equations published by Esquivel (2011), in which penetrance (P) is a function of the length of the first two segments of the labium (*a* and *b*, respectively) and the angle (θ) made as these two segments hinge during feeding (Figure 3.1):

$$cs = (a^2 + b^2 - 2ab\cos\theta)^{1/2}$$

$$P = \Sigma(a+b) - cs$$

An average length of the first two labial segments for an adult fleahopper was calculated from measurements recorded from 12 wild-caught, adult CFH from College Station. The labium acts as a sheath for the CFH's feeding stylets, the structures that penetrate the plant tissue and are directly involved in tissue laceration and uptake of nutrients. As the CFH inserts its stylets into the plant tissue, the labium hinges and bends like an elbow, to accommodate the decreasing distance between the insect's head and the substrate on which it's feeding (Figure 3.1a, b). Theta, the angle of hinge between segments *a* and *b* during feeding, was obtained from videos of CFH feeding that were recorded in the previously described behavioral assays. Using ImageJ (Rasband, 1997-2014), theta was estimated from stills derived from these videos (Figure 3.1).

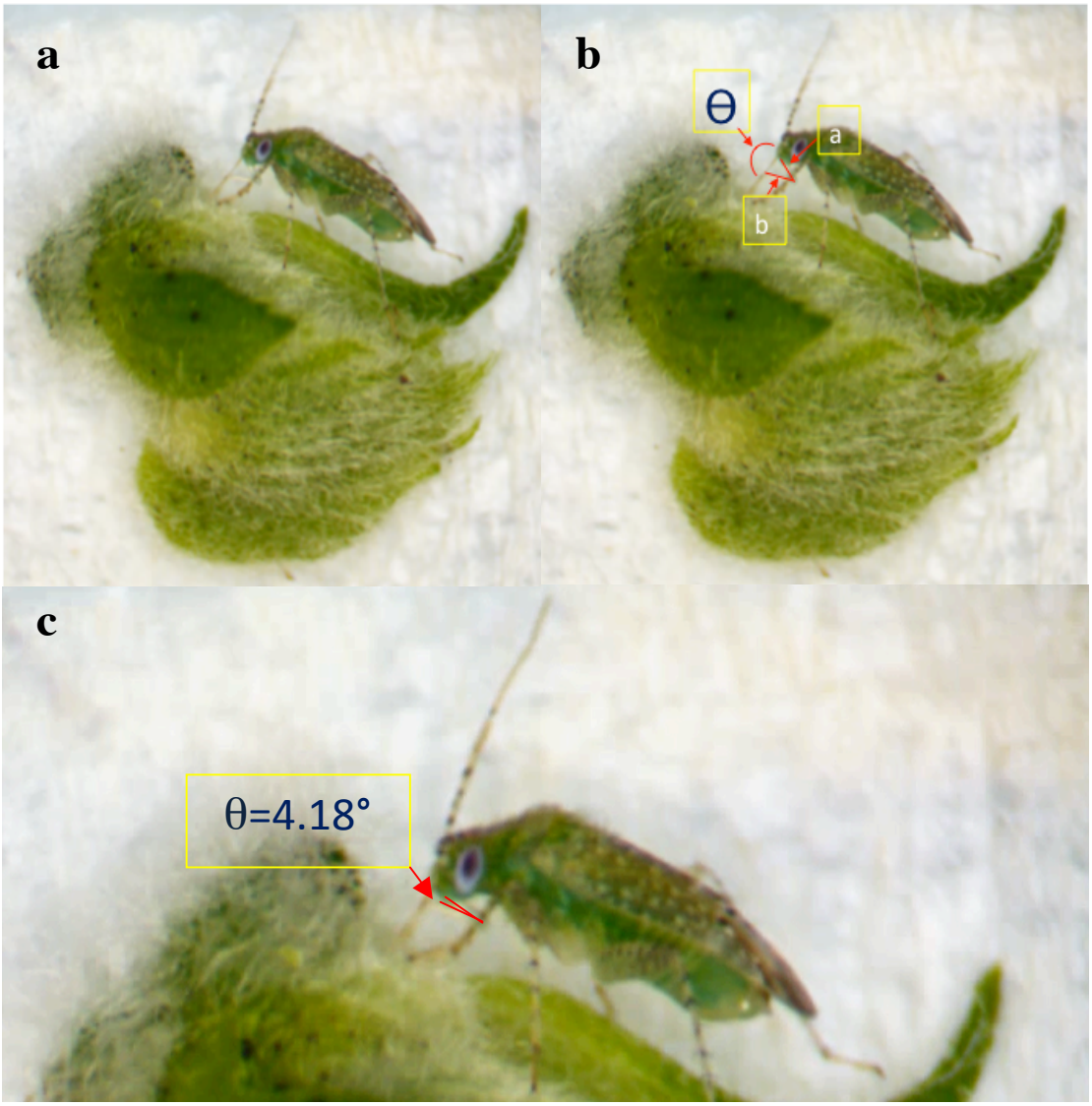

Figure 3.1. Estimation of cotton fleahopper proboscis penetration during feeding, where *a* is the length of the first labial segment, *b* is the length of the second labial segment, and *Θ* is the angle of hinge between *a* and *b*

Results and Discussion

Behavioral Assays

Evaluation of each line was based on the average time CFH spent cleaning, resting, walking, probing, or feeding. Analysis indicated that time spent feeding differed significantly between lines (Table 3.1). The insects spent the most time feeding on TAM07V-45, an average of approximately 25min, which did not differ significantly from time spent feeding on parental lines, GH13-6, TAM06WE-14, or GH20-2, but was significantly longer than time spent feeding on the remaining parental lines (GH20-1, GH18-3, GH15-2, and GH18-1) (Figure 3.2). Of the backcross progeny, the insects spent comparatively less time feeding on lines 12547 (TAM07V-45//TAM07V-45/GH13-6), 12525 (TAM06WE-14//TAM06WE-14/GH18-3), 12554 (TAM06WE-14//TAM06WE-14/GH20-2).

Significant differences in feeding time among the lines suggests that CFH prefer some cotton lines over others. For example, the insects spent almost three times longer feeding on TAM07V-45 than on GH18-1, indicating a difference in preference. Consistently, TAM07V-45 was found to be highly susceptible to CFH in choice, field evaluations (described in Chapter II). It is possible, given the data from the behavioral assays, that TAM07V-45 exhibited more CFH feeding damage in the field than the other lines because the insects preferred it and chose it as a host over the other lines.

Time spent walking, resting, probing, or cleaning did not differ significantly among lines (Table 3.1). However, it was noted that as feeding time decreased among the lines, probing and resting tended to increase (Figure 3.2). These data indicate that lines on which CFH spent more time probing and less time feeding may represent less suitable hosts. Host selection by insects is largely dependent on detection of chemical cues elicited by the host (Bernays and Chapman, 1994). Thus, prolonged probing or more time spent away from the host (walking or resting) indicate that the insect was unable to recognize the square as an acceptable host and feeding time was reduced. Time spent cleaning and walking did not vary among lines.

Table 3.1. Analysis of variance of duration of cotton fleahopper behavior during no-choice behavioral assay with parental and backcross progeny lines

	Effect	Num df, Den, df	F value
Feeding	Line	18,72	2.38 *
	Trial	4,72	2.06
Probing	Line	18,72	0.6282
	Trial	4,72	0.0009 *
Cleaning	Line	18,72	0.2657
	Trial	4,72	0.0031 *
Walking	Line	18,72	0.7791
	Trial	4,72	0.5806
Resting	Line	18,72	0.0516
	Trial	4,72	0.1717

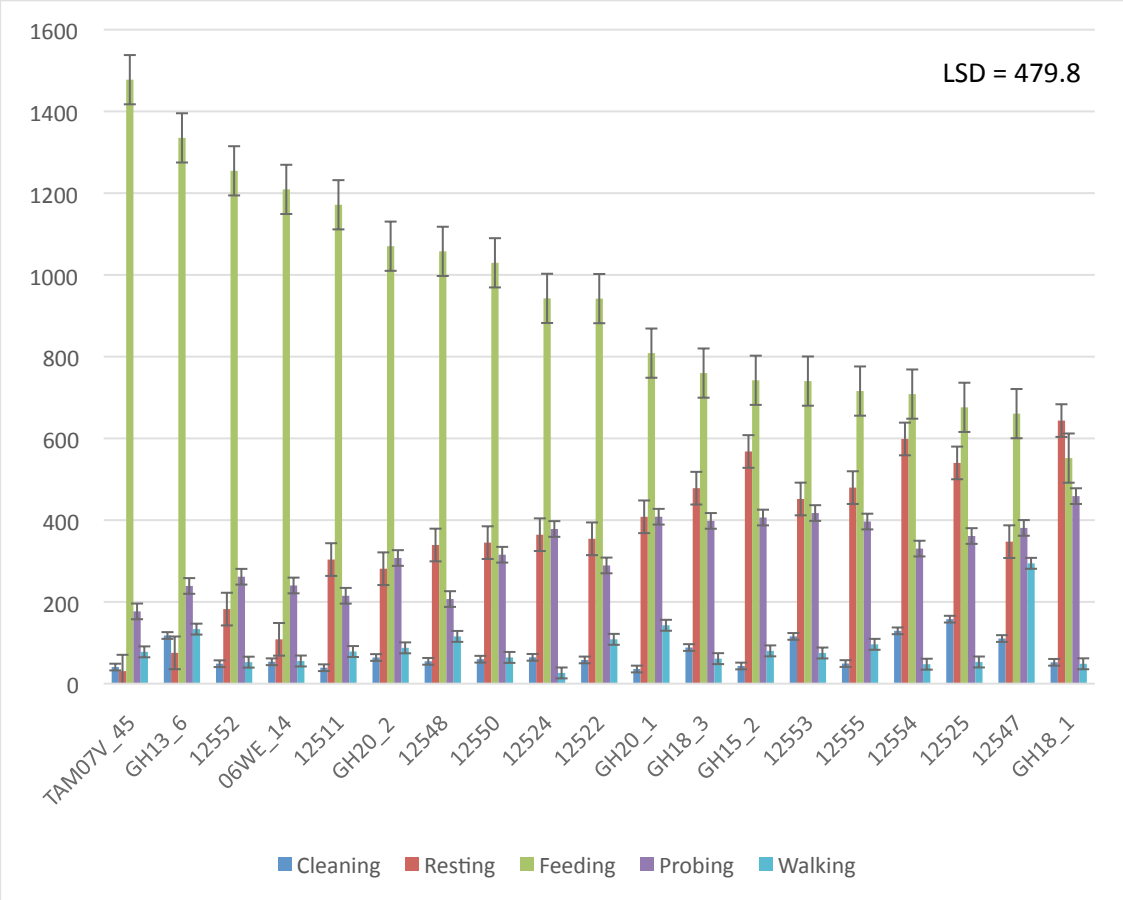

Figure 3.2. Cotton fleahopper behavior duration (sec) during no-choice behavioral assay with parental and backcross progeny lines. Error bars represent standard error.

For the parental lines, the average time CFH spent feeding in the behavioral assays was indicative of performance in the field, in terms of percent square loss, when the parental lines were ranked across two years and six weeks of data from field trials at College Station (Table 3.2). Because only CFH from College Station were used in the behavioral assays and because of noted preference differences between College Station and Corpus Christi CFH in the field trials (Chapter II), data from Corpus Christi are not

included in Table 3.2. The only notable exception to the trend of no-choice feeding time being indicative of field performance was GH13-6, which ranked second highest among parent lines for CFH feeding in the no-choice studies, but had one of the lowest injury levels across field trials in 2013 and 2014. Based on these data, apparent resistance to CFH feeding could be a manifestation of CFH preference, with the exception of GH13-6, which may possess host plant resistance mechanisms that allow it to tolerate feeding injury.

Table 3.2. Comparison of genotypes ranked by field performance (field rank), in terms of percent square loss, and cotton fleahopper preference (feeding rank), measured as duration of feeding during no-choice behavioral assay

	2013			2014					
Line	**Week 2**	**Week 3**	**Week 4**	**Week 1**	**Week 2**	**Week 3**	**Total**	**Field Rank†**	**Feeding Rank‡**
GH18-3	1	1	2	5	1	4	14	1	6
GH13-6	2	5	4	4	2	3	20	2	2
GH20-1	4	3	1	3	4	7	22	3	5
GH18-1	6	4	3	1	6	2	22	3	8
GH15-2	5	2	5	8	5	1	26	4	7
GH20-2	3	6	8	2	3	6	28	5	4
TAM07V-45	7	7	6	7	7	5	39	6	1
TAM06WE-14	8	8	7	6	8	8	45	7	3

†Field rank is based on a ranking of the total rank score across all six weeks of data collection

‡Feeding rank is a ranking of the parents based on time CFH spent feeding on the squares from each line in the no choice study

Ovary Depth and Proboscis Penetration

Significant differences in ovary depth were observed among parental and BC_1F_3 lines, and square width was found to be a significant cofactor affecting ovary depth (Table 3.3). Among the parental lines, GH15-2 had the greatest average ovary depth, and GH18-3 and TAM07V-45 had the shallowest (Table 3.4). The main interest in measuring ovary depth was to determine the ability of a feeding CFH to penetrate the ovary with its proboscis. Bell et al. (2007) reported that CFH are capable of vectoring pathogens during feeding and these pathogens, if delivered into the developing ovary, may be responsible for necrosis of ovary tissue that is characteristic of squares shed after being fed on by CFH. Additionally, digestive enzymes in the saliva of the CFH that digest plant tissues likely contribute to the plant tissue lesion characterisitic of CFH feeding (Miles, 1972; Martin et al., 1988). Based on this information, it was hypothesized that the ability of the CFH to penetrate the ovary during feeding may influence the rate at which fed upon squares are abscised. To this end, the maximum penetration depth of an adult CFH, collected in College Station, was calculated based on the average length of the labial segments of the proboscis. By measuring the length of the first and second labial segments, *a* and *b*, respectively, and estimating the angle, Θ, at which they hinge during feeding, it is possible to estimate penetration depth of the feeding stylets (Wheeler, 2001; Esquivel, 2011). A maximum penetration depth of 0.549±0.05mm was calculated using the most acute angle, 4.18°, observed in the no-choice feeding trials discussed above. At this angle, the first and second labial segments are nearly touching, and it appears they cannot be hinged any further (Figure 3.1c).

Table 3.3. Analysis of variance of ovary depth (mm) in squares of parental and backcross progeny lines, using square length and width as covariates

Effect	Num df, Den df	F-value
Line	18,236	2.81*
Length	1,236	2.49
Width	1,236	98.46**

Table 3.4. Means separation analysis of ovary depth in squares of parental and backcross progeny lines

Line	Mean	
GH15-2	0.948	a
GH18-1	0.904	ab
12552	0.855	bc
12555	0.840	bcd
GH20-2	0.836	bcd
GH13-6	0.799	cde
12554	0.784	cdef
12522	0.754	defg
12548	0.727	efgh
12525	0.725	efgh
TAM06WE-14	0.714	efgh
GH20-1	0.700	fgh
TAM07V-45	0.684	gh
12524	0.679	gh
GH18-3	0.674	gh
12511	0.667	gh
12550	0.663	h
12547	0.653	h
12553	0.640	h
LSD	**0.088**	

To determine the window of susceptibility of the ovaries in the squares of each line, in terms of the ability of the CFH to penetrate the ovary tissue during feeding, ovary depth was regressed on square width (Figures 3.3 and 3.4). Strong correlations between square width and ovary depth were found for each of the parental and backcross lines (Figures 3.3 and 3.4). A threshold of susceptibility to proboscis penetration was established at 0.549±0.05 mm (indicated by red lines in Figures 3 and 4). From the line of best fit for each set of data, the corresponding square width threshold of susceptibility can be calculated. For instance, the width threshold is 1.03 mm for GH15-2, 1.57 mm for Tam07V-45, 1.59 mm for GH18-3, and 1.62 mm for GH20-1. These data indicate that the window of susceptibility is shorter for GH15-2, compared to TAM07V-45, the squares of which must reach a comparatively larger size before the ovary wall is beyond the reach of feeding CFH. The susceptibility window for all lines was <2.0 mm in diameter (Figures 3.3 and 3.4), which is consistent with results from Knutson et al. (2013) that indicated that 99% of CFH feeding damage was observed on squares measuring ≤ 2.0 mm in diameter.

Mechanical barriers to insect feeding may confer resistance in some plants. For instance, research on jassid, *Empoasca libyca* (Homoptera: Cicadellidae) resistance in cotton indicated that trichome length is pivotal in conferring resistance (Knight, 1952). Long trichomes prevent the insects from reaching the surface of the leaf and thus prevent feeding. Ovary depth, in this case, is not exactly a mechanical barrier to feeding, but it does appear to afford the plant some protection from feeding CFH. This is evident when comparing ovary depth with performance in the field (Tables 2 and 4); there is a positive relationship between field performance, in terms of percent square loss, and ovary depth, i.e., cotton lines with greater ovary depth had lower percent square loss in field trials The notable exceptions to this trend are GH18-3 and GH20-2, for which ovary depth was not indicative of field performance.

In addition to being a physical barrier that protects the developing cotton ovary from feeding CFH, variation in ovary depth among the cotton lines examined could hypothetically influence preference of feeding CFH. Showler (2009), in a study of boll weevil feeding preference, reported on the concentration of free amino acids in the reproductive tissues (anthers, stamens, style, and ovary) and rind tissue (calyx and petals) of match-head size squares. Although CFH feed preferentially on squares < 2 mm in diameter (Knutson et al., 2013), the results of Showler (2009) are informative as to how the nutrient quality and quantity in squares could influence feeding by CFH.This study focused on 10 amino acids that are crucial to insect development: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Rose 1938; Dadd 1973; Showler 2009). Total concentration of free amino

acids did not differ significantly between reproductive and rind tissues in match-head sized squares, but the availability and concentrations of specific amino acids differed. All ten amino acids were present in reproductive tissues, but isoleucine and methionine were absent in rind tissues. Significantly higher concentrations of free alanine, glutamic acid, glycine, leucine, and proline were found in reproductive tissues. The specific nutritional requirements of CFH, in terms of amino acids, have not been determined, but in other insects, absence of certain amino acids in the diet is associated with a fitness cost. For example, absence of methionine reduces longevity of boll weevil (Showler, 2009). While amino acid availability does not dictate whether or not an insect will feed, or for how long, it may very well play a role in preference. And in the case of the genotypes included in this study, availability of nutrients is dependent on the ability of the insect to feed on certain tissues within the square.

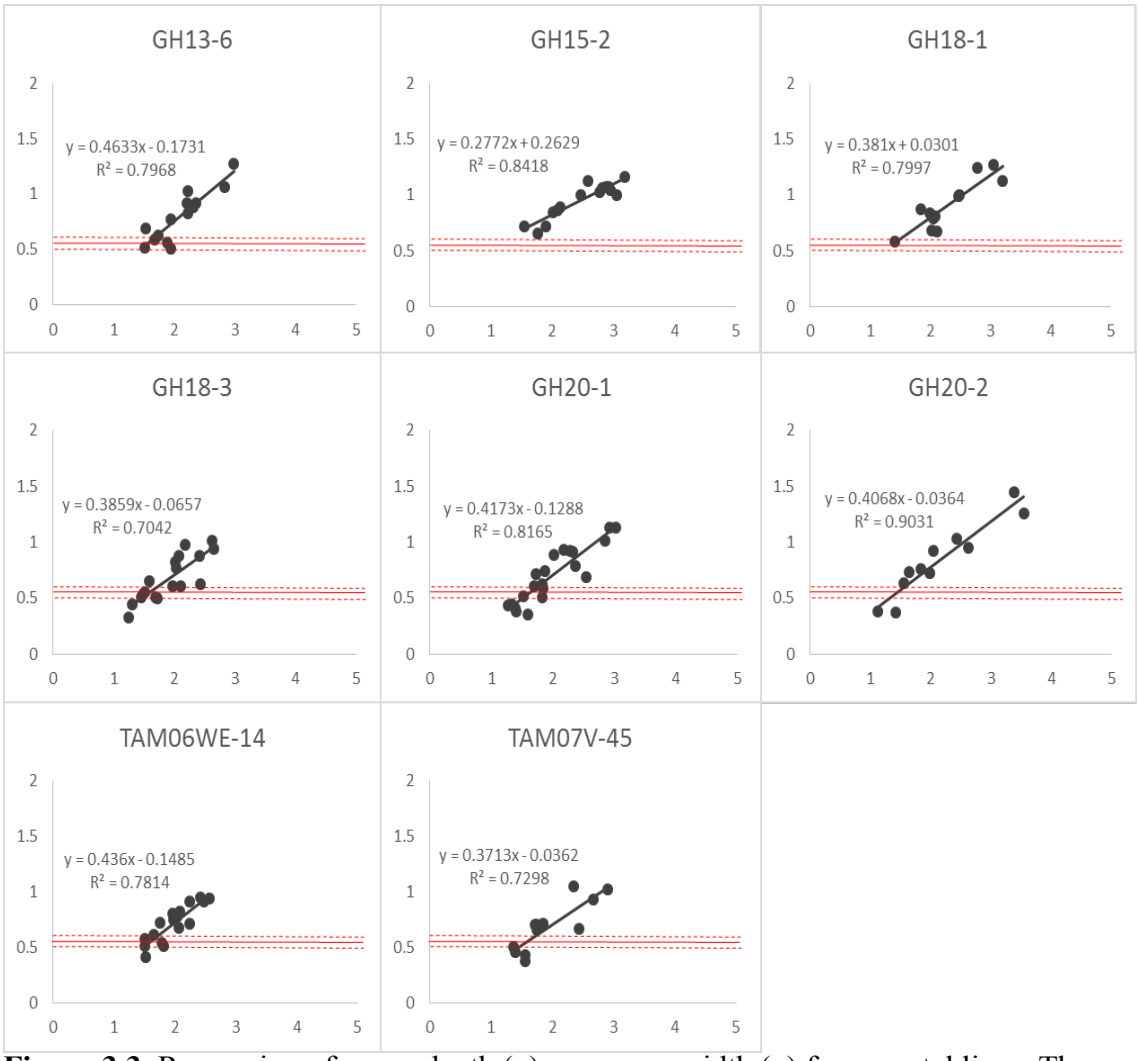

Figure 3.3. Regression of ovary depth (y) on square width (x) for parental lines. The solid red line indicates a threshold of susceptibility of 0.549±0.05 mm, determined by estimating the maximum proboscis penetration depth of adult cotton fleahoppers during feeding

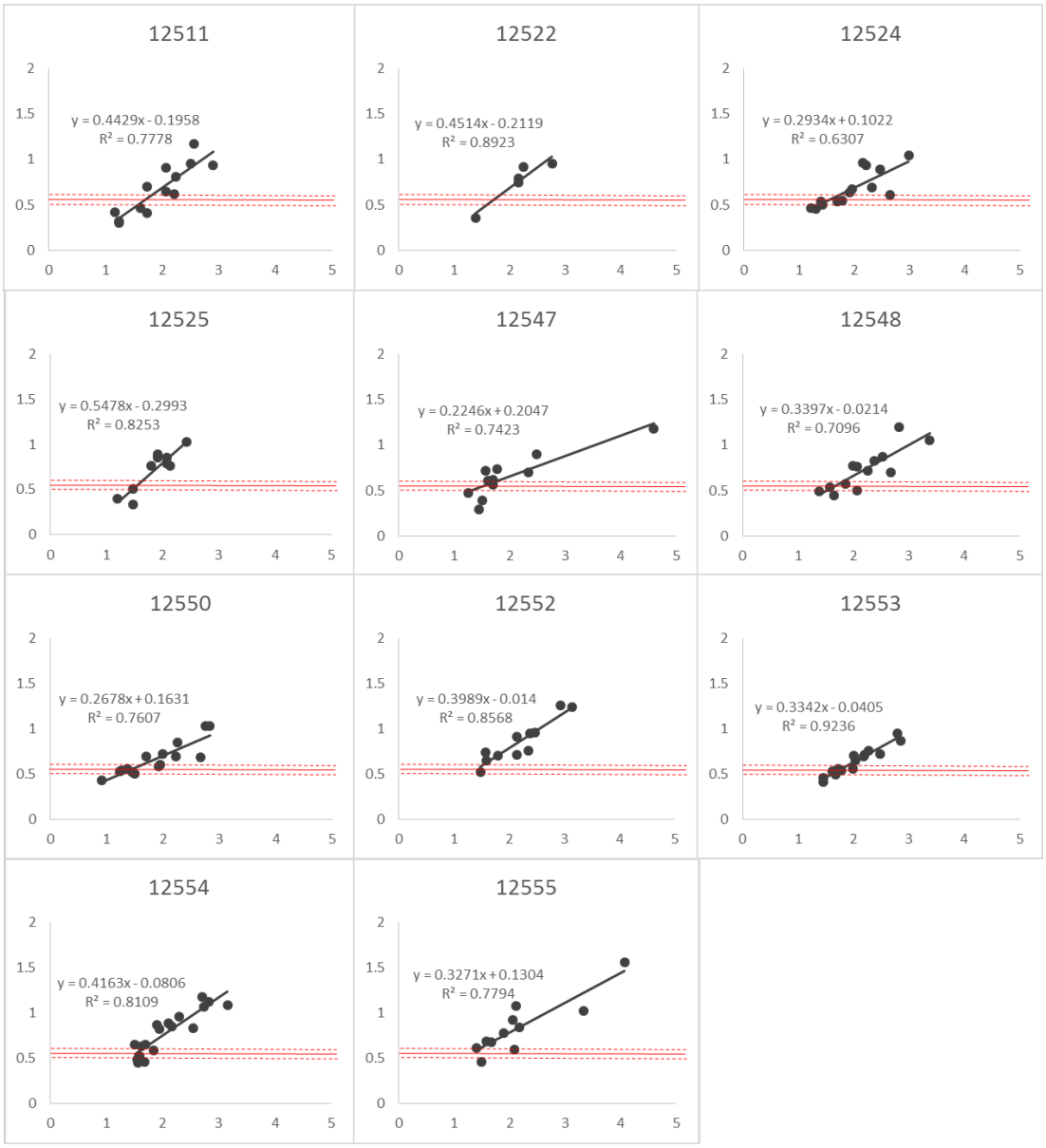

Figure 3.4. Regression of ovary depth (y) on square width (x) for backcross progeny lines. The solid red line indicates a threshold of susceptibility of 0.549±0.05 mm, determined by estimating the maximum proboscis penetration depth of adult cotton fleahoppers during feeding

Conclusions

Data from behavioral assays and ovary depth versus proboscis penetrance indicate multiple host plant resistance mechanisms may be at play in the relationship between CFH and the upland cotton lines used in this study. Analysis of CFH behavior, particularly behaviors relating to host acceptance, revealed differences in fleahopper preference among the 19 cotton lines tested. Cotton fleahoppers spent significantly more time feeding on some lines, compared to others.

Analysis of square structure from each of the 19 cotton lines indicated significant differences in the depth of the developing ovary. Presumably, based on estimations of maximum proboscis penetration, the placement of the ovary affects the ability of a feeding CFH to penetrate the developing organ with its proboscis. This finding has two important implications: 1) deeper ovaries are likely protected from digestive enzymes and direct infection with pathogens vectored during CFH feeding, which, if localized by the plant's immune system before reaching the ovary, may distinguish between susceptible plants that shed squares and resistant plants that retain squares and 2) the inability of the CFH to penetrate the ovary during feeding may impact preference to feed or duration of feeding.

CHAPTER IV

RNA-SEQ TRANSCRIPTOME PROFILING OF UPLAND COTTON UNDER COTTON FLEAHOPPER FEEDING STRESS

Introduction

Insects are often implicated in the infection of host plants with pathogenic bacteria and fungi, either as direct vectors of the pathogens or as indirect agents of infection, creating wounds via feeding through which pathogens may enter. Bacterial pathogen transmission during feeding has been reported as a contributing cause of square abscission in cotton plants fed upon by cotton fleahopper (CFH). (Bell et al., 2007). Studies have identified a cocktail of bacteria that can be isolated from the salivary glands of CFH, including known plant pathogens of the genera *Pantoea*, *Serratia*, *Xanthomonas*, and *Pseudomonas* (Duffey and Powell, 1979; Martin et al., 1987; Bell et al., 2007). Bell and colleagues (2007) reported the transmission of *Pantoea ananatis* by CFH during feeding on buds and bolls. Cotton fleahoppers acquired *P. ananatis* through feeding on infested plant tissue. *Pantoea ananatis* occurs as an epiphyte on many crops, including cotton but is also a well-known pathogen and is considered to be an emerging disease in agriculture, causing fruit and ovary rot (Coutinho and Venter, 2009). Additionally, like other Mirids, the saliva of CFH contains a pectinase, called polygalacturonase, which aids in digestion of pectins in the middle lamella (Miles, 1972; Martin et al., 1988). Martin et al. (1988) reported that injection of cotton terminal tissue with salivary isolates from the CFH caused an increase in ethylene production by the

plant and conjectured that polygalacturonase in the saliva may be responsible for the plant tissue lesion characterisitic of CFH feeding.

Plants respond to herbivory and infection through a variety of defense-related pathways that aid in the containment of invading pathogens. One type of defense response, the hypersensitive response (HR), is characterized by localized, programmed cell death (PCD) of infected tissues to limit the spread of infection and is often associated with disease resistance (Hofius et al., 2011; Lam et al., 2001). The goal of this study was to investigate the role of HR to herbivory by CFH, with particular focus on regulation of HR and attenuation of lesion formation, through whole-transcriptome analysis.

Materials and Methods

Plant Material and Tissue Collection

Four cotton lines were included in this study: TAM07V-45, GH15-2, GH18-3, GH20-1. Of these four lines, GH15-2, GH18-3, and GH20-1 are derived from crosses of Pilose by a commercial cultivar. Prior to this study, each of these lines were evaluated for resistance to CFH feeding damage under field infestation conditions for two years in College Station and Corpus Chrsiti (please see Ch. II for details). TAM07V-45 was found to be more susceptible to the CFH than the other three lines.

Two conditions were examined: 1) exposure to CFH feeding (‘Insects’) and, 2) control (‘No Insects’). From each line, four plants were grown under each condition (Figure 1). Plants were grown under growth chamber conditions (24 hr light, 50% RH). Approximately 10 days after square initiation, four adult CFH (held overnight with water

but no food) were caged for 72 hrs on the terminals of plants in the 'Insects' group. After insect exposure, a match-head sized square (approximately 2mm in diameter) was collected from the terminal of the plant and placed immediately into liquid nitrogen. Squares of similar size were collected from control plants ('No Insects') approximately 13-14 days after square initiation. Because feeding injury by the CFH can result in death and abscission of the square tissue, actual feeding by the insects on the plants in the treatment group could not be verified prior to tissue collection. However, a previous study under no choice conditions, in which CFH were similary deprived of food for 24 hrs before being introduced to an arena containing an excised square, was used to verify that the CFH would readily feed on each of the genotypes included in this study (Chapter III). Recovery of live CFH after the 72 hr caging window was also used to support the assumption that the insects fed on the plants on which they were caged. Preliminary tests indicated that CFH die after more than 24hrs without food or water.

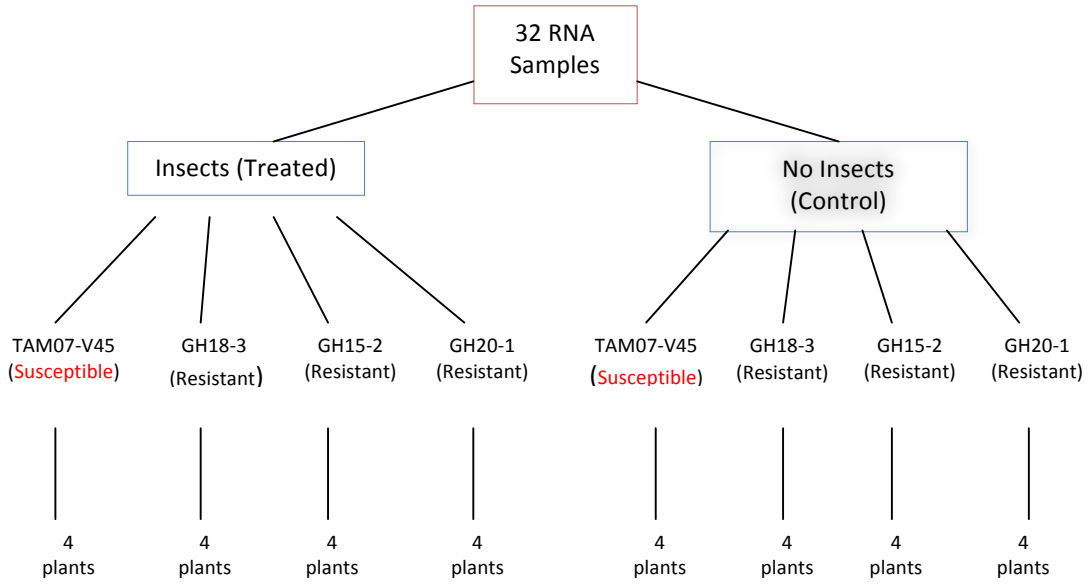

Figure 4.1. RNA-seq experimental design for feeding trials and tissue collection

RNA Isolation and Processing

Samples were stored in -80°C after collection. RNA was isolated using the Spectrum™ Plant Total RNA kit (Sigma Aldrich). Samples were ground in isolation buffer using the MP FastPre-96™ homogenizer. Isolated RNA was stored at -80°C until processing. Sample quality was verified with a Bioanalyzer and libraries were created using the Illumina TruSeq RNA kit. Samples were multiplexed and sequenced in four lanes of 100bp SE Illumina HiSeq2000.

Trimming and Mapping

Reads were trimmed with Galaxy FASTQ Quality trimmer, using a quality cutoff of 20. Reads were mapped to the *Gossypium raimondiii* (v2.1) (Paterson et al., 2012)

reference genome using CLC Genomics Workbench RNA-Seq Alignment tool (minimum length fraction: 95%, minimum similarity fraction: 95.3%)

Differential Expression Analysis

Aligned sequences were analyzed for differential expression using the Cufflinks pipeline in Galaxy (Goecks et al., 2010; Blankenberg et al., 2010; Giardine et al., 2005). Cuffdiff was used to make pairwise comparisons between the conditions, 'Insects' plants and 'No Insects' plants, using a false discovery rate (FDR) of 5%. Determination of significance was based on the Benjamini-Hochberg (1995) adjustment for multiple comparisons (Trapnell, 2014). Principal component analysis of pooled replicates under each condition for each genotype was generated in R (v.3.1.1; R Core Team, 2014), using cummeRbund (Goff et al., 2013) to visualize data quality control. Additional plots for analysis were generated in R, using ggplot2 (Wickham, 2009) and VennDiagram (Chen, 2014).

Functional Annotation

Splice variants of significant differentially expressed genes were identified through Phytozome (v10.1) (Goodstein et al., 2012). Fasta sequence files were obtained through PhytoMine in Phytozome. Significant differentially expressed genes and their splice variants were functionally annotated with Blast2GO software and mapped to gene ontologies (GO) (Conesa and Götz, 2008). An InterProScan analysis was also conducted (Quevillon et al., 2005). In addition to annotation with Blast2GO, a literature search was conducted to determine genes and proteins integral to plant immune responses to pathogens and herbivory in other plant species. Using Phytozome's resources and

InterProScan data provided by CottonGen (Yu et al., 2013), the *G. raimondii* reference was mined for transcripts with matching GO and InterProScan IDs. Expression of these transcripts was evaluated in each of the four genotypes in this experiment under both conditions by creating expression heat maps in Cummerbund.

Results and Discussion

Reads and Mapping

Four cotton germplasm lines were included in this study: TAM07V-45, GH18-3, GH20-1, and GH15-2. Samples were multiplexed by genotype for sequencing. Approximately 188.9million raw reads were generated from TAM07V-45; ~203.5million reads from GH18-3; ~204.3million reads from GH20-1; and ~170.9million reads from GH15-2. Trimmed reads were mapped to 37,331 *G. raimondii* (v.2.1) transcripts.

Expression Analysis

Initial data analysis focused on identifying similarities and differences in expression patterns among the four genotypes, TAM07V-45, GH15-2, GH18-3, and GH20-1. A summary of expression patterns in a pairwise analysis ('Insects' vs 'No Insects') for each genotype is given in Table 4.1. For all lines, the majority of significant differentially expressed genes were down regulated. Unique to GH20-1, the majority of upregulated genes were uniquely expressed in samples taken from plants exposed to CFH feeding; 91 of the 95 upregulated genes were turned on in response to herbivory.

Hierarchical clustering analysis of samples under the 'Insects' condition, performed in Cummerbund, indicated clustering of the putative resistant lines, GH15-2,

GH18-3, GH20-1, based on FPKM-adjusted expression values (Figure 2). Among the putative resistant lines, GH18-3 and GH20-1 were more related to one another than to GH15-2. This analysis indicates that the resistant lines showed more similarities in gene regulation in response to CFH herbivory, compared to the susceptible line, Tam07V-45. These results were substantiated by Venn diagram analysis of significant differentially expressed genes in a pairwise analysis of both conditions for each genotype (Figure 3). The majority of significantly regulated genes expressed by TAM07V-45 were unique to that line. Sixty four genes were commonly expressed by all four genotypes. Principal component analysis of each genotype indicated divergent expression patterns for control plants and plants on which CFH were caged (Figure 4).

Table 4.1. Summary of expression analysis of three resistant and one susceptible genotype in a pairwise comparison of plants fed-on by cotton fleahopper and plants not exposed to herbivory

Cotton Line	Significant Differentially Expressed	Up Regulated	Down Regulated	Turned On	Turned Off
TAM07V-45	1396	186	1210	39	2
GH15-2	1153	240	913	18	2
GH18-3	732	280	452	0	0
GH20-1	694	95	599	91	0

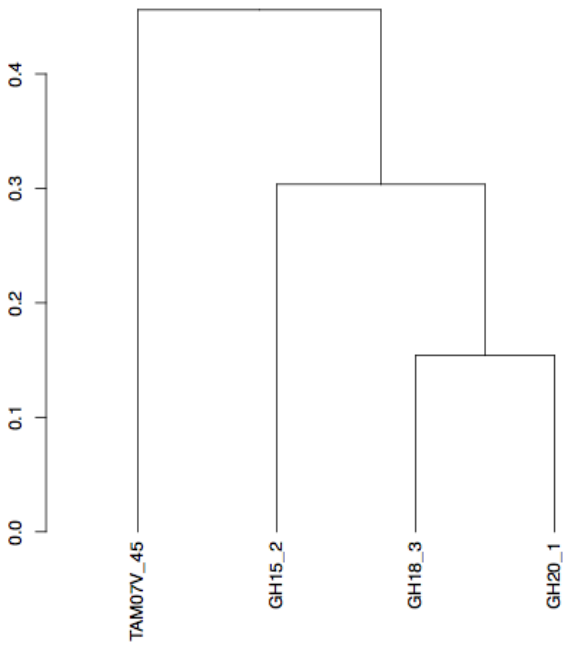

Figure 4.2. Hierarchical analysis of expression data for genotypes exposed to cotton fleahopper herbivory

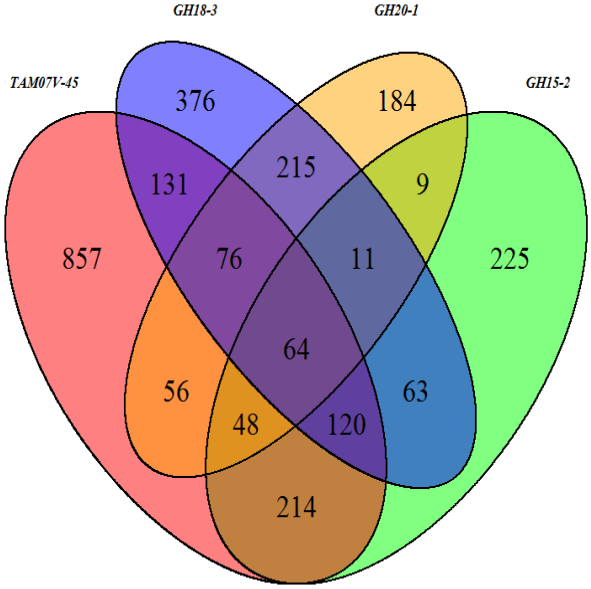

Figure 4.3. Significantly expressed genes shared among three resistant (GH18-3, GH20-1, GH15-2) and one susceptible genotype (TAM07V-45)

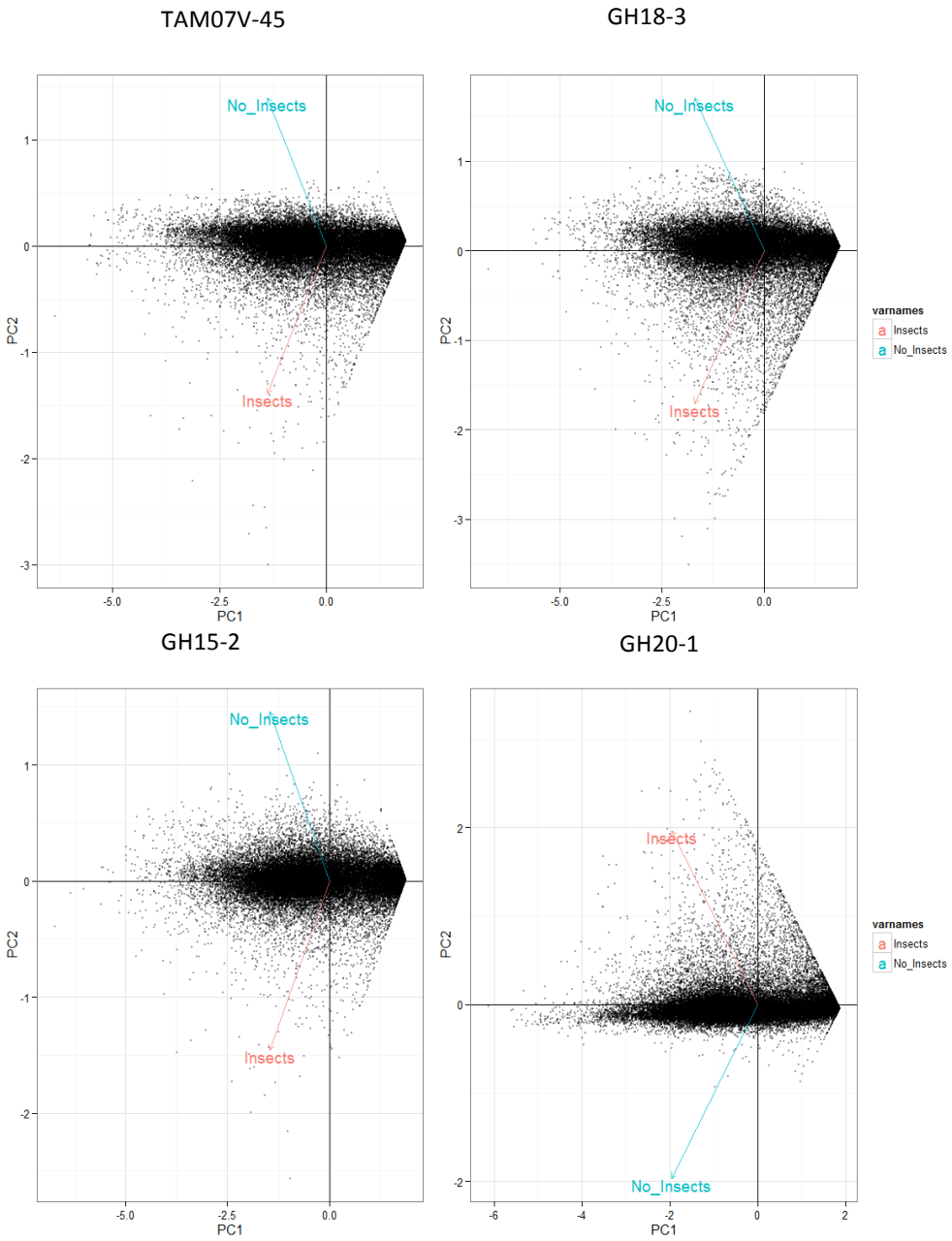

Figure 4.4. Principal component analysis of expression data for each genotype under control conditions ('No Insects') and exposure to cotton fleahopper herbivory ('Insects')

Blast Results

Phytozome (v10.1) was used to obtain splice variants for the top 30 up- and down-regulated transcripts for each genotype. Using the InterMine tool on Phytozome, fasta files of sequences for each transcript were obtained and uploaded into Blast2GO for Blast analysis. Tables of the results for each genotype are in Appendix I. Not surprisingly, Blast results showed strong upregulation of transcripts generally associated with response to wounding, herbivory, and pathogen infection. Most common among these were chitinase and protease inhibitors, such as trypsin inhibitor. Chitinase activity in plants can be induced by introduction of pathogens and is often associated with the defense response to infection (Punja and Zhang, 1993; Van Loon, 1997; Gupta et al., 2013). Protease inhibitors are anti-herbivore enzymes produced by plants to deter or inhibit insect feeding. Proteases are necessary for the digestion of plant proteins and are used by both insects and plant pathogens to digest host plant tissue (Green and Ryan, 1972; Koiwa et al., 1997). Trypsin is a digestive enzyme found in the midgut of many insects (Lopes et al., 2004). Trypsin-like enzymes were recently characterized in the saliva of *Lygus Hesperus* (Miridae), a cotton pest and an insect closely related to CFH (Zeng et al., 2002). Trypsin inhibitors produced by soybeans have been demonstrated to retard the growth of beet armyworm (*Spodoptera exigua*) larvae and are thus viewed as an important defensive compound in the host plant-insect interaction (Broadway and Duffey, 1988).

Transcripts for pectinesterases were significantly down regulated in TAM07V-45, GH18-3, GH15-2, and pectinesterase inhibitors were significantly upregulated in

GH20-1. Pectinesterases are involved in cell wall modification and can play roles in the strengthening or degradation of cell walls (Micheli, 2001). Interestingly, in other plant species, pectinesterases are upregulated in response to wounding by herbivory, and Köner et al. (2009) found that knockdown of Na*PME1*, a pectinesterase in tobacco (*Nicotiana attenuata*) improved performance of *Manduca sexta* larvae feeding on the modified plant, compared to larvae feeding on the wildtype plant. However, over expression of pectinesterase inhibitors in *Arabidopsis thaliana* increased resistance to the fungal pathogen *Botrytis cinerea* (Lionetti et al., 2007). Data from our study, in which pectinesterase was downregulated or inhibited in response to herbivory by an insect vectoring a pathogen, may indicate a tradeoff in regulation of this enzyme. Upregulation of this enzyme may have a negative impact on insect performance, but inhibition or down regulation of this enzyme may increase resistance to pathogens.

Significant upregulation among the top 30 transcripts for terpenoid or terpene biosynthesis was noted for GH18-3 and GH15-2. Gossypol, the primary terpenoid produced by cotton, has negative effects on larval development for chewing insects, such as those in the *Heliothis*/*Helicoverpa* complex (Shaver and Parrott, 1970), but has also been shown to negatively impact the fitness of *L. hesperus*. These insects, when caged on glanded or glandless (plants that lacked gossypol) cotton, grew to greater population numbers on glandless cotton, suggesting a negative impact of gossypol on nymph survival (Tingey, 1975). Cotton fleahoppers are closely related to *L. hesperus*, belonging to the same family, Miridae; it's possible that high levels of gossypol have a similar impact on CFH fitness.

Gene Ontology

Using Blast2GO, gene ontologies (GO) were obtained for the top 30 up- and down-regulated transcripts for each genotype. Gene ontologies are characterized as biological process, molecular function, or celluar component. For all genotypes, transcripts with GO-related to stress, wounding, response to stimulus and infection, and defense were significantly upregulated. The most commonly down-regulated GO were those related to general organism maintenance: DNA replication/transcription, photosynthesis, and cell cycle-related functions (Appendix I). Down regulation of transcripts involved in maintenance of the plant was not surprising, given the stress of herbivory and mobilization of resources toward responding to feeding damage. Significant upregulation of transcripts involved in defense response and response to infection indicates that the CFH were feeding on the plants on which they were caged and also indicates the likelihood that the CFH were vectoring a pathogen during feeding.

InterProScan

Using InterProScan data for *Gossypium raimondii* made available on the CottonGen website (Yu et al., 2013), *G. raimondii* transcripts with functional annotation for hypersensitive response (HR) regulators were obtained and used to generate heat maps in Cummerbund to detect differences in expression in each of the four genotypes under infested ('Insects') and non-infested ('No Insects') conditions. The hypersensitive response is a form of programmed cell death in response to injury and infection and is an integral part of resistance in plants (Lam, 2001). Three regualtors of HR were

examined: *myb*, alternative oxidase (AOX), and BAX inhibitor-1 (*BI-1*) (Lam 2001; Raffaele et al., 2008; Wang et al., 2012).

In *Arabidopsis thaliana*, the *myb* transcription factor, *AtMYB*30, acts as a positive regulator of the hypersensitive response and when overexpressed is associated with increased resistance of the plant to bacterial pathogens (Vailleau et al., 2002; Raffaele et al., 2008). Accumulation of reactive oxygen species is associated with initiation of cell death in HR (Delledonne et al., 2001) and, in plants, may be regulated by AOX, an inner-mitochondrial membrane protein that has been implicated in cell death attenuation during HR (Lam et al., 2001). Chivasa and Carr (1998) found that inhibition of AOX pathway in tobacco leaves infected with tobacco mosaic virus (TMV) resulted in larger lesions, compared with infected leaves not treated with an AOX inhibitor. Reduced lesion size is associated with overexpression of AOX but does not negatively impact disease resistance (Chivas and Carr, 1998; Ordog et al., 2002). BAX inhibitor-1 is also an important regulator of the hypersensitive response, in particular a negative regulator of cell death, but its effects on disease resistance varies with the plant/pathogen in question. In wheat, *TaBI-1* reduced BAX-initiated cell death in plants infected with stripe rust (*Puccinia striiformis*), and knockdown of *TaBI-1* increased susceptibility to the disease (Wang et al., 2012). Over expression of *BI-1* in barely infected with powdery mildew (*Blumeria graminis* f.sp. *hordei*) induced susceptibility to the fungus (Babaeizad et al., 2009), but increased resistance to *Fusarium graminearum*.

Notable differences were apparent in each of the four genotypes in expression of *myb* transcription factor, AOX and *BI-1*. Significant transcripts functionally annotated as

myb transcription factor were largely upregulated in response to herbivory in TAM07V-45, GH18-3, and GH20-1 (Figures 4.5-4.7). Only one transcript, Gorai.009G051000, was down regulated in TAM07V-45 (Figure 4.5) and GH18-3 (Figure 4.6); all transcripts were upregulated in GH20-1 (Figure 4.7). Line GH15-2 had relatively fewer significantly regulated transcripts annotated as *myb* transcription factor, and a larger portion of the transcripts were down regulated in response to herbivory (Figure 4.8).

Expression profiles for functional annotations, AOX and *BI-1*, were similar for all genotypes, with two notable exceptions. Transcript, Gorai.012G142200, annotated for AOX, was significantly upregulated in GH18-3 (Figure 4.9) and GH20-1 (Figure 4.10) in plants fed on by CFH. Little change was noted in the expression of this transcript between 'Insects' and 'No Insects' conditions for TAM07V-45 (Figure 4.11) and GH15-2 (Figure 4.12). Likewise, Gorai.004G077400, annotated for *BI-1*, was significantly upregulated in plants fed on by CFH for GH18-3 (Figure 4.13) and GH20-1 (Figure 4.14), but not TAM07V-45 (Figure 4.15) and GH15-2 (Figure 4.16).

Alternative oxidase is known to reduce the production of reactive oxygen species (ROS) by the mitochondria in plants (Maxwell et al., 1999). Accumulation of ROS, like NO, triggers cell death in HR ((Delledonne et al., 2001). Upregulation of AOX in response to herbivory by CFH, known to transmit bacterial pathogens during feeding, could attenuate HR. Hypothetically, controlling the size of the lesion produced by HR could be the determining factor in whether the fed-on square is abscised or retained. In other words, how much of the square tissue is killed in the process of containing the vectored pathogen affects the viability of the square as a developing reproductive structure and thus affects its retention by the plant. Similarly, *BI-1* regulates cell death by inhibiting BAX-induced apoptosis (Babaeizad et al., 2009; Wang et al., 2012). Again, the extent of cell death resulting from HR could distinguish susceptible plants, those that shed squares, from resistant plants, those that retain squares.

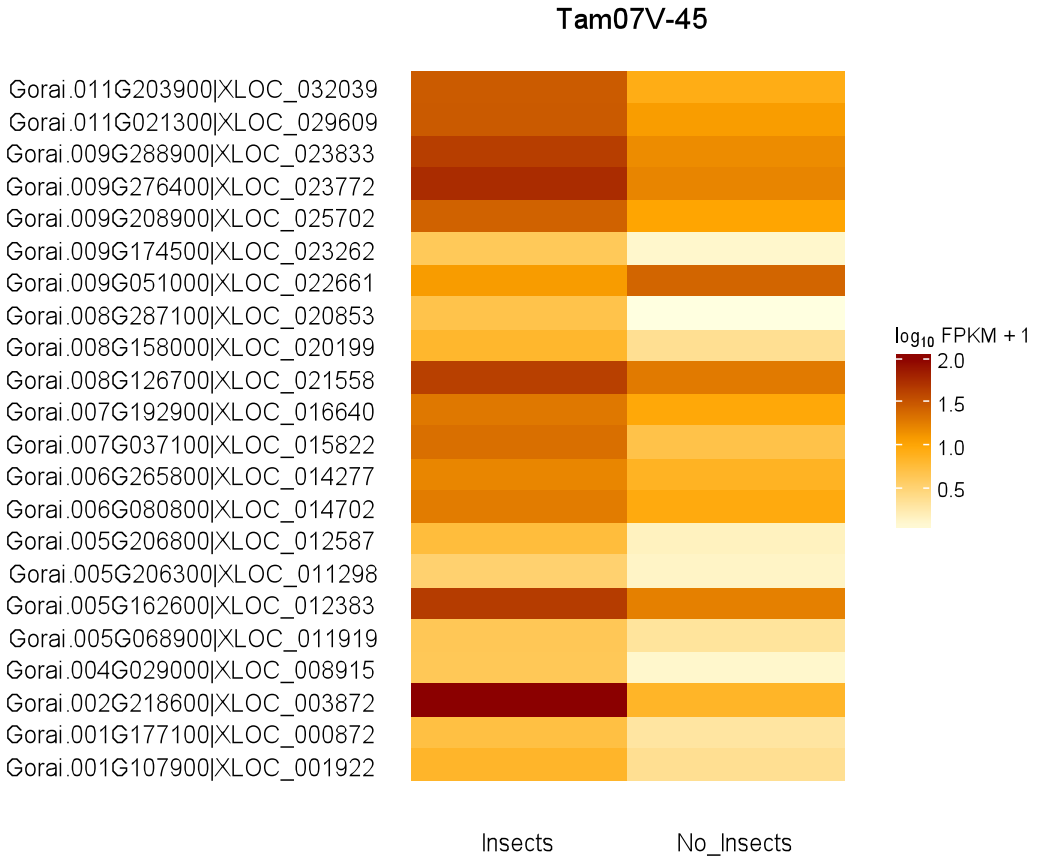

Figure 4.5. Expression of genes involved in the control of *myb* transcription factor for TAM07V-45

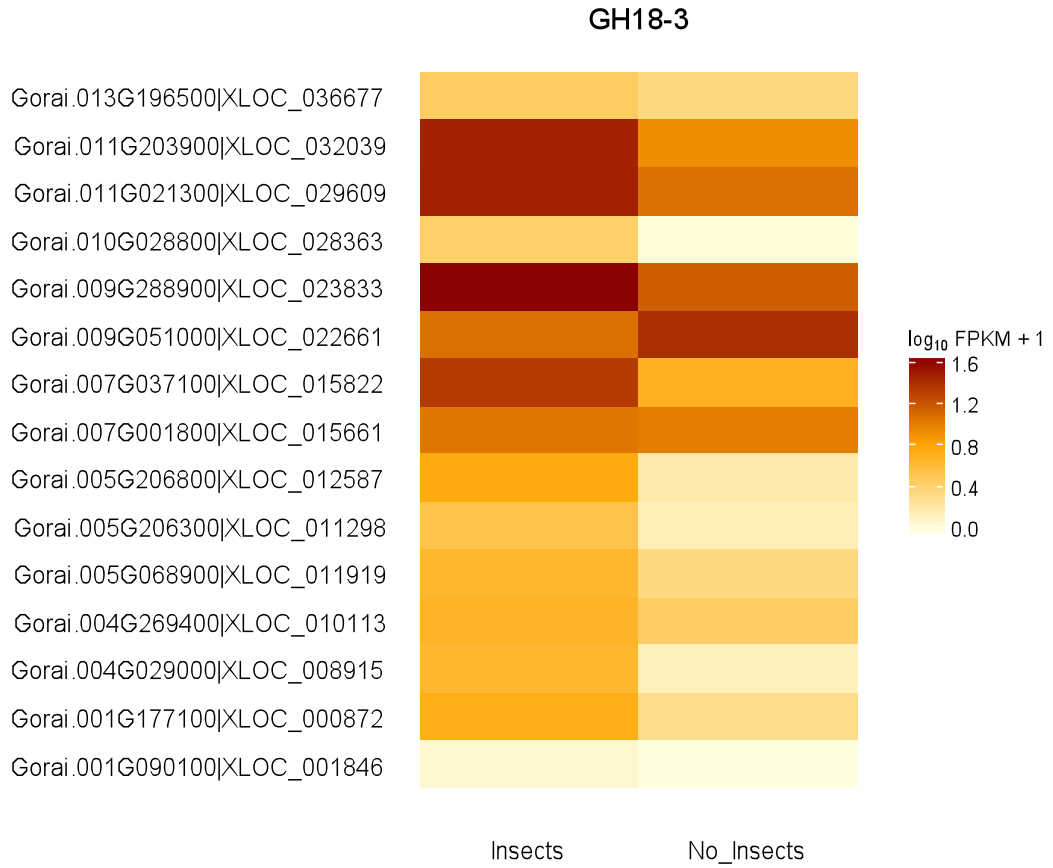

Figure 4.6. Expression of genes involved in the control of *myb* transcription factor for GH18-3

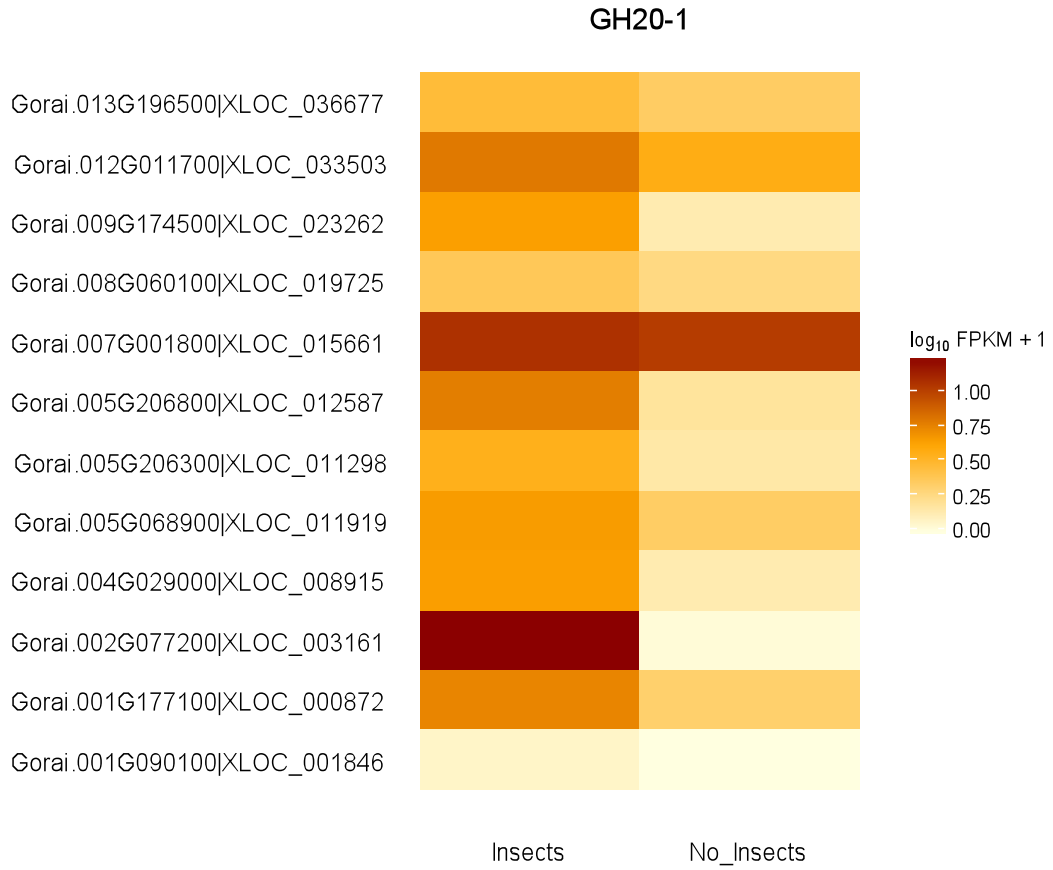

Figure 4.7. Expression of genes involved in the control of *myb* transcription factor for GH20-1

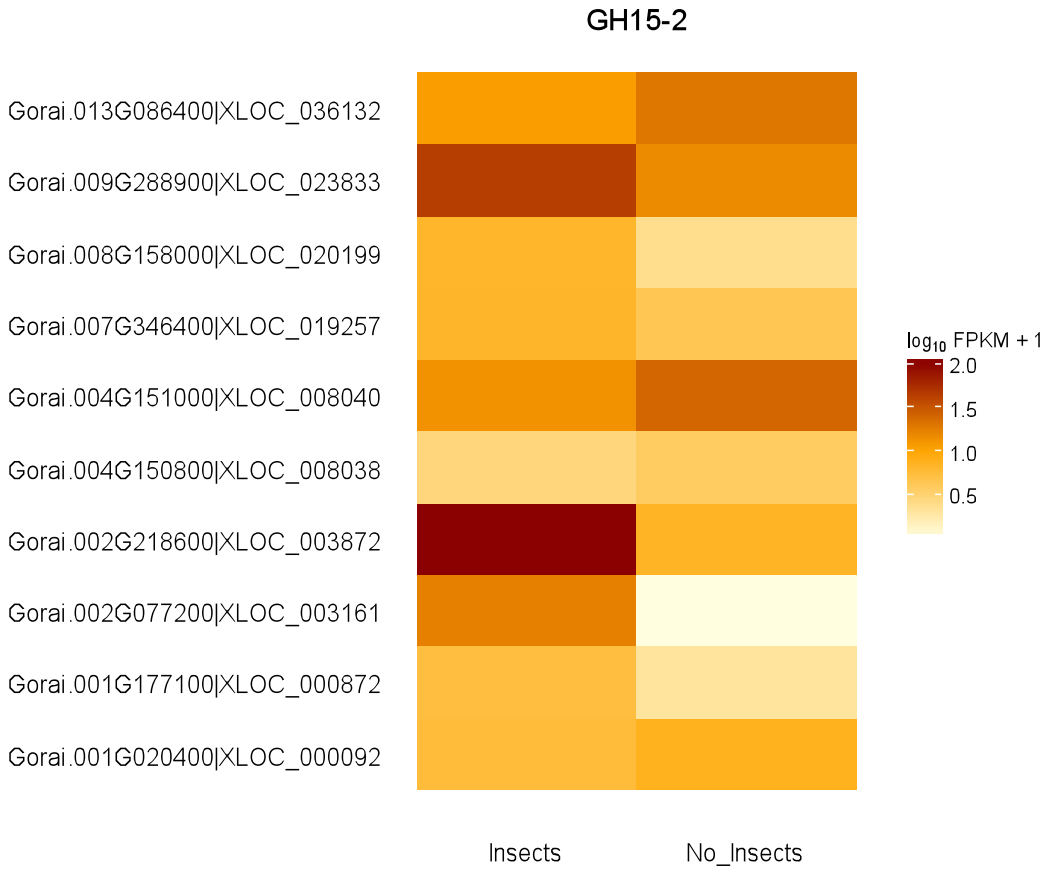

Figure 4.8. Expression of genes involved in the control of *myb* transcription factor for GH15-2

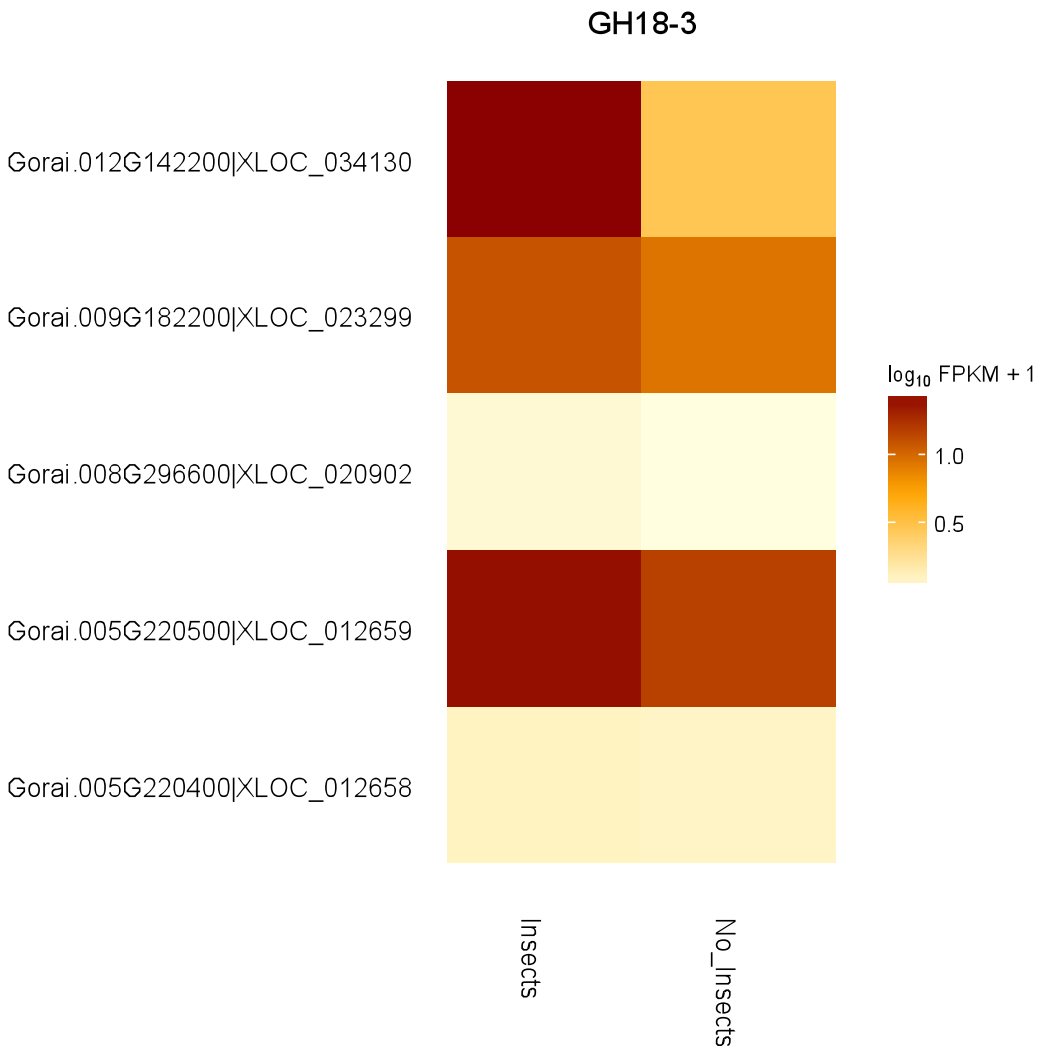

Figure 4.9. Expression of genes involved in the control of alternative oxidase (AOX) for GH18-3

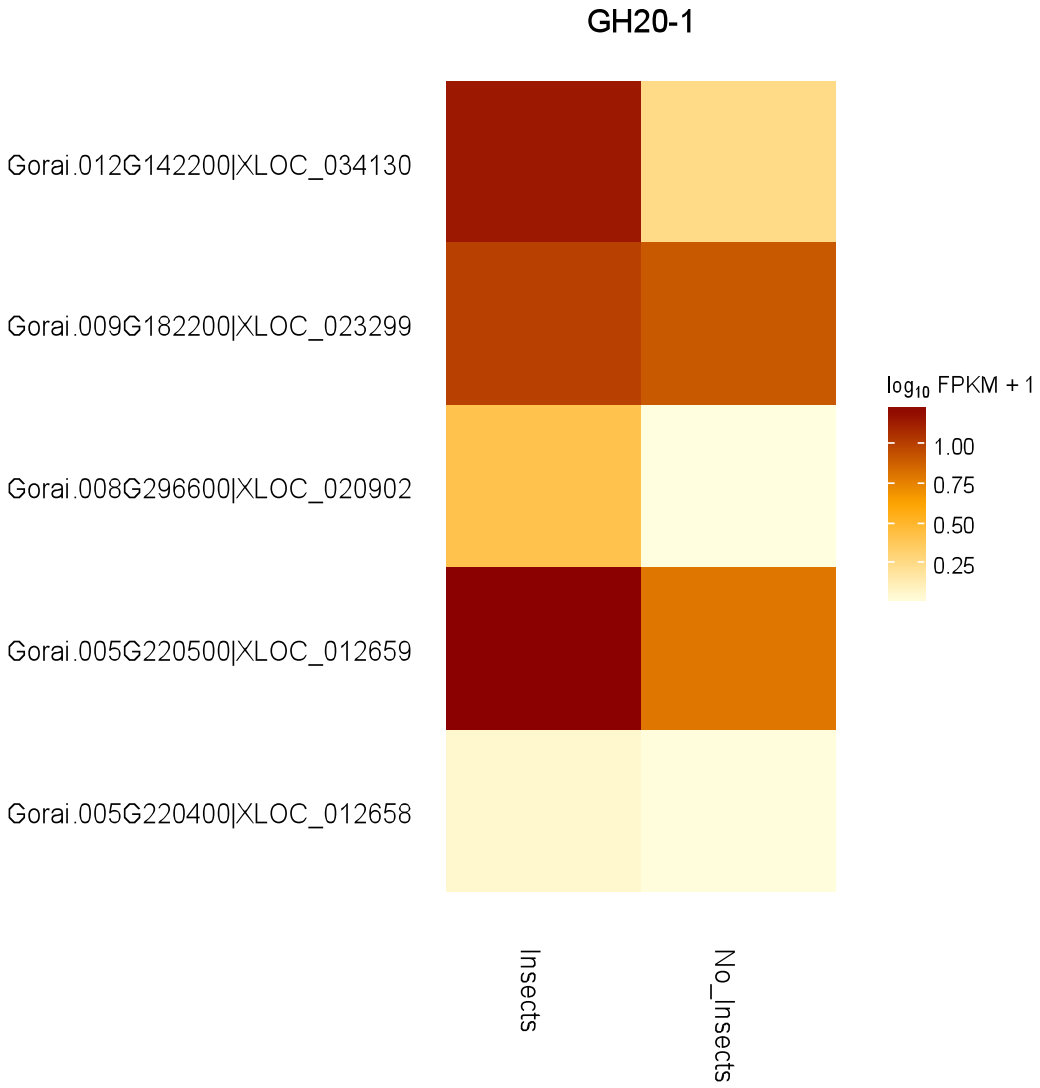

Figure 4.10. Expression of genes involved in the control of alternative oxidase (AOX) for GH20-1

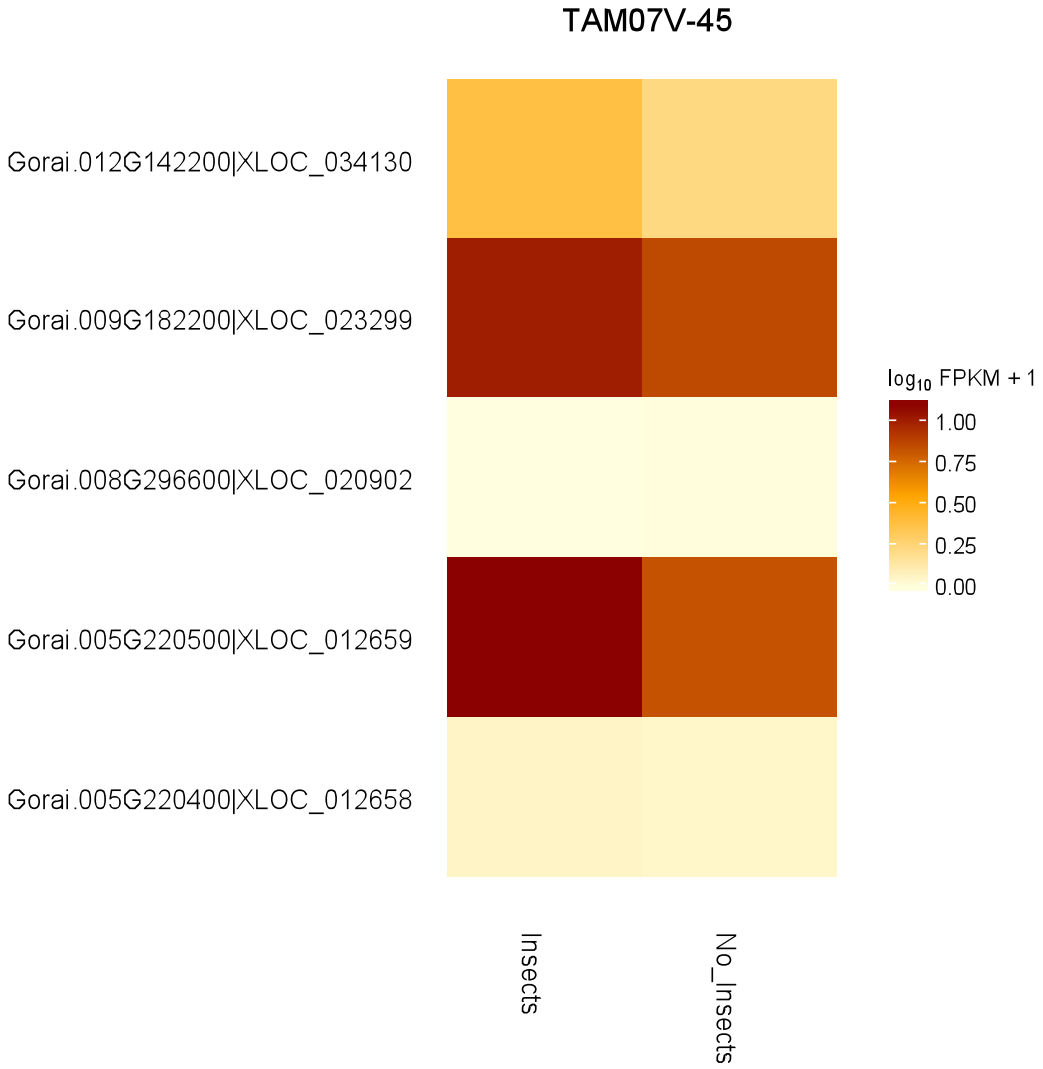

Figure 4.11. Expression of genes involved in the control of alternative oxidase (AOX) for TAM07V-45

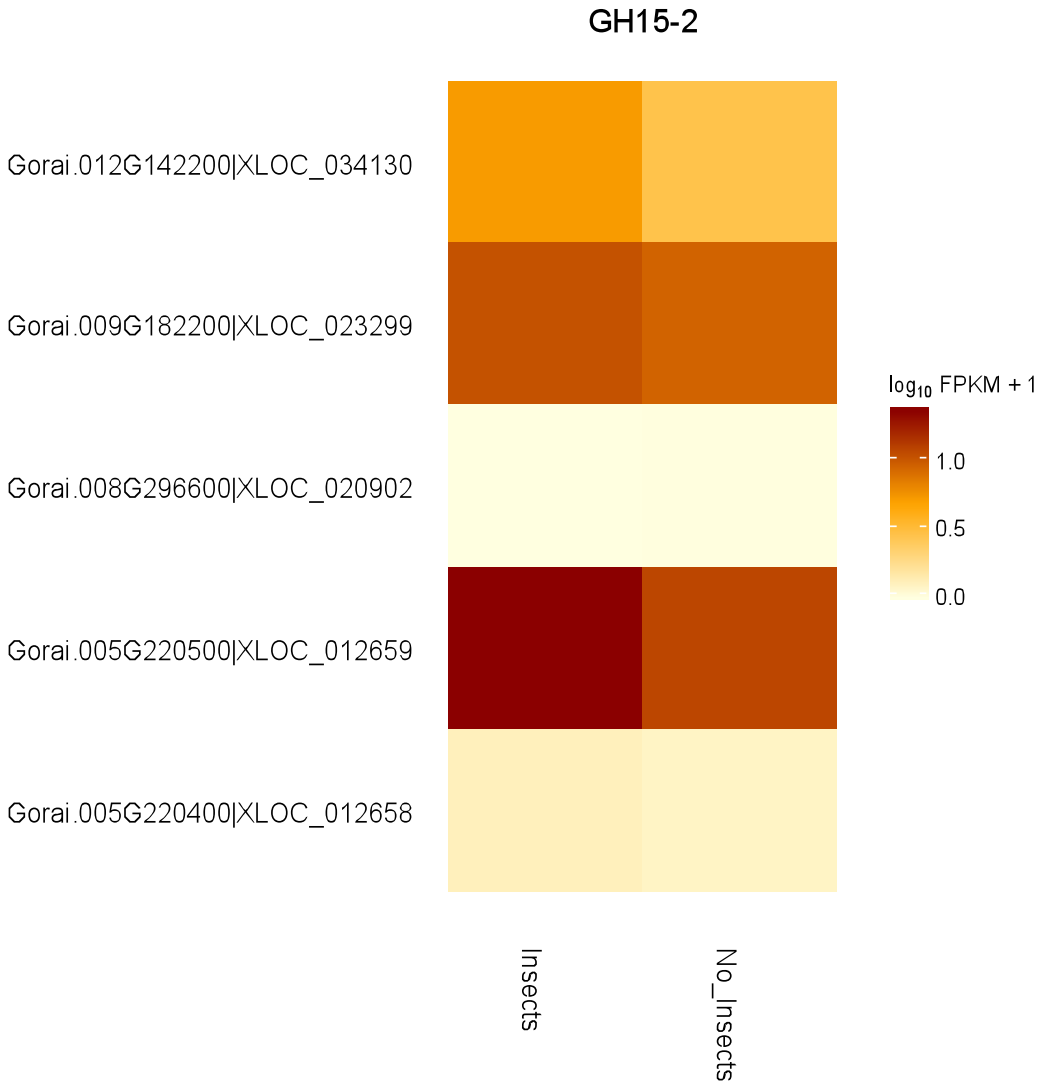

Figure 4.12. Expression of genes involved in the control of alternative oxidase (AOX) for GH15-2

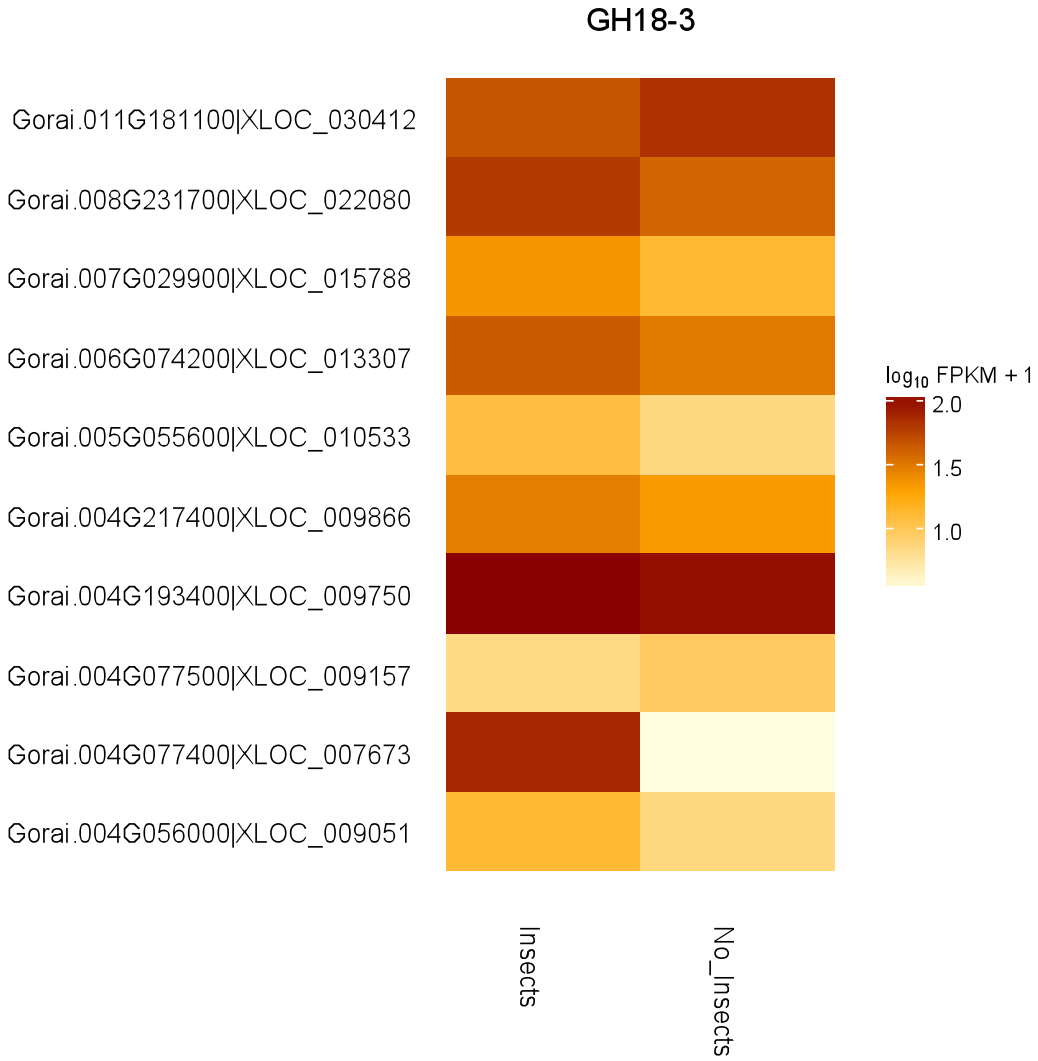

Figure 4.13. Expression of genes involved in the control of BAX inhibitor-1 (*BI-1*) for GH18-3

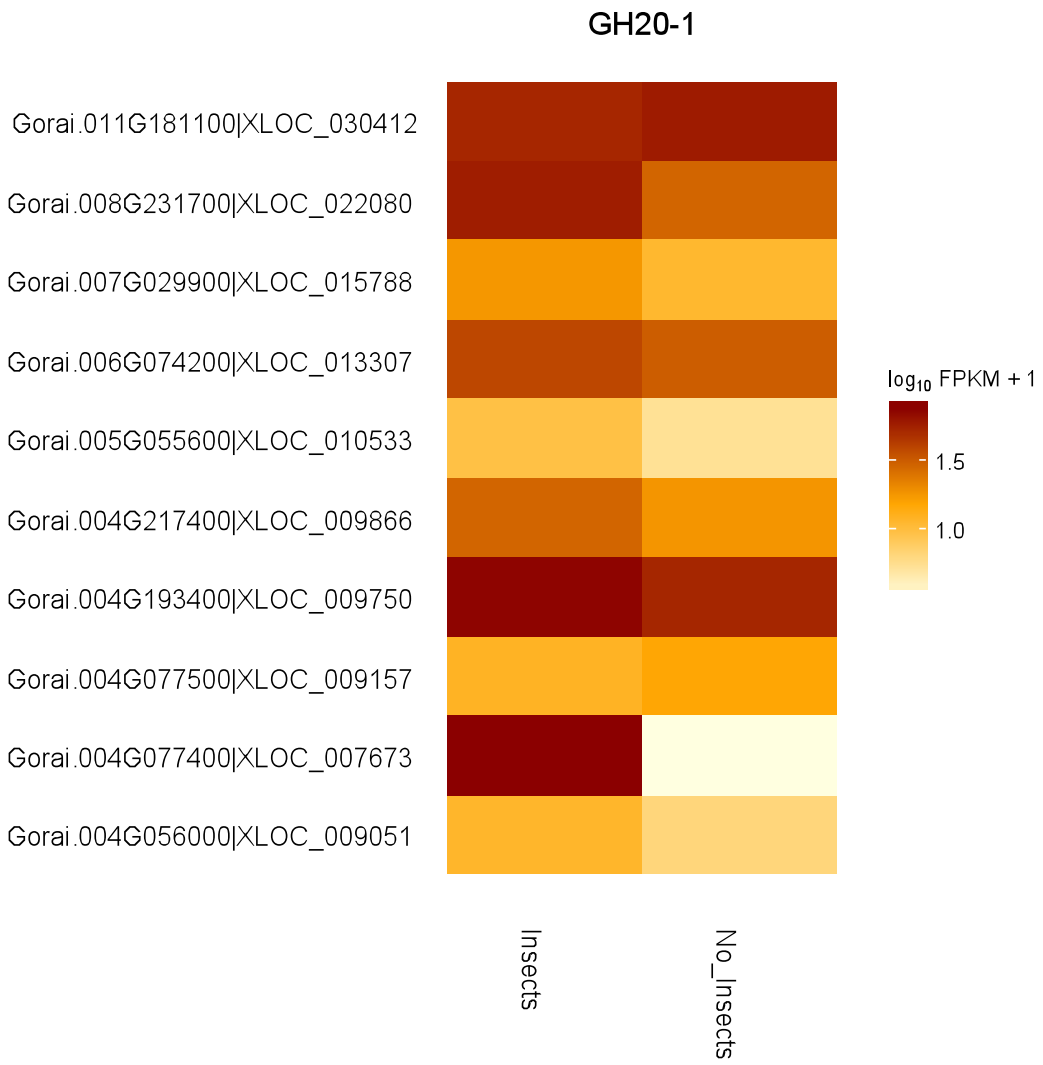

Figure 4.14. Expression of genes involved in the control of BAX inhibitor-1 (*BI-1*) for GH20-1

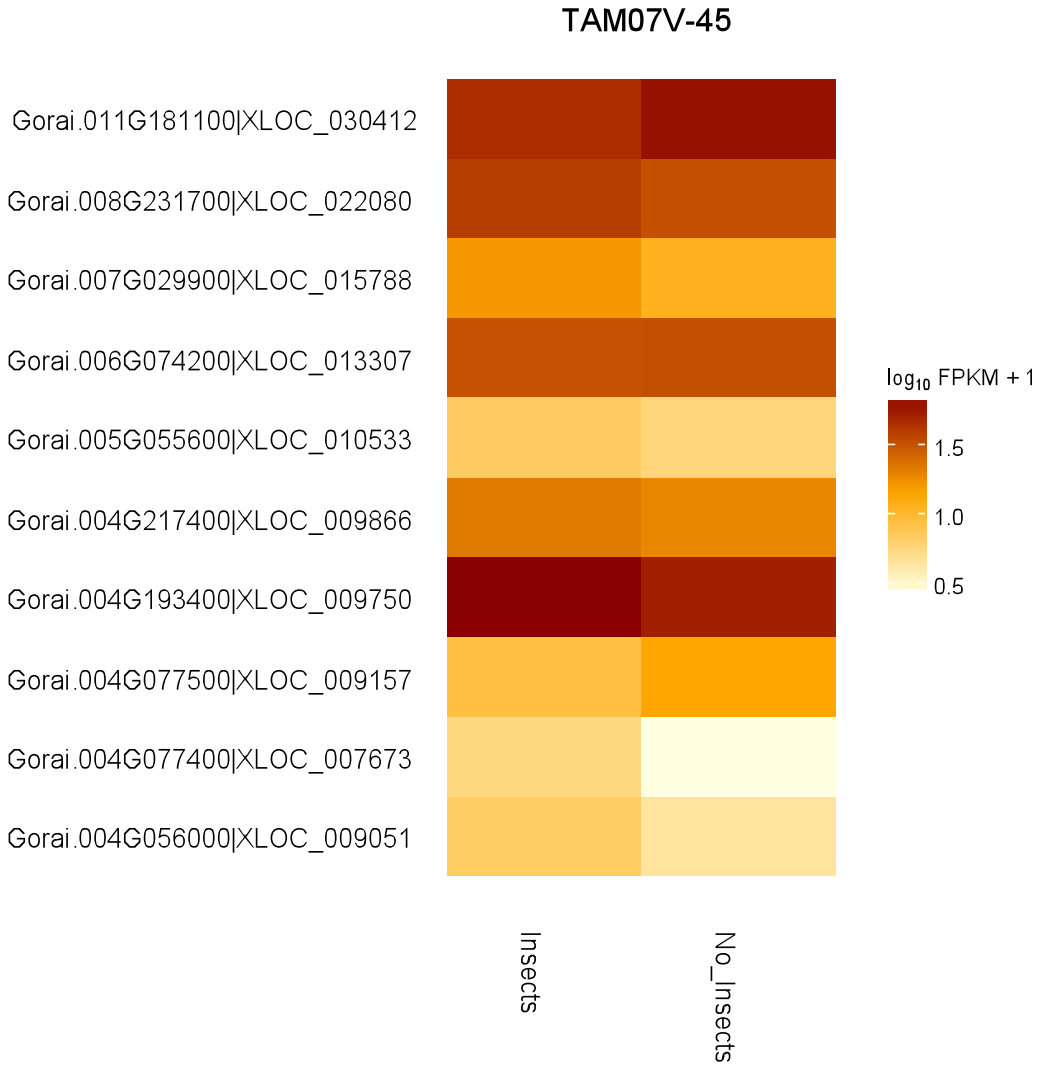

Figure 4.15. Expression of genes involved in the control of BAX inhibitor-1 (*BI-1*) for TAM07V-45

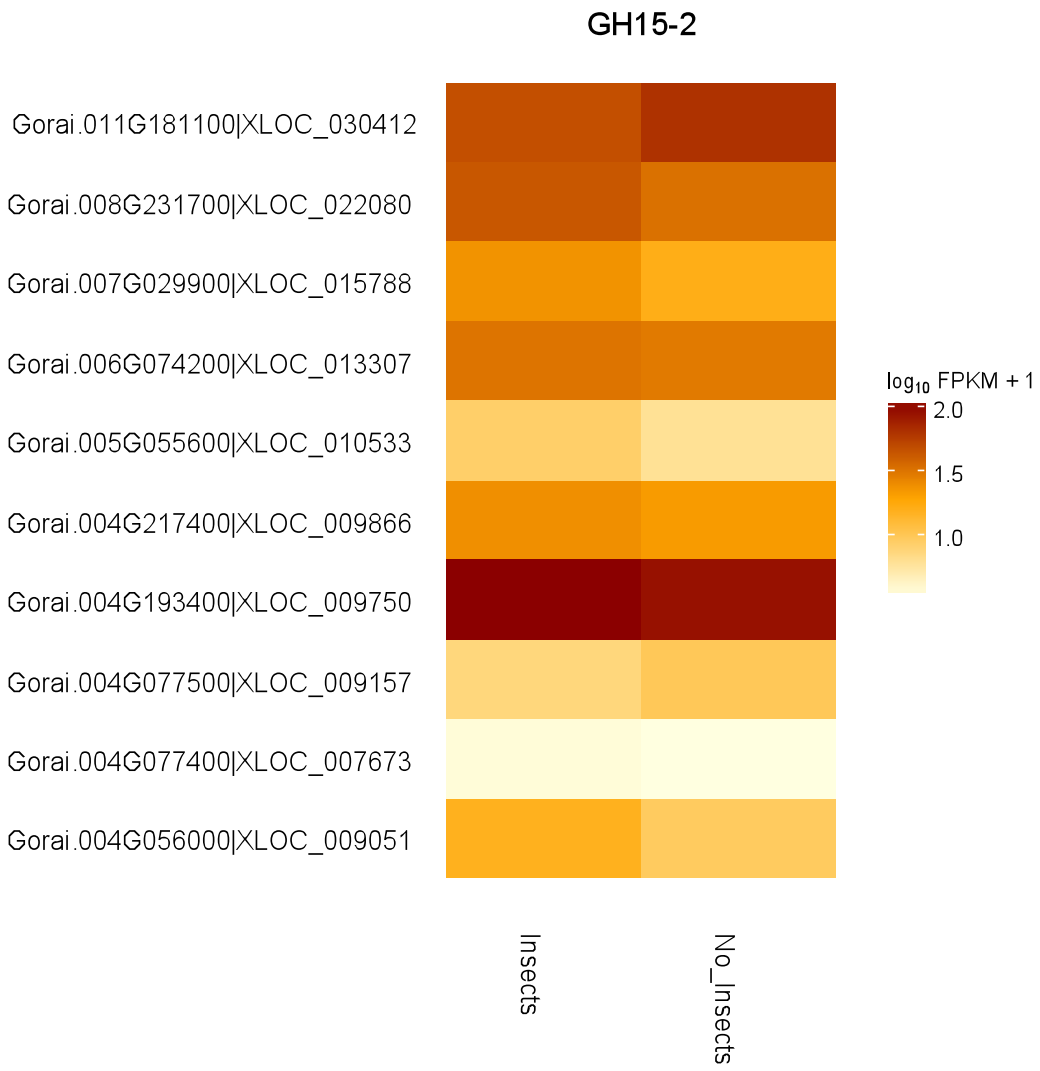

Figure 4.16. Expression of genes involved in the control of BAX inhibitor-1 (*BI-1*) for GH15-2

Conclusions

Susceptibility to CFH feeding is characterized by blasting, or abscission, of squares fed on by the insects. Pectinases in the saliva of CFH digest pectins and have been implicated as a contributing factor to formation of the lesion characterisitic of CFH feeding (Miles, 1972; Martin et al., 1988). Additionally, Bell et al. (2006; 2007) reported transmission of the bacterial pathogen, *Pantoea ananatis*, during CFH feeding and necrosis of ovary tissue in abscised squares. The role of plant immunity is likely very important in mediating the interaction between insect, host, and insect-vectored pathogen. Both susceptible and resistant genotypes included in this study showed strong upregulation of defense genes in response to herbivory by CFH. Notable among these were chitinases and protease inhibitors. Chitinases are generally upregulated in plants in response to infection by bacterial and fungal pathogens. Protease inhibitors can deter insect feeding or impact insect herbivore development through inhibition of digestive enzymes in the insect's saliva and midgut (Green and Ryan, 1972; Koiwa et al., 1997; Lopes et al., 2004). Terpenoid biosynthesis was significantly upregulated in GH18-3 and GH15-2. Terpenoid secondary metabolites, like gossypol, negatively impact insect herbivore fitness (Shaver and Parrott, 1970; Tingey 1975) and may have contributed to lower CFH preference for GH18-3 and GH15-2 in the no-choice study (please see Chapter III for review), compared with TAM07V-45, for which terpenoid biosynthesis was not strongly upregulated in response to herbivory.

Data did not indicate failure of the susceptible genotype (TAM07V-45) to mount defenses in response to herbivory by the CFH. This result was surprising but suggested an alternative explanation for performance differences noted in the field under CFH infestation (please see Chapter II for review). We hypothesized that differences in the regulation of the immune response, particularly the hypersensitive response (HR), play a key role in distinguishing susceptible and resistant genotypes. The hypersensitive response is a form of programmed cell death in response to injury and infection (Lam, 2001). Because CFH vector a pathogen during feeding and because necrosis of the fed-upon tissue is characteristic of feeding damage, it is possible that HR plays a role in the extent of the injury resulting from feeding and infection and thus influences whether squares fed on by CFH are retained or abscised.

Our analysis primarily focused on three regulators of HR: *myb* transcription factor, alternative oxidase (AOX), and BAX inhibitor-1 (*BI-1*). Differences in the regulation of all three HR factors were noted in the susceptible (TAM07V-45) and resistant genotypes (GH18-3, GH15-2, GH20-1) in response to herbivory. These differences suggest that positive control of HR is strongly upregulated in all lines, except GH15-2, in response to herbivory, but that the HR response is more tightly controlled in GH18-3 and GH20-1 than in TAM07V-45, possibly restricting the size of the lesion resulting from programmed cell death.

Few transcripts associated with *myb* transcription factor, a positive regulator of HR, were significantly upregualted in GH15-2 in response to herbivory. It is possible that infection is localized by a different mechanism in GH15-2. Another explanation

may lie in the inability of the CFH to penetrate the developing ovary in squares of GH15-2. Morphological data presented in Chapter III of this dissertation indicated that ovary depth is significantly different among the four genotypes used in this study. The ovary in GH15-2 lies deeper within the square and is presumably protected from damage during CFH feeding. Bell et al. (2007) reported that square abscission due to CFH feeding primarily results from infection of the developing ovary tissue with pathogens vectored by CFH. Perhaps because the CFH may not be able to penetrate the ovary when feeding on GH15-2, a strong immune response, like the hypersensitive response is not elicited in this genotype in respone to herbivory.

In summary, RNA-seq transcriptome profiling of one susceptible (TAM07V-45) and three resistant (GH18-3, GH20-1, GH15-2) cotton lines under CFH feeding pressure indicated that regulation of immune response may differentiate susceptible and resistant plants. All four cotton lines significantly upregulated transcripts associated with stress in response to herbivory, but differed in expression of transcripts associated with regulators of the hypersensitive response and HR-associated lesion formation.

CHAPTER V

SUMMARY AND CONCLUSIONS

The collective goals of the projects reviewed in this dissertation were to identify and characterize host plant resistance to cotton fleahopper (CFH) in the available germplasm of upland cotton. Three strategies were employed in this investigation: field screening and introgression breeding, CFH behavior and square structure analysis, and RNA-seq profiling of plant transcritpomes in response to CFH herbivory. Following is a review of the major findings of each project presented in this dissertation.

Chapter II—Identification of Resistance to Cotton Fleahopper

This project was designed to accomplish two objectives: (1) evaluate resistance to CFH feeding in eight parental lines and (2) to introgress resistance traits into high-yielding lines through backcrossing and evaluate the resistance of the progeny lines. In regards to the first objective, field evaluations over three years at College Station and Corpus Christi indicated that pilose, or densely pubescent, lines have a high resistance to CFH feeding, compared to lines with smooth or normal phenotype. However, when the CFH population approached or exceeded economic threshold levels, the pilose lines exhibited damage near or exceeding economic threshold. These data indicate that resistance can be overwhelmed by high CFH populations, but at lower populations, insecticide treatment is not necessary to maintain low levels of injury.

Data collected in 2014 indicated a difference in performance of the cotton lines, which may be indicative of a difference in preference between the College Station CFH and the Corpus Christi CFH, measured by feeding injury; lines that showed little feeding

injury at College Station showed greater feeding injury at Corpus Christi, and vice versa. Barman et al, in 2012, reported that CFH at College Station and Corpus Christi are genetically distinct as a result of host associated differentiation. Our data showed a potential difference in host preference between the two fleahopper genotypes.

The second objective of this study was to introgress resistance traits into TAM07V-45 and TAM06WE-14 (recurrent parents) using lines that showed resistance to CFH feeding in 2012 and 2013 as donor parents. Among the backcross progeny, 12525 exhibited lower injury levels than either of its parents (TAM06WE-14 and GH18-3), and resistance comparable to that of the other donor parent lines, when CFH populations were highest in 2014. Line 12525 is a normal/hairy line, indicating that resistance not linked to the pilose trait was introgressed into this line. Data also indicated parent-dependent resistance in the backcross progeny across locations, perhaps again indicating difference in host plant preference between the distinct CFH genotypes in these locations.

Chapter III—Characterization of Resistance to Cotton Fleahopper

Data from behavioral assays and an examination of the relationship of ovary depth and CFH proboscis penetrance indicated that multiple host plant resistance mechanisms may be at play in the relationship between CFH and the upland cotton lines used in this study. Analysis of fleahopper behavior, particularly behaviors relating to host acceptace, revealed differences in preference among the 19 lines tested. Cotton fleahoppers spent significantly more time feeding on some genotypes, compared to others. Morphological analysis of squares from each of the 19 lines indicated significant

differences in the depth of the developing ovary. Presumably, based on estimations of maximum proboscis penetration, the placement of the ovary affects the ability of a feeding fleahopper to penetrate the developing organ with its proboscis. This finding has two important implications: 1) deeper ovaries are likely protected from direct infection with pathogens vectored during CFH feeding, as well as digestive enzymes in the saliva of CFH and 2) the inability of the CFH to penetrate the ovary during feeding may impact preference to feed or duration of feeding.

Chapter IV—RNA-seq Trancriptome Profiling

Both susceptible (TAM07V-45) and resistant genotypes (GH18-3, GH20-1, GH15-2) included in this study showed strong upregulation of defense genes in response to herbivory by CFH. Notable among these were chitinases and protease inhibitors. Terpenoid biosynthesis was significantly upregulated in GH18-3 and GH15-2. Terpenoid secondary metabolites, like gossypol, negatively impact insect herbivore fitness (Shaver and Parrott, 1970; Tingey 1975) and may have contributed to lower CFH preference for GH18-3 and GH15-2 in the no-choice study (Chapter III), compared with TAM07V-45, for which terpenoid biosynthesis was not strongly upregulated in response to herbivory.

Our analysis primarily focused on three regulators of the hypersensitive response (HR): *myb* transcription factor, alternative oxidase (AOX), and BAX inhibitor-1 (*BI-1*). Differences in the regulation of all three HR factors were noted in the susceptible (TAM07V-45) and resistant genotypes (GH18-3, GH15-2, GH20-1). These differences suggest that positive control of HR is strongly upregulated in all lines, except GH15-2,

in response to herbivory, but that the HR response is more tightly controlled in GH18-3 and GH20-1, possibly restricting the size of the lesion resulting from programmed cell death.

Future Directions

Through these studies, many new questions were generated. For breeding, there are several important questions to consider for future research. What drives CFH preference for one genotype over another? How does maturity of the plant affect susceptibility? What are the exact pathways for processes that influence immune responses associated with resistance and can markers be developed for genes controlling these pathways? Additionally, continued efforts should be made to identify alternative sources of host plant resistance to CFH in the available germplasm to aid in breeding for resistance to this economically important pest of Texas cotton.

Many questions were also answered. Cotton fleahopper preference differences were noted among genotypes and among field trial testing locations. Location-dependent preference could strongly affect breeding programs by necessitating development of cotton lines that are regionally specific. Morphological analysis of developing squares and ovary depth provided data to support a previously published study suggesting susceptibility to CFH feeding damage is related to square size (Knutson et al., 2013). Finally, transcriptome analysis showed evidence for the importance of several immunity-related pathways that could be exploited to increase resistance to CFH feeding damage. Together, the studies conducted for this dissertation revealed a complex relationship between upland cotton and the cotton fleahopper.

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APPENDIX A

Table A.1. Top 30 significantly upregulated genes (log fold change in transcript number) in GH18-3 in response to herbivory by cotton fleahopper

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.001G176800	Gorai.001G176800.1	ethylene-responsive transcription factor 1b-like	1.46E-67	80.75%	regulation of transcription, DNA-templated; transcription, DNA-templated	DNA binding; sequence-specific DNA binding transcription factor activity	nucleus
Gorai.002G203400	Gorai.002G203400.1	cytokinin riboside 5 - monophosphate phosphoribohydrolase log5-like	7.70E-153	92.60%	metabolic process	lyase activity	cytosol
Gorai.002G203600	Gorai.002G203600.1	class i chitinase	0	87.85%	defense response; cell wall macromolecule catabolic process; polysaccharide catabolic process; carbohydrate metabolic process; metabolic process; chitin catabolic process	chitinase activity; hydrolase activity; chitin binding; hydrolase activity, acting on glycosyl bonds	vacuole
Gorai.002G234600	Gorai.002G234600.4	(+)-delta-cadinene synthase	0	90.05%	metabolic process; terpenoid biosynthetic process	metal ion binding; terpene synthase activity; magnesium ion binding; lyase activity; (+)-delta-cadinene synthase activity	
	Gorai.002G234600.1	(+)-delta-cadinene synthase	0	90.20%	metabolic process; terpenoid biosynthetic process	metal ion binding; terpene synthase activity; magnesium ion binding; lyase activity; (+)-delta-cadinene synthase activity	
	Gorai.002G234600.2	(+)-delta-cadinene synthase	0	90.15%	metabolic process; terpenoid biosynthetic process	metal ion binding; terpene synthase activity; magnesium ion binding; lyase activity; (+)-delta-cadinene synthase activity	
	Gorai.002G234600.3	(+)-delta-cadinene synthase	0	92.55%	metabolic process; terpenoid biosynthetic process	metal ion binding; terpene synthase activity; magnesium ion binding; lyase activity; (+)-delta-cadinene synthase activity	
Gorai.003G183000	Gorai.003G183000.2	platz transcription factor family protein isoform 1	2.09E-100	92.95%	-	-	-
	Gorai.003G183000.1	platz transcription factor family protein isoform 1	1.54E-140	87.40%	-	-	-

Table A.1 Continued

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.003G183500	Gorai.003G183500.1	like cupins superfamily protein	0	66.20%	-	nutrient reservoir activity	-
	Gorai.003G183500.2	glutelin type-a 3-like	9.28E-149	65.10%	-	nutrient reservoir activity	-
	Gorai.003G183500.3	glutelin type-a 3-like	1.14E-139	65.35%	-	nutrient reservoir activity	-
Gorai.004G081800	Gorai.004G081800.1	basic 7s globulin 2-like	0	82.85%	proteolysis; response to salt stress	aspartic-type endopeptidase activity	plasmodesma; plant-type cell wall; membrane; Golgi apparatus; cell wall; plasma membrane
Gorai.004G123100	Gorai.004G123100.1	homeobox-leucine zipper protein athb-7	2.07E-103	73.05%	regulation of transcription, DNA-templated; transcription, DNA-templated	sequence-specific DNA binding; transcription regulatory region sequence-specific DNA binding; sequence-specific DNA binding transcription factor activity; DNA binding	nucleus
Gorai.007G079900	Gorai.007G079900.2	nac12 l-protein	2.30E-175	94.35%	regulation of transcription, DNA-templated	DNA binding	
	Gorai.007G079900.1	nac12 l-protein	0	94.85%	regulation of transcription, DNA-templated	DNA binding	
Gorai.007G145800	Gorai.007G145800.1	ethylene-responsive transcription factor 1b-like	6.19E-135	76.55%	regulation of transcription, DNA-templated; transcription, DNA-templated	sequence-specific DNA binding transcription factor activity; DNA binding	nucleus
Gorai.007G170100	Gorai.007G170100.1	1-aminocyclopropane-1-carboxylate oxidase	0	90.95%	oxidation-reduction process; response to fungus; cellular response to fatty acid	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors; oxidoreductase activity; iron ion binding	
Gorai.007G267900	Gorai.007G267900.2	nac transcription factor 29-like	0	79.20%	regulation of transcription, DNA-templated	DNA binding	
	Gorai.007G267900.1	nac transcription factor 29-like	0	73.45%	regulation of transcription, DNA-templated	DNA binding	

Table A.1 Continued

Gene ID	Splice Variant	Seq. Description	Min. eValue	Mean Similarity	Gene Ontology		
					Biological Process	Molecular Function	Cellular Component
Gorai.008G010600	Gorai.008G010600.1	desiccation-related protein pcc13-62-like	5.14E-76	55.95%	-	-	-
	Gorai.008G014700.2	nac domain-containing protein 68	4.10E-110	82.90%	regulation of transcription, DNA-templated; regulation of programmed cell death; xylem development; shoot system development	DNA binding	
Gorai.008G014700	Gorai.008G014700.3	nac domain-containing protein 68	3.98E-89	89.60%	regulation of transcription, DNA-templated; regulation of programmed cell death; xylem development; shoot system development	DNA binding	
	Gorai.008G014700.1	nac domain-containing protein 68	1.68E-112	84.00%	regulation of transcription, DNA-templated; regulation of programmed cell death; xylem development; shoot system development	DNA binding	
Gorai.008G065900	Gorai.008G065900.1	salicylate o-methyltransferase	0	86.10%	methylation; metabolic process	methyltransferase activity; transferase activity; jasmonate O-methyltransferase activity	
Gorai.008G203000	Gorai.008G203000.1	flavonol sulfotransferase-like	3.20E-76	53.20%	metabolic process	transferase activity; sulfotransferase activity; alcohol sulfotransferase activity; estrone sulfotransferase activity	
Gorai.008G245000	Gorai.008G245000.1	osmotin 34	4.28E-148	89.70%	defense response to fungus, incompatible interaction; response to salt stress		
	Gorai.008G276700.2	par1 protein	1.13E-126	85.10%	-	-	-
	Gorai.008G276700.3	par1 protein	4.60E-127	83.70%	-	-	-
Gorai.008G276700	Gorai.008G276700.1	par1 protein	2.94E-127	83.00%	-	-	-

Table A.1 Continued

Gene ID	Splice Variant	Seq. Description	Min. eValue	Mean Similarity	Gene Ontology		
					Biological Process	Molecular Function	Cellular Component
Gorai.009G124000	Gorai.009G124000.1	probable wrky transcription factor 40	0	80.30%	regulation of transcription, DNA-templated; response to molecule of bacterial origin; response to wounding; regulation of defense response to virus by host; defense response to fungus; defense response to bacterium; response to chitin; response to salicylic acid; regulation of defense response	sequence-specific DNA binding transcription factor activity; sequence-specific DNA binding	
Gorai.009G211600	Gorai.009G211600.2	alpha amylase family protein	0	90.20%	fatty acid beta-oxidation; seed germination; glyoxylate metabolic process; carbohydrate metabolic process; metabolic process	cation binding; hydrolase activity; alpha-amylase activity; catalytic activity; hydrolase activity, acting on glycosyl bonds; calcium ion binding	peroxisome
	Gorai.009G211600.1	alpha amylase family protein	0	88.25%	metabolic process; carbohydrate metabolic process; fatty acid beta-oxidation; seed germination; glyoxylate metabolic process	calcium ion binding; hydrolase activity; cation binding; hydrolase activity, acting on glycosyl bonds; catalytic activity; alpha-amylase activity	peroxisome
Gorai.009G223000	Gorai.009G223000.3	branched-chain-amino-acid aminotransferase	0	84.10%	branched-chain amino acid metabolic process; metabolic process	transferase activity; transaminase activity; branched-chain-amino-acid transaminase activity; catalytic activity;L-isoleucine transaminase activity; L-valine transaminase activity; L-leucine transaminase activity	
	Gorai.009G223000.1	branched-chain-amino-acid aminotransferase chloroplastic-like isoform x1	0	84.65%	branched-chain amino acid metabolic process; metabolic process	transferase activity; transaminase activity; branched-chain-amino-acid transaminase activity; catalytic activity; L-isoleucine transaminase activity; L-valine transaminase activity; L-leucine transaminase activity	

Table A.1 Continued

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.009G223000	Gorai.009G223000.2	branched-chain-amino-acid aminotransferase chloroplastic-like isoform x1	0	85.15%	branched-chain amino acid metabolic process; metabolic process	transferase activity; transaminase activity; branched-chain-amino-acid transaminase activity; catalytic activity; L-isoleucine transaminase activity; L-valine transaminase activity; L-leucine transaminase activity	
Gorai.010G131500	Gorai.010G131500.1	ethylene-responsive transcription factor abr1-like isoform x1	1.25E-92	67.95%	regulation of transcription, DNA-templated; transcription, DNA-templated; positive regulation of transcription, DNA-templated; regulation of timing of transition from vegetative to reproductive phase	sequence-specific DNA binding transcription factor activity; DNA binding;	C:nucleus;
	Gorai.010G131500.2	ethylene-responsive transcription factor abr1-like isoform x2	3.90E-79	68.25%	regulation of transcription, DNA-templated; transcription, DNA-templated; regulation of timing of transition from vegetative to reproductive phase;P:positive regulation of transcription, DNA-templated;	sequence-specific DNA binding transcription factor activity; DNA binding;	nucleus
Gorai.011G254400	Gorai.011G254400.1	trypsin inhibitor	5.42E-84	78.00%	negative regulation of endopeptidase activity	endopeptidase inhibitor activity	
Gorai.011G254500	Gorai.011G254500.1	trypsin inhibitor	1.99E-73	75.65%	negative regulation of endopeptidase activity	endopeptidase inhibitor activity	
Gorai.011G254700	Gorai.011G254700.1	trypsin inhibitor	1.95E-79	69.60%	negative regulation of endopeptidase activity	endopeptidase inhibitor activity	
Gorai.011G254800	Gorai.011G254800.1	trypsin inhibitor	2.92E-77	68.95%	negative regulation of endopeptidase activity	endopeptidase inhibitor activity	
Gorai.012G016200	Gorai.012G016200.2	peroxidase 4-like	0	91.65%	response to oxidative stress; oxidation-reduction process	metal ion binding; heme binding; oxidoreductase activity; peroxidase activity	apoplast; Golgi apparatus; cell wall; cytosol; extracellular region
	Gorai.012G016200.1	peroxidase 4-like	0	89.35%	oxidation-reduction process; response to oxidative stress	metal ion binding; heme binding; oxidoreductase activity; peroxidase activity	extracellular region; apoplast; Golgi apparatus; cell wall; cytosol
	Gorai.012G016200.3	peroxidase 4-like	0	90.90%	oxidation-reduction process; response to oxidative stress	metal ion binding; heme binding; oxidoreductase activity; peroxidase activity	extracellular region; apoplast; Golgi apparatus; cell wall; cytosol

Table A.1 Continued

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.012G081000	Gorai.012G081000.1	cytokinin dehydrogenase 6-like	0	87.80%	oxidation-reduction process; cytokinin metabolic process	oxidoreductase activity; UDP-N-acetylmuramate dehydrogenase activity; catalytic activity; cytokinin dehydrogenase activity; oxidoreductase activity, acting on CH-OH group of donors; flavin adenine dinucleotide binding; primary amine oxidase activity	endoplasmic reticulum lumen
Gorai.013G190700	Gorai.013G190700.1	tyrosine decarboxylase 1-like	0	83.90%	cellular amino acid metabolic process; carboxylic acid metabolic process	catalytic activity; carboxy-lyase activity; pyridoxal phosphate binding; lyase activity	
Gorai.013G208700	Gorai.013G208700.1	wat1-related protein at1g09380	0	85.90%	membrane		

Table A.2. Top 30 significantly down regulated genes (log fold change in transcript number) in GH18-3 in response to herbivory by cotton fleahopper

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.001G211300	Gorai.001G211300.1	gdsl esterase lipase at2g42990-like	1.02E-166	78.00%	lipid metabolic process;	hydrolase activity, acting on ester bonds; hydrolase activity	
Gorai.001G225300	Gorai.001G225300.1	portal 56	0	81.30%	-		
	Gorai.001G225300.2	portal 56	0	90.15%	-		
Gorai.002G019900	Gorai.002G019900.1	targeting protein for isoform 1	0	77.35%	mitotic nuclear division;		spindle; microtubule
	Gorai.002G019900.2	targeting protein for isoform 1	0	77.70%	mitotic nuclear division;		spindle; microtubule
Gorai.002G105300	Gorai.002G105300.1	Uncharacterized protein TCM_032572	2.33E-104	88.90%			plasmodesma
Gorai.002G112300	Gorai.002G112300.1	cyclin-dependent kinase	0	94.60%	protein phosphorylation; F:RNA polymerase II carboxy-terminal domain kinase activity; phosphorylation; protein autophosphorylation; histone phosphorylation; hormone-mediated signaling pathway; regulation of G2/M transition of mitotic cell cycle; regulation of meristem structural organization	ATP binding; protein kinase activity; transferase activity; nucleotide binding; transferase activity, transferring phosphorus-containing groups; protein serine/threonine kinase activity; kinase activity;	cyclin-dependent protein kinase holoenzyme complex;
	Gorai.002G112300.2	cyclin-dependent kinase	3.36E-167	88.40%	protein phosphorylation; phosphorylation	ATP binding; protein kinase activity; transferase activity; nucleotide binding; RNA polymerase II carboxy-terminal domain kinase activity; transferase activity, transferring phosphorus-containing groups; protein serine/threonine kinase activity; kinase activity;	
	Gorai.002G112300.3	cyclin-dependent kinase	6.61E-175	80.50%	protein phosphorylation; phosphorylation; protein autophosphorylation; histone phosphorylation; hormone-mediated signaling pathway; regulation of G2/M transition of mitotic cell cycle; regulation of meristem structural organization	ATP binding; protein kinase activity; transferase activity; nucleotide binding; RNA polymerase II carboxy-terminal domain kinase activity; transferase activity, transferring phosphorus-containing groups; protein serine/threonine kinase activity; kinase activity;	cyclin-dependent protein kinase holoenzyme complex;

Table A.2 Continued

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.002G112300	Gorai.002G112300.4	cyclin-dependent kinase	9.82E-130	94.10%	protein phosphorylation; phosphorylation	ATP binding; protein kinase activity; transferase activity; nucleotide binding; RNA polymerase II carboxy-terminal domain kinase activity; transferase activity, transferring phosphorus-containing groups; protein serine/threonine kinase activity; kinase activity;	
Gorai.002G224300	Gorai.002G224300.1	Uncharacterized protein TCM_024909	4.02E-67	67.80%	-		
Gorai.003G003000	Gorai.003G003000.1	g2 mitotic-specific cyclin-1-like	0	81.15%	regulation of cell cycle; cell cycle; cell division; regulation of cyclin-dependent protein serine/threonine kinase activity;	protein kinase binding;	nucleus
	Gorai.003G003000.2	g2 mitotic-specific cyclin-1-like	0	81.40%	regulation of cell cycle; cell cycle; cell division; regulation of cyclin-dependent protein serine/threonine kinase activity;	protein kinase binding;	nucleus
Gorai.003G061500	Gorai.003G061500.1	protein iq-domain 14-like	0	76.80%	-		
	Gorai.003G061500.2	protein iq-domain 14-like	0	76.80%	-		
Gorai.003G171400	Gorai.003G171400.1	probable pectinesterase 68	0	89.30%	cell wall modification; metabolic process;	aspartyl esterase activity; pectinesterase activity; hydrolase activity;	cell wall;
	Gorai.003G171400.2	probable pectinesterase 68	2.33E-170	91.95%	cell wall modification; metabolic process;	aspartyl esterase activity; pectinesterase activity; hydrolase activity;	cell wall;
Gorai.004G156500	Gorai.004G156500.1	vacuolar protein 8	0	90.40%	metabolic process	ligase activity;	
Gorai.004G259400	Gorai.004G259400.1	low quality protein: dentin sialophosphoprotein	3.82E-145	57.40%	-		
	Gorai.004G259400.2	low quality protein: dentin sialophosphoprotein	1.15E-146	57.40%	-		
	Gorai.004G259400.3	low quality protein: dentin sialophosphoprotein	1.82E-144	56.90%	-		
	Gorai.004G259400.4	low quality protein: dentin sialophosphoprotein	6.94E-108	52.20%	-		

Table A.2 Continued

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.005G008800	Gorai.005G008800.1	Uncharacterized protein TCM_019849	4.08E-80	77.05%	-		
Gorai.006G011300	Gorai.006G011300.1	tpx2 protein	3.60E-171	74.25%	mitotic nuclear division;		spindle; microtubule
Gorai.006G065100	Gorai.006G065100.1	65-kda microtubule-associated protein 3	0	84.80%	cytokinesis; microtubule cytoskeleton organization; cytokinesis by cell plate formation; microtubule polymerization; formation by symbiont of syncytium involving giant cell for nutrient acquisition from host; nuclear division; response to nematode;	microtubule binding;	preprophase band; cortical microtubule
	Gorai.006G065100.2	65-kda microtubule-associated protein 3	0	84.80%	cytokinesis; microtubule cytoskeleton organization; cytokinesis by cell plate formation; microtubule polymerization; formation by symbiont of syncytium involving giant cell for nutrient acquisition from host; nuclear division; response to nematode;	microtubule binding;	preprophase band; cortical microtubule
Gorai.006G232300	Gorai.006G232300.1	Uncharacterized protein TCM_019849	4.93E-108	79.10%	-		
Gorai.007G082600	Gorai.007G082600.1	circumsporozoite protein	4.42E-173	60.35%	transport		integral component of membrane; membrane;
Gorai.007G082700	Gorai.007G082700.1	hydroxyproline-rich glycoprotein family protein	3.79E-51	76.85%	cytokinesis by cell plate formation; response to cyclopentenone; microtubule cytoskeleton organization;	molecular_function;	nucleus
	Gorai.007G082700.2	hydroxyproline-rich glycoprotein family protein	6.07E-41	76.30%	-		
Gorai.007G172600	Gorai.007G172600.1	uncharacterized loc101221004	1.52E-104	67.70%	-		
Gorai.007G192600	Gorai.007G192600.1	tetratricopeptide repeat-like superfamily protein isoform 1	0	84.90%	-		
Gorai.007G192700	Gorai.007G192700.1	tetratricopeptide repeat-like superfamily protein isoform 1	0	84.70%	-		

Table A.2 Continued

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.007G351700	Gorai.007G351700.1	cyclin family isoform partial	0	77.20%	regulation of cell growth; regulation of cell cycle;cell division; regulation of cyclin-dependent protein serine/threonine kinase activity; cell cycle; response to gamma radiation	protein kinase binding;	nucleus; cytoplasm;
	Gorai.007G351700.2	cyclin family isoform partial	0	83.65%	regulation of cell growth; regulation of cell cycle;cell division; regulation of cyclin-dependent protein serine/threonine kinase activity; cell cycle; response to gamma radiation	protein kinase binding;	nucleus; cytoplasm;
Gorai.008G093800	Gorai.008G093800.1	di-glucose binding protein with kinesin motor domain isoform 1	0	85.15%	metabolic process; microtubule-based movement;	ATP binding;nucleotide binding; microtubule motor activity; microtubule binding;	kinesin complex;microtubule
Gorai.008G287300	Gorai.008G287300.1	protein endosperm defective 1-like	0	77.20%	-		
Gorai.009G248300	Gorai.009G248300.1	cyclin-a1-1-like	0	80.90%	regulation of cell cycle; cell cycle; cell division; regulation of cyclin-dependent protein serine/threonine kinase activity; regulation of meiotic cell cycle; microsporogenesis; male meiosis; meiosis II	protein kinase binding;	nucleus;cytoplasm;
Gorai.009G423900	Gorai.009G423900.1	125 kda kinesin-related protein	0	84.35%	metabolic process; microtubule-based movement;	ATP binding; nucleotide binding; microtubule motor activity; microtubule binding;	kinesin complex; microtubule
	Gorai.009G423900.2	125 kda kinesin-related protein	0	84.30%	metabolic process; microtubule-based movement;	ATP binding; nucleotide binding; microtubule motor activity; microtubule binding;	kinesin complex; microtubule
Gorai.009G454200	Gorai.009G454200.1	rna polymerase ii elongation factor ell3 isoform 1	1.10E-124	83.55%	-		
	Gorai.009G454200.2	rna polymerase ii elongation factor ell3 isoform 1	1.10E-124	83.55%	-		
Gorai.011G070600	Gorai.011G070600.1	syntaxin-124 protein	0	57.95%	-		
Gorai.011G151700	Gorai.011G151700.1	sigma non-opioid intracellular receptor 1	0	73.55%	lipid transport		
	Gorai.011G151700.2	sigma non-opioid intracellular receptor 1	2.79E-148	75.60%	lipid transport		

Table A.2 Continued

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.013G118200	Gorai.013G118200.1	syntaxin-related protein knolle	0	90.35%	intracellular protein transport; vesicle-mediated transport;	SNAP receptor activity;	plasmodesma; cell plate; membrane; phragmoplast; endomembrane system; plasma membrane
Gorai.013G118200	Gorai.013G118200.2	syntaxin-related protein knolle	0	90.00%	intracellular protein transport; vesicle-mediated transport;	SNAP receptor activity;	plasmodesma; cell plate; membrane; phragmoplast; endomembrane system; plasma membrane
Gorai.013G250600	Gorai.013G250600.1	golgin subfamily a member	0	69.50%	-		
	Gorai.013G250600.2	golgin subfamily a member	0	67.85%	-		

Table A.3. Top 30 significantly upregulated genes (log fold change in transcript number) in GH20-1in response to herbivory by cotton fleahopper

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.001G060900	Gorai.001G060900.1	malate glyoxysomal	0	93.05%	tricarboxylic acid cycle; glyoxylate cycle;	transferase activity; catalytic activity; malate synthase activity; transferase activity, transferring acyl groups	peroxisome; glyoxysome;
Gorai.002G077200	Gorai.002G077200.1	transcription factor myb39	6.94E-112	78.00%	response to abscisic acid; response to ethylene; response to salt stress; response to osmotic stress; response to wounding; response to jasmonic acid	chromatin binding; DNA binding;	
Gorai.002G219600	Gorai.002G219600.1	heavy metal-associated isoprenylated plant protein 26-like	4.87E-96	91.70%	metal ion transport	metal ion binding;	
Gorai.002G219600	Gorai.002G219600.2	heavy metal-associated isoprenylated plant protein 26-like	4.87E-96	91.70%	metal ion transport	metal ion binding;	
Gorai.003G117600	Gorai.003G117600.1	nfu1 iron-sulfur mitochondrial	2.44E-105	73.15%	-		
Gorai.003G160400	Gorai.003G160400.1	protein p21-like	3.92E-125	87.00%	-		
Gorai.005G063300	Gorai.005G063300.1	major allergen pru ar 1	4.32E-111	75.25%	response to biotic stimulus; defense response		
Gorai.005G172200	Gorai.005G172200.1	b12d protein	5.36E-58	93.20%	-		
Gorai.006G105200	Gorai.006G105200.1	asparagine synthetase	0	94.60%	L-asparagine biosynthetic process; asparagine biosynthetic process; metabolic process; cellular amino acid biosynthetic process; cellular response to sucrose starvation; response to sucrose; response to glucose; response to fructose	ATP binding; ligase activity; nucleotide binding;asparagine synthase (glutamine-hydrolyzing) activity;	
	Gorai.006G105200.3	asparagine synthetase	0	96.50%	L-asparagine biosynthetic process; asparagine biosynthetic process; metabolic process; cellular amino acid biosynthetic process; cellular response to sucrose starvation; response to sucrose; response to glucose; response to fructose	ATP binding; ligase activity; nucleotide binding; asparagine synthase (glutamine-hydrolyzing) activity;	

	Gorai.006G105200.4	asparagine synthetase	0	96.75%	L-asparagine biosynthetic process; asparagine biosynthetic process; metabolic process; cellular amino acid biosynthetic process; cellular response to sucrose starvation; response to sucrose; response to glucose; response to fructose	ATP binding; ligase activity; nucleotide binding; asparagine synthase (glutamine-hydrolyzing) activity;	

Table A.3 Continued

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.006G105200	Gorai.006G105200.2	asparagine synthetase	0	94.50%	L-asparagine biosynthetic process; asparagine biosynthetic process; metabolic process; cellular amino acid biosynthetic process; cellular response to sucrose starvation; response to sucrose; response to glucose; response to fructose	ATP binding; ligase activity; nucleotide binding; asparagine synthase (glutamine-hydrolyzing) activity;	
Gorai.006G130400	Gorai.006G130400.1	protein yls9	4.35E-82	71.30%	salicylic acid mediated signaling pathway; defense response, incompatible interaction		
Gorai.007G079900	Gorai.007G079900.2	nac12 l-protein	2.30E-175	94.35%	regulation of transcription, DNA-templated;	DNA binding	
	Gorai.007G079900.1	nac12 l-protein	0	94.85%	regulation of transcription, DNA-templated;	DNA binding	
Gorai.007G101800	Gorai.007G101800.2	ferric reduction oxidase 2	0	78.85%	oxidation-reduction process;	oxidoreductase activity; NAD(P)H oxidase activity	integral component of membrane; membrane;
	Gorai.007G101800.1	ferric reduction oxidase 2	0	75.85%	oxidation-reduction process;	oxidoreductase activity; NAD(P)H oxidase activity	integral component of membrane; membrane;
Gorai.007G126200	Gorai.007G126200.1	protein exordium-like 2	2.03E-161	71.00%	-		
Gorai.007G170100	Gorai.007G170100.1	1-aminocyclopropane-1-carboxylate oxidase	0	90.95%	oxidation-reduction process; response to fungus; cellular response to fatty acid	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors; oxidoreductase activity; iron ion binding;	

Gorai.008G020700	Gorai.008G020700.1	zinc finger protein zat10	6.53E-124	62.30%	regulation of root development; hyperosmotic salinity response; response to abscisic acid; negative regulation of transcription, DNA-templated; embryo development ending in seed dormancy; response to water deprivation; phosphate ion homeostasis; response to chitin; photoprotection; response to oxidative stress; response to wounding; multicellular organism growth; photosynthesis; response to cold; response to salt stress; response to high light intensity	metal ion binding; sequence-specific DNA binding;	

Table A.3 Continued

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.008G143800	Gorai.008G143800.1	probable pectinesterase pectinesterase inhibitor 7	0	87.25%	pectin catabolic process; cell wall modification;negative regulation of catalytic activity; response to brassinosteroid; response to cold	hydrolase activity; aspartyl esterase activity;enzyme inhibitor activity; pectinesterase activity;	cell wall;
	Gorai.008G143800.2	probable pectinesterase pectinesterase inhibitor 7	0	80.10%	pectin catabolic process; cell wall modification;negative regulation of catalytic activity; response to brassinosteroid; response to cold	hydrolase activity; aspartyl esterase activity;enzyme inhibitor activity; pectinesterase activity;	cell wall;
Gorai.008G187700	Gorai.008G187700.1	protein ida	7.13E-31	71.50%	metabolic process	NAD+ ADP-ribosyltransferase activity;	
Gorai.008G245000	Gorai.008G245000.1	osmotin 34	4.28E-148	89.70%	defense response to fungus, incompatible interaction; response to salt stress		
Gorai.009G223000	Gorai.009G223000.3	branched-chain-amino-acid aminotransferase	0	84.10%	branched-chain amino acid metabolic process; metabolic process;	transferase activity; transaminase activity; branched-chain-amino-acid transaminase activity; catalytic activity; L-isoleucine transaminase activity; L-valine transaminase activity; L-leucine transaminase activity	
Gorai.009G223000	Gorai.009G223000.1	branched-chain-amino-acid aminotransferase chloroplastic-like isoform x1	0	84.65%	branched-chain amino acid metabolic process; metabolic process;	transferase activity; transaminase activity; branched-chain-amino-acid transaminase activity; catalytic activity; L-isoleucine transaminase activity; L-valine transaminase activity; L-leucine transaminase activity	

Gorai.009G223000	Gorai.009G223000.2	branched-chain-amino-acid aminotransferase chloroplastic-like isoform x1	0	85.15%	branched-chain amino acid metabolic process; metabolic process;	transferase activity; transaminase activity; branched-chain-amino-acid transaminase activity; catalytic activity; L-isoleucine transaminase activity; L-valine transaminase activity; L-leucine transaminase activity

Table A.3 Continued

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.009G272400	Gorai.009G272400.1	dna polymerase epsilon catalytic subunit	1.24E-94	67.75%	-		
Gorai.009G277800	Gorai.009G277800.2	branched-chain-amino-acid aminotransferase chloroplastic-like isoform x1	0	87.90%	branched-chain amino acid metabolic process; metabolic process;	transferase activity; transaminase activity; branched-chain-amino-acid transaminase activity; catalytic activity; L-isoleucine transaminase activity; L-valine transaminase activity; L-leucine transaminase activity	
Gorai.009G277800	Gorai.009G277800.1	branched-chain-amino-acid aminotransferase chloroplastic-like isoform x1	0	82.95%	branched-chain amino acid metabolic process; metabolic process;	transferase activity; transaminase activity; branched-chain-amino-acid transaminase activity; catalytic activity; L-isoleucine transaminase activity; L-valine transaminase activity; L-leucine transaminase activity	
Gorai.009G298600	Gorai.009G298600.1	mic-3	1.62E-55	54.60%	-		
Gorai.009G429700	Gorai.009G429700.1	vacuolar sorting-associated protein 62	0	82.70%	-		
Gorai.010G089100	Gorai.010G089100.1	protein lurp-one-related 6-like	1.86E-146	86.50%	-		
Gorai.010G168800	Gorai.010G168800.1	protein phloem protein 2-like a9-like	8.58E-99	67.35%	-		

Gorai.011G075500	Gorai.011G075500.1	serine threonine-protein kinase	0	66.55%	phosphorylation;protein phosphorylation;recognition of pollen	transferase activity; protein serine/threonine kinase activity; protein kinase activity; nucleotide binding; ATP binding; kinase activity; transferase activity, transferring phosphorus-containing groups;	
Gorai.011G082000	Gorai.011G082000.1	eg45-like domain containing protein 2	6.00E-82	83.75%	alternative respiration		cell wall; apoplast;
Gorai.011G168100	Gorai.011G168100.1	arm repeat superfamily protein	0	85.70%	-		

Table A.3 Continued

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.011G231300	Gorai.011G231300.1	orcinol o-methyltransferase	0	81.10%	methylation;	methyltransferase activity; transferase activity; protein dimerization activity; O-methyltransferase activity; catechol O-methyltransferase activity	
Gorai.012G081000	Gorai.012G081000.1	cytokinin dehydrogenase 6-like	0	87.80%	oxidation-reduction process; cytokinin metabolic process;	oxidoreductase activity; UDP-N-acetylmuramate dehydrogenase activity; catalytic activity; cytokinin dehydrogenase activity; oxidoreductase activity, acting on CH-OH group of donors; flavin adenine dinucleotide binding; primary amine oxidase activity;	endoplasmic reticulum lumen
Gorai.013G218200	Gorai.013G218200.1	potassium channel kat3	0	84.50%	potassium ion transport; ion transport; transport; high-affinity potassium ion import; response to nematode; transmembrane transport; potassium ion transmembrane transport; ion transmembrane transport	ion channel activity; voltage-gated potassium channel activity;	integral component of membrane; membrane; endoplasmic reticulum; plasma membrane;

Table A.4. Significantly down regulated genes (log fold change in transcript number) in GH20-1in response to herbivory by cotton fleahopper

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.007G052900	Gorai.007G052900.3	l-ascorbate oxidase homolog	0	89.60%	P:oxidation-reduction process; P:response to karrikin	F:oxidoreductase activity; F:copper ion binding; F:L-ascorbate oxidase activity;	C:cell wall; C:plant-type cell wall;
	Gorai.007G052900.2	l-ascorbate oxidase homolog	0	89.60%	P:oxidation-reduction process; P:response to karrikin	F:oxidoreductase activity; F:copper ion binding; F:L-ascorbate oxidase activity;	C:cell wall; C:plant-type cell wall;
Gorai.006G197200	Gorai.006G197200.1	ethylene-responsive transcription factor erf027-like	2.01E-122	74.75%	P:regulation of transcription, DNA-templated; P:transcription, DNA-templated; P:defense response to fungus	F:sequence-specific DNA binding transcription factor activity; F:DNA binding;	C:nucleus;
Gorai.013G114100	Gorai.013G114100.1	f-box family	0	53.55%	-		
Gorai.007G220200	Gorai.007G220200.1	gibberellin-regulated protein	3.25E-28	87.35%	-		
Gorai.007G052900	Gorai.007G052900.1	l-ascorbate oxidase homolog	0	89.60%	P:oxidation-reduction process; P:response to karrikin	F:oxidoreductase activity; F:copper ion binding; F:L-ascorbate oxidase activity;	C:cell wall; C:plant-type cell wall;
Gorai.002G170000	Gorai.002G170000.1	lipid transfer protein	6.37E-61	84.40%	P:proteolysis;	F:peptidase activity	

Table A.5. Top 30 significantly upregulated genes (log fold change in transcript number) in TAM07V-45 in response to herbivory by cotton fleahopper

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.001G009200	Gorai.001G009200.1	multidrug and toxin extrusion protein 2-like	0	84.95%	drug transmembrane transport	drug transmembrane transporter activity; antiporter activity	integral component of membrane
Gorai.002G077200	Gorai.002G077200.1	transcription factor myb39	6.94E-112	78.00%	response to wounding; response to salt stress; response to ethylene; response to abscisic acid; response to jasmonic acid	DNA binding; chromatin binding	-
Gorai.002G203600	Gorai.002G203600.1	class i chitinase	0	87.85%	polysaccharide catabolic process; chitin catabolic process; defense response; cell wall macromolecule catabolic process	chitinase activity; chitin binding;	vacuole
Gorai.004G081800	Gorai.004G081800.1	basic 7s globulin 2-like	0	82.85%	proteolysis; response to salt stress	aspartic-type endopeptidase activity	Golgi apparatus; plasma membrane; plant-type cell wall; plasmodesma
Gorai.004G186200	Gorai.004G186200.1	seed maturation protein	2.83E-41	73.55%	embryo development	-	-
Gorai.004G192500	Gorai.004G192500.1	expansin-like b1	1.52E-155	83.65%	sexual reproduction	-	extracellular region
Gorai.004G217500	Gorai.004G217500.1	chlorophyllase 1	6.79E-119	61.70%	chlorophyll catabolic process	chlorophyllase activity	plastid
Gorai.005G047100	Gorai.005G047100.1	cellulose synthase like g2	0	77.50%	cellulose biosynthetic process	cellulose synthase (UDP-forming) activity	membrane;
Gorai.005G063300	Gorai.005G063300.1	major allergen pru ar 1	4.32E-111	75.25%	defense response; response to biotic stimulus		-
Gorai.005G150300	Gorai.005G150300.1	isocitrate lyase	0	94.00%	glyoxylate cycle; tricarboxylic acid cycle	isocitrate lyase activity	glyoxysome
Gorai.006G096500	Gorai.006G096500.1	oxidative stress isoform 2	2.17E-37	67.50%	response to cadmium ion; response to oxidative stress	-	nuclear speck;
Gorai.006G159500	Gorai.006G159500.1	embryonic dc-8	0	53.95%	-	-	cell wall; cytoplasm; extracellular region
Gorai.007G110000	Gorai.007G110000.1	lob domain-containing protein 1-like	1.70E-92	76.80%	-	-	-
Gorai.007G126200	Gorai.007G126200.1	protein exordium-like 2	2.03E-161	71.00%	-		-
Gorai.007G267900	Gorai.007G267900.1	nac transcription factor 29-like	0	73.45%	regulation of transcription, DNA-templated	DNA binding	-
	Gorai.007G267900.2	nac transcription factor 29-like	0	79.20%	regulation of transcription, DNA-templated	DNA binding	-
Gorai.008G022700	Gorai.008G022700.1	hypothetical protein JCGZ_15465	2.08E-10	70.55%	-	-	-

Gorai.008G202900	Gorai.008G202900.1	flavonol 4 -	9.96E-135	70.15%	metabolic process	sulfotransferase activity	-

Table A.5 Continued

					Gene Ontology		
Gene ID	**Splice Variant**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.008G258900	Gorai.008G258900.1	homeobox-leucine zipper protein athb-7	8.63E-124	63.90%	response to stimulus; regulation of transcription, DNA-templated;	sequence-specific DNA binding transcription factor activity; sequence-specific DNA binding	nucleus
Gorai.008G277500	Gorai.008G277500.1	low-temperature-induced 65 kda	0	58.85%	-	-	-
Gorai.009G209800	Gorai.009G209800.1	Uncharacterized protein isoform 1	1.08E-16	57.67%	-	-	-
Gorai.010G058900	Gorai.010G058900.1	basic endochitinase-like	0	89.30%	carbohydrate metabolic process; P:chitin catabolic process; P:cell wall macromolecule catabolic process	chitinase activity; chitin binding	-
Gorai.011G254400	Gorai.011G254400.1	trypsin inhibitor	5.42E-84	78.00%	-	endopeptidase inhibitor activity	-
Gorai.011G254500	Gorai.011G254500.1	trypsin inhibitor	1.99E-73	75.65%	-	endopeptidase inhibitor activity	-
Gorai.011G254600	Gorai.011G254600.1	trypsin inhibitor	1.72E-82	77.70%	-	endopeptidase inhibitor activity	-
Gorai.011G254700	Gorai.011G254700.1	trypsin inhibitor	1.95E-79	69.60%	-	endopeptidase inhibitor activity	-
Gorai.011G254800	Gorai.011G254800.1	trypsin inhibitor	2.92E-77	68.95%	-	endopeptidase inhibitor activity	-
Gorai.012G125700	Gorai.012G125700.1	ap2 domain-containing transcription factor family protein	6.44E-94	63.75%	regulation of transcription, DNA-templated	sequence-specific DNA binding transcription factor activity; DNA binding	-
Gorai.013G005900	Gorai.013G005900.1	lob domain-containing protein 15-like	2.70E-103	87.05%	-	-	nucleus
Gorai.013G132800	Gorai.013G132800.1	o-acyltransferase wsd1-like	0	70.55%	glycerolipid biosynthetic process	diacylglycerol O-acyltransferase activity	-
	Gorai.013G132800.2	o-acyltransferase wsd1-like	2.98E-160	67.55%	glycerolipid biosynthetic process	diacylglycerol O-acyltransferase activity	-
Gorai.N025100	Gorai.N025100.1	par1 protein	4.08E-121	81.85%	-	-	-

Table A.6. Top 30 significantly down regulated genes (log fold change in transcript number) in TAM07V-45 in response to herbivory by cotton fleahopper

					Gene Ontology		
Gene ID	**Splice VariantID**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.001G080700	Gorai.001G080700.1	tyrosine decarboxylase 1-like	0	82.35%	cellular amino acid metabolic process; carboxylic acid metabolic process	catalytic activity; carboxy-lyase activity; pyridoxal phosphate binding; lyase activity	-
Gorai.002G097100	Gorai.002G097100.1	cytochrome p450	0	78.80%	oxidation-reduction process	metal ion binding; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; heme binding; oxidoreductase activity; iron ion binding; monooxygenase activity	-
Gorai.002G100000	Gorai.002G100000.1	Uncharacterized protein TCM_030646	1.55E-04	78.00%	-	-	-
Gorai.002G105300	Gorai.002G105300.1	Uncharacterized protein TCM_032572	2.33E-104	88.90%	-	-	plasmodesma
Gorai.002G236500	Gorai.002G236500.1	transcription repressor ofp13-like	4.57E-75	64.35%	negative regulation of transcription, DNA-templated	-	-
Gorai.003G109200	Gorai.003G109200.1	adenine guanine permease azg2	0	86.30%	transmembrane transport; transport; guanine transport; adenine transport; purine nucleobase transport	transporter activity; purine nucleobase transmembrane transporter activity	membrane
Gorai.003G171400	Gorai.003G171400.1	probable pectinesterase 68	0	89.30%	cell wall modification; metabolic process	hydrolase activity; aspartyl esterase activity; pectinesterase activity	cell wall
	Gorai.003G171400.2	probable pectinesterase 68	2.33E-170	91.95%	cell wall modification; metabolic process;	aspartyl esterase activity; pectinesterase activity; hydrolase activity	cell wall
Gorai.005G008800	Gorai.005G008800.1	Uncharacterized protein TCM_019849	4.08E-80	77.05%	-	-	-
Gorai.005G034500	Gorai.005G034500.1	-			-	-	-
Gorai.005G121300	Gorai.005G121300.1	sec14p-like phosphatidylinositol transfer family protein	0	77.95%	transport	transporter activity	integral component of membrane; cytosol; plasma membrane; nucleus; intracellular

Gorai.006G207800	Gorai.006G207800.1	ethylene-responsive transcription factor erf061-like	4.10E-121	70.35%	regulation of transcription, DNA-templated; transcription, DNA-templated	sequence-specific DNA binding transcription factor activity; DNA binding	nucleus

Table A.6 Continued

					Gene Ontology		
Gene ID	**Splice VariantID**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.006G232300	Gorai.006G232300.1	Uncharacterized protein TCM_019849	4.93E-108	79.10%	-	-	-
Gorai.006G242600	Gorai.006G242600.1	early light-induced protein chloroplastic-like	1.95E-105	78.70%	response to UV-B; photoprotection; response to red light; response to far red light; cellular response to UV-A; regulation of chlorophyll biosynthetic process; cellular response to heat; cellular response to red light; cellular response to far red light; positive regulation of seed germination; cellular response to high light intensity; cellular response to blue light; response to karrikin; response to cold	-	-
Gorai.007G082600	Gorai.007G082600.1	circumsporozoite protein	4.42E-173	60.35%	transport	-	integral component of membrane; membrane
Gorai.007G172600	Gorai.007G172600.1	uncharacterized loc101221004	1.52E-104	67.70%	-	-	-
Gorai.007G248900	Gorai.007G248900.1	f-box kelch-repeat protein skip25-like	1.39E-178	72.60%	-	-	-
Gorai.007G359300	Gorai.007G359300.1	---NA---			-	-	-
Gorai.008G142900	Gorai.008G142900.1	cellulose synthase-like protein d5	0	91.65%	cellulose biosynthetic process; shoot system development;response to osmotic stress; response to water deprivation; response to salt stress; mannosylation; microtubule cytoskeleton organization; cytokinesis by cell plate formation; cell wall biogenesis; double-strand break repair via homologous recombination; polysaccharide biosynthetic process; response to cyclopentenone; glucosyltransferase activity;	transferase activity; cellulose synthase (UDP-forming) activity; transferase activity, transferring glycosyl groups; mannan synthase activity	integral component of membrane; membrane; Golgi apparatus

					plant-type cell wall biogenesis; leaf morphogenesis; regulation of cell proliferation		

Table A.6 Continued

					Gene Ontology		
Gene ID	**Splice VariantID**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.008G255600	Gorai.008G255600.1	phosphatidylinositol 4-kinase gamma 2-like	0	84.10%	metabolic process; phosphorylation	phosphotransferase activity, alcohol group as acceptor; transferase activity, transferring phosphorus-containing groups; kinase activity	
Gorai.008G270700	Gorai.008G270700.1	transcription factor speechless-like	2.53E-125	75.30%	stomatal complex development	protein dimerization activity	-
Gorai.009G115700	Gorai.009G115700.1	tetratricopeptide repeat-like superfamily	0	64.45%	-	-	-
Gorai.009G115700	Gorai.009G115700.2	tetratricopeptide repeat-like superfamily	0	63.75%	-	-	-
Gorai.009G127900	Gorai.009G127900.1	protein too many mouths	0	82.65%	phosphorylation; response to abscisic acid; asymmetric cell division; oxidation-reduction process	kinase activity; oxidoreductase activity; 2-alkenal reductase [NAD(P)] activity	-
Gorai.009G136500	Gorai.009G136500.1	ethylene-responsive transcription factor tiny-like	1.28E-89	73.60%	regulation of transcription, DNA-templated; transcription, DNA-templated	sequence-specific DNA binding transcription factor activity; DNA binding	nucleus
Gorai.009G169200	Gorai.009G169200.1	transcription factor bhlh36-like	2.78E-79	70.35%		protein dimerization activity; DNA binding	
Gorai.009G169200	Gorai.009G169200.3	transcription factor bhlh36-like	6.48E-60	69.65%	-	protein dimerization activity; DNA binding	-
Gorai.009G169200	Gorai.009G169200.2	transcription factor bhlh36-like	1.08E-80	71.45%	-	protein dimerization activity; DNA binding	-

Gorai.009G287400	Gorai.009G287400.1	protein epidermal patterning factor 2-like	2.78E-53	73.40%	epidermis morphogenesis; guard cell differentiation; negative regulation of stomatal complex development; stomatal complex development	-	-
Gorai.009G436500	Gorai.009G436500.1	cc-nbs-lrr resistance	0	57.75%	P:defense response; P:metabolic process;P:dephosphorylation;	F:ADP binding; F:ATP binding; F:nucleotide binding; F:nucleoside-triphosphatase activity; F:phosphoprotein phosphatase activity; F:hydrolase activity	-

Table A.6 Continued

					Gene Ontology		
Gene ID	**Splice VariantID**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.010G194100	Gorai.010G194100.2	probably inactive leucine-rich repeat receptor-like protein kinase imk2-like	0	83.55%	phosphorylation	ATP binding; protein kinase activity; transferase activity, transferring phosphorus-containing groups; kinase activity	integral component of membrane; membrane
Gorai.010G194100	Gorai.010G194100.1	probably inactive leucine-rich repeat receptor-like protein kinase imk2-like	0	84.10%	protein phosphorylation; phosphorylation	ATP binding; protein kinase activity; kinase activity; transferase activity, transferring phosphorus-containing groups	integral component of membrane; membrane
Gorai.011G103800	Gorai.011G103800.1	ap2 b3-like transcriptional factor family	7.36E-97	48.40%	regulation of transcription, DNA-templated; transcription, DNA-templated	DNA binding	nucleus
Gorai.011G250800	Gorai.011G250800.1	ankyrin repeat-containing protein	0	64.30%	-	-	-
Gorai.012G038300	Gorai.012G038300.1	probable carboxylesterase 18-like	4.44E-148	74.05%	metabolic process; pollen tube growth	hydrolase activity; carboxylic ester hydrolase activity	nucleus

Table A.7. Top 30 significantly upregulated genes (log fold change in transcript number) in GH15-2 in response to herbivory by cotton fleahopper

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.001G009600	Gorai.001G009600.1	oxidative stress 3	1.08E-30	73.75%	response to cadmium ion; response to oxidative stress	-	nuclear speck;
Gorai.002G203600	Gorai.002G203600.1	class i chitinase	0	87.85%	defense response; cell wall macromolecule catabolic process; polysaccharide catabolic process; carbohydrate metabolic process; metabolic process; chitin catabolic process;	chitinase activity; hydrolase activity; chitin binding; hydrolase activity, acting on glycosyl bonds;	vacuole
Gorai.002G263100	Gorai.002G263100.1	leucoanthocyanidin dioxygenase-like	0	85.20%	oxidation-reduction process;	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors; oxidoreductase activity; iron ion binding; dioxygenase activity; leucocyanidin oxygenase activity	-
Gorai.003G183500	Gorai.003G183500.1	like cupins superfamily protein	0	66.20%	-	nutrient reservoir activity	-
	Gorai.003G183500.2	glutelin type-a 3-like	9.28E-149	65.10%	-	nutrient reservoir activity	-
	Gorai.003G183500.3	glutelin type-a 3-like	1.14E-139	65.35%	-	nutrient reservoir activity	-
Gorai.004G129600	Gorai.004G129600.1	transcription factor myc2-like	0	70.70%	oxidation-reduction process;	protein dimerization activity; oxidoreductase activity; 2-alkenal reductase [NAD(P)] activity	-
Gorai.005G047100	Gorai.005G047100.1	cellulose synthase like g2	0	77.50%	cellulose biosynthetic process;	transferase activity; cellulose synthase (UDP-forming) activity; transferase activity, transferring glycosyl groups	integral component of membrane; membrane;
Gorai.005G104200	Gorai.005G104200.1	patatin-like protein 2	0	81.45%	lipid metabolic process; metabolic process; cell death; cellular response to hypoxia; plant-type hypersensitive response; oxylipin biosynthetic process; defense response to virus; response to cadmium ion	lipase activity;	membrane; cytoplasm;
Gorai.005G142300	Gorai.005G142300.1	nac domain-containing protein 29	0	80.80%	regulation of transcription, DNA-templated; leaf senescence; flower development;	DNA binding;	

multidimensional cell growth

Table A.7 Continued

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.005G253100	Gorai.005G253100.1	chaperone protein dnaj chloroplastic-like	2.92E-49	72.90%	-	-	-
	Gorai.005G253100.2	chaperone protein dnaj chloroplastic-like	2.92E-49	72.90%	-	-	-
Gorai.006G087400	Gorai.006G087400.1	pesticidal crystal cry1ag	0	78.95%	-	-	-
Gorai.006G087500	Gorai.006G087500.1	pesticidal crystal cry1ag	4.49E-172	78.05%	-	-	-
Gorai.006G105200	Gorai.006G105200.1	asparagine synthetase	0	94.60%	L-asparagine biosynthetic process; asparagine biosynthetic process; metabolic process; cellular amino acid biosynthetic process; cellular response to sucrose starvation; response to sucrose; response to glucose; response to fructose	ATP binding; ligase activity; nucleotide binding; asparagine synthase (glutamine-hydrolyzing) activity;	-
	Gorai.006G105200.2	asparagine synthetase	0	94.50%	L-asparagine biosynthetic process; asparagine biosynthetic process; metabolic process; cellular amino acid biosynthetic process; cellular response to sucrose starvation; response to sucrose; response to glucose; response to fructose	ATP binding; ligase activity; nucleotide binding; asparagine synthase (glutamine-hydrolyzing) activity;	-
	Gorai.006G105200.3	asparagine synthetase	0	96.50%	L-asparagine biosynthetic process; asparagine biosynthetic process; metabolic process; cellular amino acid biosynthetic process; cellular response to sucrose starvation; response to sucrose; response to glucose; response to fructose	ATP binding; ligase activity; nucleotide binding; asparagine synthase (glutamine-hydrolyzing) activity;	-
	Gorai.006G105200.4	asparagine synthetase	0	96.75%	L-asparagine biosynthetic process; asparagine biosynthetic process; metabolic process; cellular amino acid biosynthetic process; cellular response to sucrose starvation; response to sucrose; response to glucose; response to fructose	ATP binding; ligase activity; nucleotide binding; asparagine synthase (glutamine-hydrolyzing) activity;	-

Gorai.006G159500	Gorai.006G159500.1	embryonic dc-8	0	53.95%	-	-	cell wall; cytoplasm; extracellular region

Table A.7 Continued

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.007G126200	Gorai.007G126200.1	protein exordium-like 2	2.03E-161	71.00%	-	-	-
Gorai.007G170100	Gorai.007G170100.1	1-aminocyclopropane-1-carboxylate oxidase	0	90.95%	oxidation-reduction process;response to fungus; cellular response to fatty acid	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors; oxidoreductase activity; iron ion binding;	-
Gorai.008G066100	Gorai.008G066100.1	salicylate o-methyltransferase	0	85.60%	methylation;	methyltransferase activity; transferase activity; jasmonate O-methyltransferase activity; nicotinate N-methyltransferase activity	-
Gorai.008G254300	Gorai.008G254300.1	ring u-box superfamily protein	4.11E-90	56.30%	-	zinc ion binding; polysaccharide binding; metal ion binding	-
Gorai.009G170800	Gorai.009G170800.1	p -nerolidol ( )-geranyl linalool synthase	0	73.30%	F:magnesium ion binding; metabolic process; response to singlet oxygen; diterpenoid biosynthetic process; response to wounding; response to herbivore; response to jasmonic acid; response to bacterium	metal ion binding; terpene synthase activity; lyase activity;(E,E)-geranyllinalool synthase activity;	-
Gorai.009G363000	Gorai.009G363000.1	myrcene chloroplastic	0	79.05%	metabolic process; terpenoid biosynthetic process;	metal ion binding; terpene synthase activity; magnesium ion binding; lyase activity; myrcene synthase activity;	plastid; chloroplast
Gorai.009G363300	Gorai.009G363300.1	myrcene chloroplastic	0	77.75%	metabolic process; terpenoid biosynthetic process; sesquiterpene biosynthetic process;	lyase activity;sesquiterpene synthase activity; myrcene synthase activity;	plastid; chloroplast
Gorai.010G215800	Gorai.010G215800.1	ribonuclease	3.13E-54	51.45%	RNA phosphodiester bond hydrolysis, endonucleolytic; metabolic process; response to salt stress	RNA binding; ribonuclease T2 activity; hydrolase activity;	-
Gorai.011G141300	Gorai.011G141300.1	23 kda jasmonate-induced	1.46E-69	59.25%	-	-	-
Gorai.011G173200	Gorai.011G173200.1	Uncharacterized protein TCM_020877	3.50E-21	61.00%	-	-	-
Gorai.011G254400	Gorai.011G254400.1	trypsin inhibitor	5.42E-84	78.00%	negative regulation of endopeptidase activity;	endopeptidase inhibitor activity	-
Gorai.011G254500	Gorai.011G254500.1	trypsin inhibitor	1.99E-73	75.65%	negative regulation of endopeptidase activity;	endopeptidase inhibitor activity	-

Gorai.011G254700	Gorai.011G254700.1	trypsin inhibitor	1.95E-79	69.60%	negative regulation of endopeptidase activity;	endopeptidase inhibitor activity	-

Table A.7 Continued

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.011G254800	Gorai.011G254800.1	trypsin inhibitor	2.92E-77	68.95%	negative regulation of endopeptidase activity;	endopeptidase inhibitor activity	-
Gorai.012G115200	Gorai.012G115200.1	transcription factor hbp-1b -like	5.35E-114	69.55%	transcription, DNA-templated;	sequence-specific DNA binding	-
Gorai.012G132700	Gorai.012G132700.1	beta-ocimene synthase	0	73.40%	metabolic process;	metal ion binding; terpene synthase activity; magnesium ion binding; lyase activity; isoprene synthase activity;	plastid; chloroplast
Gorai.012G132800	Gorai.012G132800.1	isoprene synthase	0	71.80%	metabolic process;	metal ion binding; terpene synthase activity; magnesium ion binding; lyase activity; isoprene synthase activity	-

Table A.8. Top 30 significantly down regulated genes (log fold change in transcript number) in GH15-2 in response to herbivory by cotton fleahopper

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.001G034700	Gorai.001G034700.1	14 kda proline-rich protein	3.36E-47	93.60%	systemic acquired resistance; defense response to fungus	-	plasmodesma
Gorai.001G080900	Gorai.001G080900.1	---NA---	-	-	-	-	-
Gorai.001G271500	Gorai.001G271500.1	ribonucleoside-diphosphate reductase small chain	0	80.85%	oxidation-reduction process; deoxyribonucleoside diphosphate metabolic process; regulation of cell cycle; programmed cell death; multicellular organismal development; DNA replication	oxidoreductase activity; ribonucleoside-diphosphate reductase activity, thioredoxin disulfide as acceptor; metal ion binding;	cytoplasm
Gorai.002G097100	Gorai.002G097100.1	cytochrome p450	0	78.80%	oxidation-reduction process	metal ion binding; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; heme binding; oxidoreductase activity; iron ion binding; monooxygenase activity	-
Gorai.002G114000	Gorai.002G114000.1	glyceraldehyde-3-phosphate dehydrogenase chloroplastic	0	95.75%	oxidation-reduction process; glucose metabolic process; response to sucrose; response to light stimulus; response to cold; response to cadmium ion	oxidoreductase activity; oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor; NAD binding; NADP binding	chloroplast thylakoid membrane; stromule; apoplast; chloroplast envelope
Gorai.002G236500	Gorai.002G236500.1	transcription repressor ofp13-like	4.57E-75	64.35%	negative regulation of transcription, DNA-templated	-	-
Gorai.003G141500	Gorai.003G141500.1	Uncharacterized protein TCM_042052	1.27E-21	60.85%	-	-	-
Gorai.003G171400	Gorai.003G171400.1	probable pectinesterase 68	0	89.30%	cell wall modification; metabolic process;	hydrolase activity; aspartyl esterase activity; pectinesterase activity	cell wall
	Gorai.003G171400.2	probable pectinesterase 68	2.33E-170	91.95%	cell wall modification; metabolic process;	hydrolase activity;aspartyl esterase activity; pectinesterase activity	cell wall
Gorai.004G150800	Gorai.004G150800.1	myb-related protein 308-like	1.38E-152	71.60%	-	chromatin binding; DNA binding	-
Gorai.004G151000	Gorai.004G151000.1	myb-related protein 308-like	1.65E-129	69.25%	-	chromatin binding; DNA binding	-
Gorai.004G214500	Gorai.004G214500.1	leucine-rich repeat family protein	0	85.40%	oxidation-reduction process; phosphorylation	kinase activity; oxidoreductase activity;2-alkenal reductase [NAD(P)] activity	-

Gorai.005G202200	Gorai.005G202200.1	expansin beta isoform 1	8.20E-140	86.60%	sexual reproduction; syncytium formation	-	plasmodesma; extracellular region

Table A.8 Continued

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.006G163600	Gorai.006G163600.1	saur-like auxin-responsive protein	5.72E-47	69.20%	-	-	-
Gorai.006G228900	Gorai.006G228900.1	defensin-like protein 1	1.14E-30	77.75%	defense response	-	-
Gorai.007G027500	Gorai.007G027500.1	14 kda proline-rich protein	5.48E-35	86.65%	-	-	-
Gorai.007G082600	Gorai.007G082600.1	circumsporozoite protein	4.42E-173	60.35%	transport	-	integral component of membrane; membrane
Gorai.007G172600	Gorai.007G172600.1	uncharacterized loc101221004	1.52E-104	67.70%	-	-	-
Gorai.007G181300	Gorai.007G181300.1	ribulose- -bisphosphate carboxylase oxygenase small subunit	1.13E-119	98.75%	photorespiration; photosynthesis; carbon fixation; oxidation-reduction process; reductive pentose-phosphate cycle	lyase activity; oxidoreductase activity; ribulose-bisphosphate carboxylase activity; monooxygenase activity	chloroplast; plastid
	Gorai.007G181300.2	ribulose- -bisphosphate carboxylase oxygenase small subunit	4.72E-117	98.10%	photorespiration; photosynthesis; carbon fixation; oxidation-reduction process; reductive pentose-phosphate cycle	lyase activity; oxidoreductase activity; ribulose-bisphosphate carboxylase activity; monooxygenase activity	chloroplast; plastid
Gorai.007G181400	Gorai.007G181400.1	ribulose- -bisphosphate carboxylase oxygenase small subunit	1.13E-119	98.75%	photorespiration; photosynthesis; carbon fixation; oxidation-reduction process; reductive pentose-phosphate cycle	lyase activity; oxidoreductase activity; ribulose-bisphosphate carboxylase activity; monooxygenase activity	chloroplast; plastid
Gorai.008G064600	Gorai.008G064600.1	Uncharacterized protein TCM_000260	9.33E-12	75.00%	-	-	
Gorai.008G153800	Gorai.008G153800.1	expansin-b3-like	1.93E-180	90.50%	sexual reproduction	-	extracellular region
	Gorai.008G153800.2	expansin beta isoform partial	4.25E-130	90.35%	sexual reproduction	-	extracellular region
Gorai.009G035500	Gorai.009G035500.1	germin protein subfamily 3 member 3	1.46E-101	87.30%	response to cold	metal ion binding; nutrient reservoir activity; manganese ion binding	extracellular region; plant-type cell wall; nucleus; extracellular matrix; cell wall
Gorai.009G063200	Gorai.009G063200.1	early nodulin-like protein 1	2.31E-95	88.60%	-	electron carrier activity; copper ion binding	plasmodesma; anchored component of plasma membrane
Gorai.009G065800	Gorai.009G065800.1	protein glutamine dumper 5-like	1.67E-40	72.15%	-	-	-
Gorai.010G165100	Gorai.010G165100.1	chlorophyll a-b binding chloroplastic	0	96.10%	photosynthesis; protein-chromophore linkage; photosynthesis, light harvesting	chlorophyll binding; metal ion binding	integral component of membrane; membrane; thylakoid; photosystem II; chloroplast; plastid;

chloroplast thylakoid membrane; photosystem I

Table A.8 Continued

					Gene Ontology		
Gene ID	**Seq. Name**	**Seq. Description**	**Min. eValue**	**Mean Similarity**	**Biological Process**	**Molecular Function**	**Cellular Component**
Gorai.011G051800	Gorai.011G051800.1	PREDICTED: uncharacterized protein LOC100803585	1.05E-17	66.35%	biological_process	molecular_function	plasma membrane; extracellular region
Gorai.011G192200	Gorai.011G192200.1	mate efflux family protein 5-like	0	81.85%	drug transmembrane transport; transmembrane transport	drug transmembrane transporter activity; antiporter activity	integral component of membrane; membrane
Gorai.011G253100	Gorai.011G253100.1	aquaporin tip1-3	1.32E-162	93.45%	transport; urea transmembrane transport; water transport; urea transport	transporter activity;urea transmembrane transporter activity; water channel activity	integral component of membrane; membrane; cytoplasm
	Gorai.011G253100.2	aquaporin tip1-3-like	4.01E-132	94.10%	transport;urea transmembrane transport; water transport; urea transport	transporter activity; urea transmembrane transporter activity	integral component of membrane; membrane
Gorai.013G003000	Gorai.013G003000.1	glutaredoxin family	9.78E-137	68.55%	oxidation-reduction process; cell redox homeostasis	electron carrier activity; protein disulfide oxidoreductase activity	-
Gorai.013G211900	Gorai.013G211900.1	ankyrin repeat and kh domain-containing mask	1.74E-166	89.55%	-	-	-
	Gorai.013G211900.2	plant f12b17-70 protein	1.08E-122	91.00%	-	-	-