THE STUDY OF *MELANAPHIS SACCHARI* (HEMIPTERA: APHIDIDAE) (ZEHTNER) (SUGARCANE APHID) AND *SORGHUM BICOLOR* (CYPERALES: POACEAE) (L.) MOENCH (SORGHUM) INTERACTIONS

A Thesis

by

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ABSTRACT

Sorghum bicolor (L.) Moench (Cyperales: Poacea) is a cereal grain crop grown worldwide. National Agriculture Statistics Service reported that in 2014 and 2015 over 6 million hectares of sorghum were planted in the United States and the value of grain sorghum was over $1.6 billion. Sorghum is an important staple crop for many countries and is used as mainly fodder for livestock. *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), the sugarcane aphid, is a new invasive pest of sorghum in southern U.S. It was first observed in sorghum in 2013 in Texas, but high infestations caused important losses in 2014 in several southern states (Louisiana, Mississippi, and Oklahoma). The novelty and the speed of invasion of this pest caught the industry by surprise, and today few control options are available. In order to develop sustainable control strategies a life table analysis was first conducted of the sugarcane aphid on five sorghum hybrids. These included both grain, sweet and photoperiod sensitive sorghum hybrids. Only the sugarcane aphid overall survivorship was significantly different between hybrids ATx645/R07007 (grain sorghum) and A0535-C53-6F/UMBRELLA (sweet sorghum). Genetic resistance was then evaluated to sugarcane aphid in 16 commercial grain sorghum hybrids in Burleson County, TX on the Texas A&M University farm. No resistant hybrid to sugarcane aphid was determined because the aphid population was below the threshold that would affect sorghum yield. Finally sorghum defenses against sugarcane aphid were evaluated through a transcriptome analysis post insect feeding.

Plants recognized aphid attack, mounted defenses, but those responses were insufficient in deterring feeding.

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NOMENCLATURE

HPR	Host Plant Resistance
IPM	Integrated Pest Management
SPAD	Soil Plant Analysis Development
NDVI	Normalized Difference Vegetation Index

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Sugarcane aphid biology

The sugarcane aphid *Melanaphis Sacchari* (Zehntner) (Hemiptera: Aphididae) is a new invasive pest on *Sorghum bicolor* (L.) Moench (Cyperales: Poaceae) in the southern United States. Aphids are cyclic parthenogenetic organisms, and parthenogenetic females are viviparous. Aphid's life cycle is short: it is characterized by short generation time and generation telescopage (i.e., asexual females carry their babies which in turn carry their own babies, potentially two generations simultaneously existing in one female). These characteristics can result in exponential increase of aphid populations. The sugarcane aphid host-range includes 20 graminous species (Singh et al. 2004b).

Sorghum

Among the major cereal crops cultivated worldwide, sorghum is unique in being used efficiently for food, fuel, feed and fiber production (Paterson et al. 2009). These multiple usages, together with its highly efficient C4 photosynthesis system, strong tolerance to abiotic stresses and high yield potential, make sorghum an increasingly important crop for many countries in dealing with shortages of natural resources and climate changes (Palmer 1992, Paterson et al. 2009). The 2014 United States national average for sorghum yield was 67.6 bushels/acre for a production of $1.7 billion (USDA 2015).

Sugarcane aphid as an invasive pest

The sugarcane aphid first invaded the continental USA in 1977 via Florida (Hall 1987, 1988). This insect was mainly a pest of sugarcane and made its way westward, reaching Louisiana in 1999 (White et al. 2001). A problem in grain sorghum fields was first reported in 2013 in southern Texas, Louisiana, Oklahoma, and Mississippi where the insect was detected in 38 counties and parishes (Knutson 2015).

Early season infestations of grain sorghum fields had significant impacts on plant growth and development, resulting in significant loss (up to 50%) for some producers. In addition, large amounts of honeydew produced by late season aphid infestations can clog combines and result in indirect yield loss (Armstrong et al. 2015). In northern states, the sugarcane aphid has appeared late in the season, causing damage only in late-planted sorghum (Stewart 2015). By 2014, the area affected by this insect had expanded to include all southern states from Texas to Florida, including the northeast of Mexico and as far north as Kansas with more than 150 counties and parishes affected (Knutson 2015). During 2015, the sugarcane aphids have been found as far north as Illinois.

Host plant resistance

Presently, the only effective control mechanism for sugarcane aphids relies on the use of systemic neonicotinoid insecticides such as Sulfoxaflor (Transform) and Flupyradifurone (Sivanto). However, pesticide applications are not a sustainable and long-term control strategy. Host plant resistance (HPR), one of the pillars of integrated pest management (IPM), remains an option of choice to control pests. Host plant resistance is defined as the heritable plant characteristics that may reduce the utilization

of the plant as a host by an insect (Dent 2000). Antixenosis, antibiosis, and tolerance are the three categories of host plant resistance currently used (Painter 1951). Antixenosis (non-preference) is the unfavorable effects by the plant on the pest behavior. Characteristics such as color, palatability, waxiness, trichomes which can deter the pest from consuming the plant in favor of an alternate host plant are examples of antixenosis (Van Emden and Harrington 2007). Antibiosis is the unfavorable effects by the plant on the pests' life table (Painter 1951). Antibiosis can be conferred by glandular trichomes, toxins, or nutritional factors that alter survival rate, growth rate, fecundity, and development time of the pest (Van Emden and Harrington 2007). Tolerance is the ability of the plant to recover from pest injury or decrease damage to plant fitness and/or yield loss (Painter 1951, Stout 2013).

Stout proposed a new dichotomous framework for the host plant resistance model first proposed by Painter. The framework focuses on the categories of resistance and tolerance. Resistance encompasses characteristics/processes of the given plant that reduces the injury done by an herbivore (Stout 2013). Resistance can then be further divided into 4 subcategories (constitutive, inducible, direct, and indirect) (Stout 2013). Constitutive resistance is expressed without the need of injury and inducible resistance is expressed in response to injury (Stout 2013). Direct resistance affects the herbivores' behavior and indirect resistance is dependent on natural enemies (Stout 2013).

Plant defense response

Signaling pathways associated with HPR to arthropod attacks are driven by the phytohormones salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid

(ABA) (Smith 2005). These signal transduction pathways are interconnected, crosstalking with one another and leading to downstream effects inducing defense genes and production of defense proteins (Li et al. 2006). Bacterial and fungal pathogen defense genes have been reported to be activated in response to aphid-plant interactions (Zhu-Salzman et al. 2004). Previous studies have shown that JA and SA are induced in response to aphid attacks (Kusnierczyk et al. 2008). Many studies have been completed on *Arabidopsis thaliana*, but only few have been completed in sorghum. Understanding the defense pathways that are involved in sorghum-sugarcane aphid interactions may provide new information and tools for management of sugarcane aphids in sorghum.

Research objectives

A life table comparison between *Schizaphis graminum* (greenbug) and the sugarcane aphid was completed on known greenbug resistant sorghum hybrids (Bayoumy et al. 2015). Recently, a screening was conducted in the southern United States to evaluate suitability of graminous plants as sugarcane aphid hosts, and to screen for host plant resistance (tolerance and antixenosis) to the sugarcane aphid in different sorghum types (forage, energy, grain, sweet) including genotypes resistant to *Schizaphis graminum* (Armstrong et al. 2015). The objectives of my thesis, listed below, complement those and other on-going studies to design sustainable strategies to control sugarcane aphids on sorghum.

The long-term objective was to investigate the sugarcane aphid biology on sorghum as a first step towards better understanding the biology of this aphid to develop economically and ecologically sustainable control strategies. The specific objectives are

as follows, (1) complete life table analysis of the sugarcane aphid on different sorghum hybrids, (2) evaluate sorghum germplasm in a field trial, and (3) evaluate sorghum defenses against the sugarcane aphid.

CHAPTER II

LIFE TABLE ANALYSIS OF *MELANAPHIS SACCHARI* (ZHETNER) (HEMIPTERA: APHIDIDAE) ON DIFFERENT HYBRIDS OF *SORGHUM BICOLOR* (L.) MOENCH (CYPERALES: POACEAE)

Introduction

Few control options are available against the sugarcane aphid and they rely on pesticide applications. In order to develop long-lasting control strategies, a better understanding of this insect biology is needed. In this study, a life table analysis of the sugarcane aphid was performed on five sorghum hybrids in the laboratory. Recently, a screening was conducted in the southern United States to evaluate suitability of graminous plants as sugarcane aphid hosts, and to screen for HPR (tolerance and antixenosis) to the sugarcane aphid in different sorghum lines including genotypes resistant to *Schizaphis graminum* (Armstrong et al. 2015).

Where that previous study focused on tolerance and antixenosis (Armstrong et al. 2015), our study focused on antibiosis through the analysis of sugarcane aphid life tables. The objective of the present study was to investigate the sugarcane aphid biology on different sorghum hybrids (photoperiod sensitive, grain, and sweet) as a first step towards the identification of resistant hybrids since life tables allow comparing insect's performance on different plant cultivars to measure host plant resistance. Five sorghum hybrids were chosen based on field observations of potential tolerance against this pest as well as on the availability of insecticide treatment-free seeds.

Methods and materials

***Plant Material*:** Seeds of five sorghum hybrids, M81E (sweet), ATx631/RTx436 (grain), ATx645/R07007 (photoperiod sensitive energy), A05035-C53-6F/UMBRELLA (sweet), and ATx631/R10781 (photoperiod sensitive energy), were obtained from the Texas A&M Sorghum Breeding program. Seeds were planted individually in cone-tainers (SC7, Greenhouse Megastore, white cells, Danville, IL) in Metro-Mix (900, Sun-Gro Horticulture, Agawam, MA) in the laboratory. The plants were watered every two days after an initial application of 0.7 cm^3 of soluble fertilizer (20-20-20) L^{-1} of distilled water (Miracle Gro, Marysville, OH). Plants were kept under 40 watts T12 plant/aquarium bulbs (General Electric, Houston, TX) set at a long photoperiod of 16:8 (L : D) h by a15-AMP light timer (Utilitech, San Francisco, CA). Once the sorghum was one month old (about 25 cm height), a 3.81 x 30.48 centimeters clear plastic tube (Uline, Pleasant Prairie, WI) with high density polyethylene insect mesh on the top opening was placed over each plant and the plants were placed in a Percival Incubator (Percival, Boone, IA) at 16:8 L : D and 23°C for 24 hours prior to aphid infestation.

***Aphids*:** An aphid colony was maintained on M81E sweet sorghum in a growth chambers (Percival, Boone, IA) at 16:8 L : D and 23°C. Multiple aphids were collected from a field in Corpus Christi, Texas to begin the initial laboratory colony.

***Experimental Procedure*:** A single adult female was placed on each sorghum plant and allowed to nymphoposit overnight. The following day, the adult and all nymphs except one were removed. Every day, aphids were monitored to register survival and nymphal stages. After aphids reached adulthood, the number of progeny were

counted and removed. Adult aphid survival was monitored daily. The experiment was carried out in growth chambers (Percival, Boone, IA) at 23°C and a photoperiod of 16:8 (L : D).

***Life Table Parameters*:** Mean durations were measured in days for each nymphal instar for each hybrid. Nymphal development was averaged across the four instars to obtain the development time. Nymphal instars were determined by morphology and when present the shedding of the exoskeleton after molting. Adult longevity was the number of days from pre-reproductive (adult till first day of reproduction) till death. The following formulas were used to calculate the number of aphids that survived daily (survivorship: l_x) and the average reproductive rate for all adult females on a singular hybrid (R_0) (Price et al. 2011):

x = age interval or number of days

l_x (survivorship) = a_x (number of individuals) / a_0 (original number of individuals)

R_0 (reproductive rate) = l_x * m_x (average number of female progeny)

The estimated intrinsic rate of increase (r_m), a function of aphid generation time (T) and reproductive rate (R_0), represents the increase within a natural population (Vranken and Heip 1983). A reduced intrinsic rate can be due to a prolonged generation time (nymphal development and adult longevity) and/or a low reproductive rate (Price et al. 2011). Therefore, the intrinsic rate of increase is a good measure of plant resistance. The intrinsic rate of increase was calculated as follows (Wyatt and White 1977):

r_m (estimated intrinsic rate of increase) = ln (R_0) / T (mean generation time)

Average mortality rates were calculated using the survivorship values obtained across all life stages of all aphids for each hybrid. The mortality rate was calculated daily and averaged as follows (Price et al. 2011):

d_x (mortality) = l_x - l_{x+1}

q_x (mortality rate) = d_x / l_x

***Data Analysis*:** Thirty replicates per sorghum hybrid were analyzed, except for hybrid ATx631/RTx436, for which 3 plants died before the end of the experiment. All the statistical analyses were conducted using the open source software R (R_Core_Team 2014). ANOVA was performed using the aov function in the stats library to determine significant differences in aphid life table parameters. Post-hoc analyses were performed by multiple comparison tests after Kruskal-Wallis using the function kruskalmc in the pgirmess library. Survival analysis was performed in R using the function surv. Post hoc analyses were performed by multiple comparison tests.

Results

Sugarcane aphids developed through four instars on each tested sorghum hybrid. No significant differences in nymphal development time among hybrids were detected for the first ($F = 0.598$; $df = 4$; $P = 0.6643$), second ($F = 0.895$; $df = 4$; $P = 0.486$), third ($F = 0.827$; $df = 4$; $P = 0.5077$), and fourth instars ($F = 1.431$; $df = 4$; $P = 0.2208$) (Table 1).

Table 1 Duration in days of each nymphal instar in each sorghum hybrid. No significant differences in nymphal development time among hybrids were detected for any instar.

Nymphal Development (mean± SE)					
	Sorghum Hybrid				
Instar	M81E	ATx631/RTx436	ATx645/R07007	A05035-C53-6F/UMBRELLA	ATx631/R10781
I	1.3 ± 0.2	1.5 ± 0.2	1.2 ± 0.1	1.5 ± 0.3	1.4 ± 0.2
II	1.7 ± 0.2	1.6 ± 0.2	1.9 ± 0.2	1.7 ± 0.3	1.5 ± 0.2
III	1.8 ± 0.3	1.3 ± 0.2	1.2 ± 0.1	1.3 ± 0.3	1.4 ± 0.2
IV	1.7 ± 0.3	1.4 ± 0.2	1.2 ± 0.1	1.1 ± 0.2	1.4 ± 0.1

Similarly, no significant differences were detected when comparing the overall nymphal development time among hybrids ($F = 0.382$; $df = 4$; $P = 0.819$) (Table 2). The overall nymphal development across the four instars varied between 5.6 ± 0.3 days on hybrid ATx645/R07007 and Atx631/E10781 to 6.2 ± 0.7 days on hybrid M81E. On average the overall nymphal development was 5.8 ± 0.1 days across hybrids (Table 2).

No significant differences of adult longevity (number of days as an adult) were detected among sorghum hybrids ($F = 0.798$; $df = 4$; $P = 0.6643$) (Table 2). The adult longevity ranged from 14.9 ± 2.9 on hybrid ATx645/R07007 to 21.7 ± 2.2 on hybrid A05035-C53-6F/UMBRELLA with an average adult longevity of 17.5 ± 1.2 days across all hybrids (Table 2).

No significant differences in overall longevity (number of days from birth until death) were measured ($F = 0.96$; $df = 4$; $P = 0.455$). Overall longevity ranged from 20.4 ± 3.1 on hybrid ATx645/R07007 to 27.6 ± 2.3 on hybrid A05035-C53-6F/UMBRELLA

(Table 2). Overall longevity averaged 23.5 ± 1.3 days across all hybrids (Table 2). Overall longevity included those aphids that died during nymphal development.

No significant differences in nymphal mortality ($F = 1.609$; $df = 4$; $P = 0.169$) among hybrids were detected (Table 2). The average nymphal mortality ranged between 0.02 ± 0.0 on hybrid ATx631/R10781 and 0.23 ± 0.1 on hybrid M81E (Table 2). The average adult mortality ranged between 0.41 ± 0.1 on hybrid ATx631/R10781 0.53 ± 0.0 on hybrid A05035-C53-6F/UMBRELLA; no significant differences ($F = 1.249$; $df = 4$; $P = 0.328$) among hybrids were observed (Table 2).

Table 2 Development data among different sorghum hybrids. Nymphal development, adult and overall longevity, and nymphal and adult mortality rates of sugarcane aphid reared on five sorghum hybrids were not significantly different.

Developmental Data (mean± SE)					
Sorghum Hybrid	Overall Nymphal development (days)	Adult longevity (days)	Overall Longevity (days)	Nymphal mortality rate	Adult mortality rate
M81E	6.2 ± 0.7	18.3 ± 5.2	25.9 ± 4.8	0.23 ± 0.1	0.43 ± 0.1
ATx631/RTx436	5.7 ± 0.3	16.9 ± 2.2	22.7 ± 2.5	0.12 ± 0.1	0.46 ± 0.1
ATx645/R07007	5.6± 0.3	14.8± 2.9	20.4 ± 3.1	0.08 ± 0.0	0.48 ± 0.0
A05035-C53-6F/UMBRELLA	5.7± 0.3	21.7 ± 4.1	27.6 ± 5.2	0.06 ± 0.0	0.53 ± 0.0
ATx631/R10781	5.6 ± 0.3	15.6 ± 1.9	21.2 ± 2.2	0.02 ± 0.0	0.41 ± 0.1
AVERAGE	5.8 ± 0.1	17.3 ± 1.3	23.2 ± 1.4	0.10 ± 0.0	0.46 ± 0.0

No significant differences were detected for the female pre-reproductive period among hybrids ($F = 0.286$; $df = 4$; $P = 0.883$) (Table 3). On average, adults reproduced 2.7 ± 0.1 days after adult emergence across all hybrids (pre-reproductive period). No significant differences in total number of nymphs per female were detected among hybrids ($F = 0.988$; $df = 4$; $P = 0.6643$) (Table 3). The total number of nymphs per female ranged from 27.6 ± 6.2 on ATx631/RTx436 to 45.9 ± 9.0 on A05035-C53-6F/UMBRELLA. On average, adults produced 35.2 ± 3.6 nymphs across all hybrids (Table 3). The number of nymphs per female per day was not significantly different among hybrids ($F = 0.988$; $df = 4$; $P = 0.6643$) (Table 3). The number of new nymphs per day ranged from 1.8 ± 0.4 on hybrid ATx631/R10781 to 2.5 ± 0.2 on hybrid M81E. On average 2.0 ± 0.1 nymphs per female per day were born across all hybrids. In general, the reproduction periods lasted as long as the adult lived.

There were no significant differences among reproductive rates ($F = 0.615$; $df = 4$; $P = 0.6643$) or the intrinsic rate of increase ($F = 0.3344$; $df = 4$; $P = 0.855$) across the 5 different hybrids (Table 3). The reproductive rates ranged between 23.3 ± 0.7 on ATx631/RTx436 and 35.9 ± 5.7 on A05035-C53-6F/UMBRELLA and the overall average was 31.0 ± 2.8 across all hybrids (Table 3). The intrinsic rate of increase ranged between 0.12 ± 0.0 on M81E, ATx631/RTx436, ATx645/R07007, and ATx631/R10781 and 0.13 ± 0.0 on A05035-C53-6F/UMBRELLA and the average was 0.12 ± 0.0 across all hybrids (Table 3).

Table 3 No significant differences detected in the reproductive data. Pre-reproductive period, progeny, reproductive rate, and estimated intrinsic rate of increase of the sugarcane aphid on five sorghum hybrids.

	Reproductive Data (mean ± SE)				
Sorghum Hybrid	Pre-reproductive period	Mean total number of nymphs (±SE) per female	Mean number of nymphs (±SE) per female per day	Reproductive rate (R_0) (±SE)	Estimated intrinsic rate of increase (r_m) (±SE)
M81E	2.5 ± 0.4	43.1 ± 14.8	2.5 ± 0.2	35.8 ± 18.5	0.12 ± 0.0
ATx631/RTx436	2.9 ± 0.2	27.6 ± 6.2	2.1 ± 0.1	23.3 ± 7.0	0.12 ± 0.0
ATx645/R07007	2.7 ± 0.3	32.5 ± 7.0	2.0 ± 0.2	25.3 ± 8.9	0.12 ± 0.0
A05035-C53-6F/UMBRELLA	2.1 ± 0.4	45.9 ± 9.0	2.1 ± 0.4	35.9 ± 5.7	0.13 ± 0.0
ATx631/R10781	2.7 ± 0.0	29.1 ± 5.0	1.8 ± 0.4	34.5 ± 5.5	0.12 ± 0.0
Average	2.7 ± 0.1	35.2 ± 3.6	2.0 ± 0.1	31.0 ± 2.8	0.12 ± 0.0

Aphid survivorship was measured from birth until death. Overall survivorship showed significant differences (F=2.413; df=4; P=0.04672) among hybrids. Post-hoc analyses identified differences between hybrids ATx645/R07007 and A05035-C53-6F/UMBRELLA (Figure 1).

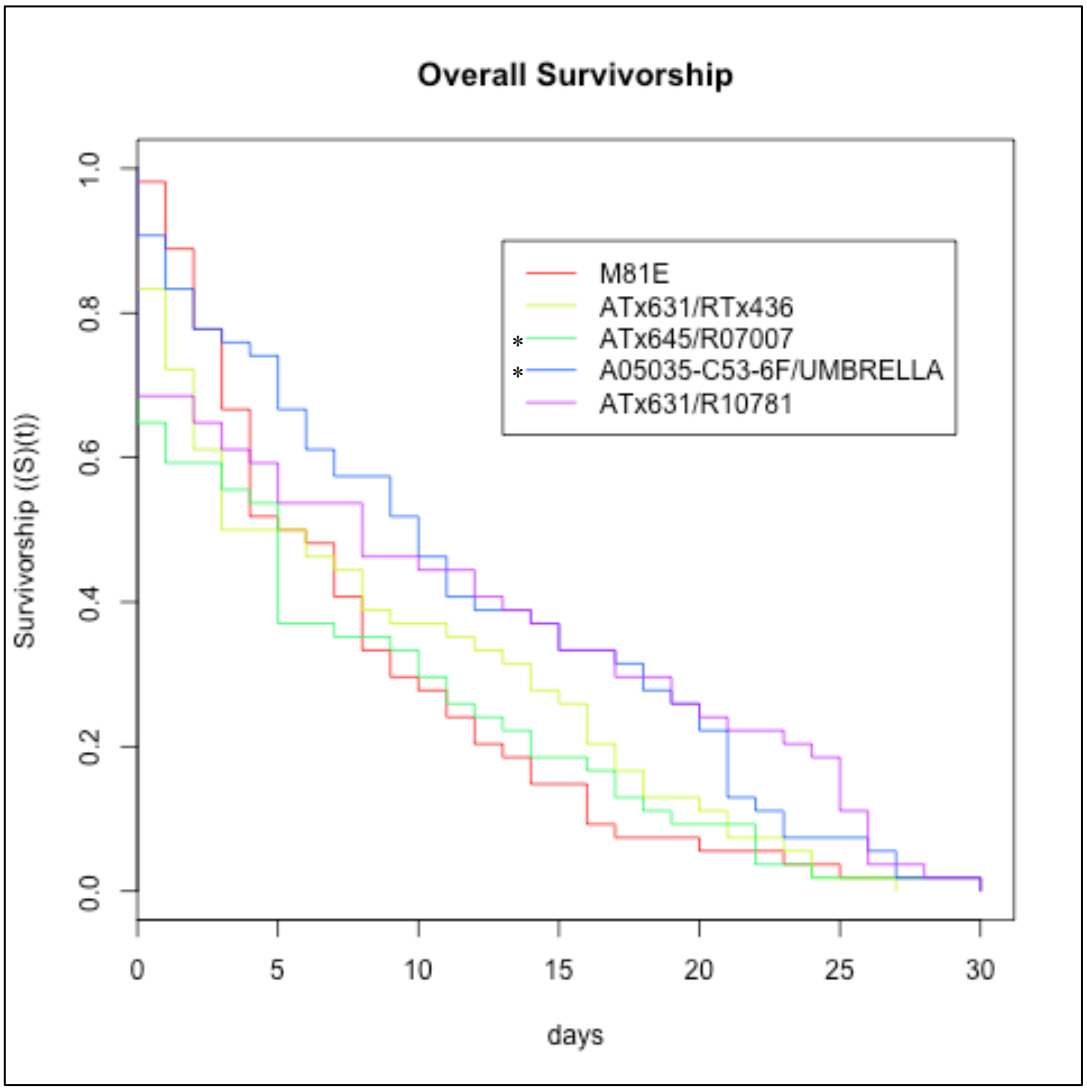

Figure 1 The overall survivorship of the sugarcane aphid. Comparisons among five different sorghum hybrids. Significant differences depicted by asterisks.

Discussion

The sugarcane aphid is an emerging pest of sorghum in the United States, however, it is a known pest of graminous plants worldwide (Singh et al. 2004b). Host plant resistance is an environmentally sustainable solution to control pests. Resistant

plants are those that can avoid, tolerate, or recover from damage due to insect feeding or oviposition (Dent 2000, Berenbaum et al. 2013). Life table analyses can be used to evaluate the reproductive potential of an insect on different cultivars in order to plan and deploy the most adapted strategies to control the pest. Laboratory age-specific life tables also provide standard life table data of the pest than can be used to evaluate host plat resistance (Ruggle and Gutierrez 1995). The amount of time spent at different life stages is directly affected by the amount of food and the climate (Speight et al. 1999). The life table analyses in this study focused on the quality of the hybrids as food since the study was conducted under controlled conditions.

The focus of this study was on sugarcane aphid performance in five different sorghum hybrids. Different sorghum hybrids can display different levels of resistance due to the variety of genetically inherited qualities, such as nutritional differences and plant defenses mechanisms, making some hybrids more susceptible than others (Smith 2005, Van Emden and Harrington 2007). Previous studies on the pea aphid have shown that a lower quality of food (higher resistant plant) resulted in longer development time (Speight et al. 1999). Patterns of resistance were evaluated across the five hybrids upon analyzing insect life table traits such as development and population dynamics (Van Emden and Harrington 2007). No differences in aphid development and reproduction were measured among the five selected hybrids. The hybrids chosen in this study represent different crop uses (photoperiod sensitive, sweet, and grain); therefore the characteristics of these plants are different. Since no differences in aphid development time and reproduction were measured among the five hybrids, the values obtained in this

study provide a standard life table data of this pest. Therefore, this life table analysis is a base for the population growth on sorghum for future studies assessing the temperature effect on sugarcane aphid life table parameters and field studies to test sorghum resistance and/or tolerance.

Differences in aphid overall survivorship were found between hybrids ATx645/R07007 and A05035-C53-6F/UMBRELLA. Aphids had the lowest survivorship on hybrid ATx645/R07007. The hybrid ATx645/R07007 (grain sorghum) is a cross between the female ATx645 (grain sorghum) and male R07007 (high-biomass sorghum pollinator). In a previous study, both parents had evidenced high yield (PACKER 2011, Ben-Israel et al. 2012). This high-biomass sorghum, ATx645/R07007, showed a higher level of insect resistance to the sugarcane aphids when compared to A05035-C53-6F/UMBRELLA providing some insight into discovering additional sources of resistance. Resistance to other cereal aphids exist within sorghum germplasm (Andrews et al. 1993), and are being tested for sugarcane aphid resistance (Bayoumy et al. 2015). Our results are encouraging and show that further testing of sorghum germplasm needs to be conducted to identify resistance against the sugarcane aphid. Future work being completed will analyze genetic resistance across a greater number of hybrids, including those resistant hybrids identified after completion of this study. Similarly in the future trials in field conditions will be completed.

CHAPTER III

FIELD TRIAL EVALUATION OF *SORGHUM BICOLOR* (L.) MOENCH (CYPERALES: POACEAE) GERMPLASM RESISTANCE TO *MELANAPHIS SACCHARI* (ZHETNER) (HEMIPTERA: APHIDIDAE)

Introduction

Sorghum bicolor (L.) Moench (Cyperales: Ponaceae) is both a cereal and fodder crop, and is in the top five grains grown worldwide (Ghani et al. 2015). *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), or the sugarcane aphid is a new invasive pest of sorghum in southern U.S. The novelty and the speed of invasion of this pest caught the industry by surprise, and today few control options are available, mainly relying on chemical control.

Discovery of resistant sorghum hybrids to sugarcane aphid are important to find longer lasting control strategies. The use of resistant and tolerant hybrids is an important Integrated Pest Management (IPM) tool. To be considered resistant, a hybrid must produce a greater yield when the insect pest is present than the susceptible, or if the pest is absent the yield needs to be greater than or equal to that of the susceptible plant (Farrell 1977). Yields produced by resistant graminous plants have shown to provide a higher return per dollar than those that use pesticides to control for insects (Smith 2005). Sorghum hybrids resistant to other cereal aphids such as the greenbug *Schizaphis graminum* have been identified (Armstrong et al. 2015). The objective of the present field study was to identify resistant hybrids to the sugarcane aphid among 12 hybrids in field conditions and under natural insect pressure.

Methods and materials

***Land Preparation*:** The field plot was located in Burleson County, TX on the Texas A&M University farm. BH-Genetics and Golden Acres gave us a total of 14 hybrids to test to identify resistant hybrids to sugarcane aphid. Hybrids 101 through 112 are commercially available grain sorghum hybrids. Four hybrids, ATx3408/RTx2783 ATx3408/RTx436, ATx3409/R12169, and ATx3409/RTx436, were included as SCA tolerant checks. Parents, Tx2783, Tx3408, and Tx3409, are registered as sugarcane aphid tolerant lines (Mbulwe et al. 2016). Hybrids developed with Tx436 are believed to be tolerant, but the level of tolerance is uncertain (Mbulwe et al. 2016). Greenhouse non-choice test and a field study showed that RTx2783 was a good resistant genotype against sugarcane aphids (Armstrong et al. 2015).

After conventional tillage, a pre-plant application of glyphosate (1 qt/acre) and atrazine (1 qt/acre) was performed. Orange flag stakes (Home Depot, Atlanta, GA) were used to map out the sorghum plots within the field for planting hybrid seeds. Sorghum hybrids were planted in 4 replicate paired two-row plots using a 2-row John Deere Max Emerge Plus planter fitted with belt cone seed meter (John Deere, Moline, IL). The plots were planted on beds with 30 inch spacing, 30 ft. in length with 4-foot alleys. The seeding rate was 55,000 seeds/acre. Hybrid Dekalb DKS 53-53 served as a two-row border separating the paired hybrids, this allowed for application of insecticide to aphid-free checks. On May 15th, 90 lbs/acre of liquid urea ammonium nitrate was applied. On July 3rd, Transform® (Dow AgroSciences, Indianapolis, IN) was applied at an active ingredient rate of 1.0 ounce/acre to aphid-free checks. On July 20th, Sivanto® (Bayer

CropSciences, Leverkusun, Germany) was applied at an active rate of 8.0 ounces/acre on half of the plots, later called controlled aphid plots. The rest of the plants remained untreated for the entire experiment, later called experimental plots.

***Experimental Procedures*:** Three weeks after seeds were planted, five plants per plot were chosen at random and marked with 1 in. orange flagging tape (Home depot, Atlanta, GA) to mark which plants would be used for recording aphid pressure, and soil plant analysis development (SPAD) meter readings. A leaf from both the bottom and the top of each flagged plant were chosen at random, and the number of aphids present on both sides of the leaves was recorded for both the controlled aphid plots and the experimental plots. Aphids were counted using a quick check observation guide (Table 4) and recorded weekly (Bowling 2015). The crop developmental stage for each plot was recorded weekly (Vanderlip and Reeves 1972) (Figure 2).

Table 4 Visual assessment of the number of sugarcane aphids per leaf. Estimates were used for data analysis.

Quick Check – Number of Aphids	
Actual Count	Estimate
0-10	----
11-25	18
26-50	38
Threshold to begin control	
51-100	75
101-500	300
501-1000	750
>1001	1500

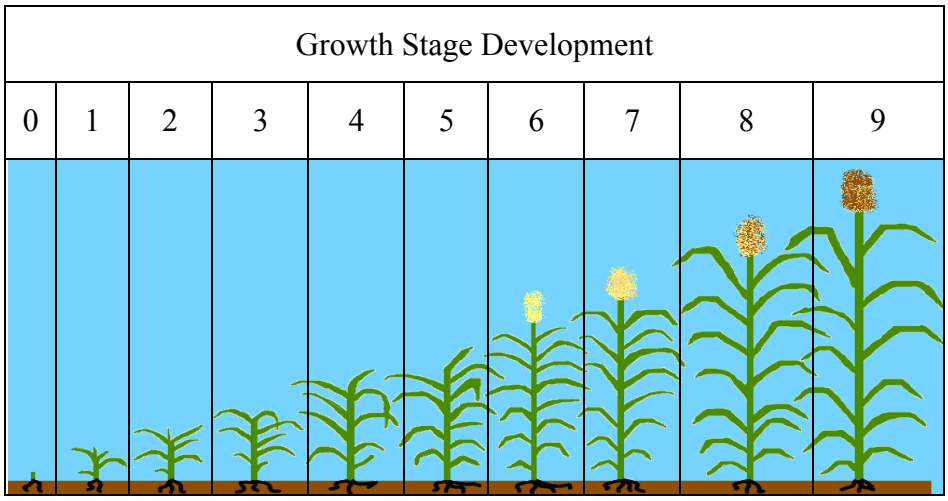

Figure 2 Pictorial guide depicting the growth stages of *Sorghum bicolor*

A SPAD -502 Plus Chlorophyll Meter (Spectrum Technologies, Bridgend, United Kingdom) was used to take SPAD readings on both a top and bottom leaves, chosen at random, of each flagged plant weekly. SPAD readings were taken to estimate the chlorophyll concentration as an indicator of plant health (SPAD is an acronym for soil plant analysis development)(Wood et al. 1992). The SPAD meter recorded the "greenness" of plants which is used as an indication of chlorophyll concentration for a plant (Babar et al. 2006). Chlorophyll content is a good indicator of stress in a plant, because it is related to nutritional status (Filella and Penuelas 1994).

Canopy reflectance was measured weekly from June 5th until harvest. The average canopy reflectance reading was taken for each plot using a MSR87 unit (CropScan, Rochester, MN). CropScan MSR software was used to calculate percent reflectance of each band (460-810 nm). Normalized difference vegetation index (NDVI)

was calculated using the canopy reflectance data. NDVI estimates the photosynthetic area of the canopy, measuring the visible and near infrared reflectance from the sun off the sorghum hybrids (Chen et al. 2003, Babar et al. 2006). The healthier the vegetation, the higher the NDVI and SPAD values (Chen et al. 2003). NDVI was calculated based off of the visible light spectrum and near infrared using the following formula (Babar et al. 2006):

n = wavelength

R_n = Reflectance

$NDVI = (R_{780} - R_{670}) / (R_{780} + R_{670})$

Temperature readings were taken using a Fluke 62 MAX handheld Infrared Thermometer (Fluke Corporation, Everett, WA) for each plot. The rainfall, temperature, humidity, wind speed, and wind direction was recorded using a WatchDog 2000 weather station (Spectrum Technologies, Inc., Aurora, IL). A nearby weather station was used to collect data until the WatchDog was installed on June 17th.

Harvesting was performed on August 4th, 2015 using a John Deere 3300 combine (John Deere, Moline, IL), and the average grain weight, moisture content, and test weight was recorded for each plot using Harvest Master Classic GrainGage (Juniper Systems, Sunnyvale, CA). Grain yields were calculated and corrected for moisture, which was 14%.

***Data Analysis*:** To evaluate if sugarcane aphid had an effect on sorghum production characteristics (hybrid tolerance), four replicates per sorghum hybrid were analyzed for aphid population, growth development stages, SPAD readings, NDVI

readings, yield, and percent moisture content for both treatments (pesticide treated and untreated sorghum plants) using T-tests, t.test function in stats library of JMP.

Harvest Master function of Mirus Harvest Software was used to analyze the moisture and yield of each sorghum plot.

Results

***Treatment Analysis*:** Within each hybrid the numbers of aphids were compared between the controlled aphid and experimental sorghum plants. The average number of aphids per plant was not significantly different ($F = 1.51$; $df = 1$; $P = 0.221$) between treatments. Overall, a higher number of aphids were counted on the untreated plants (Figure 3).

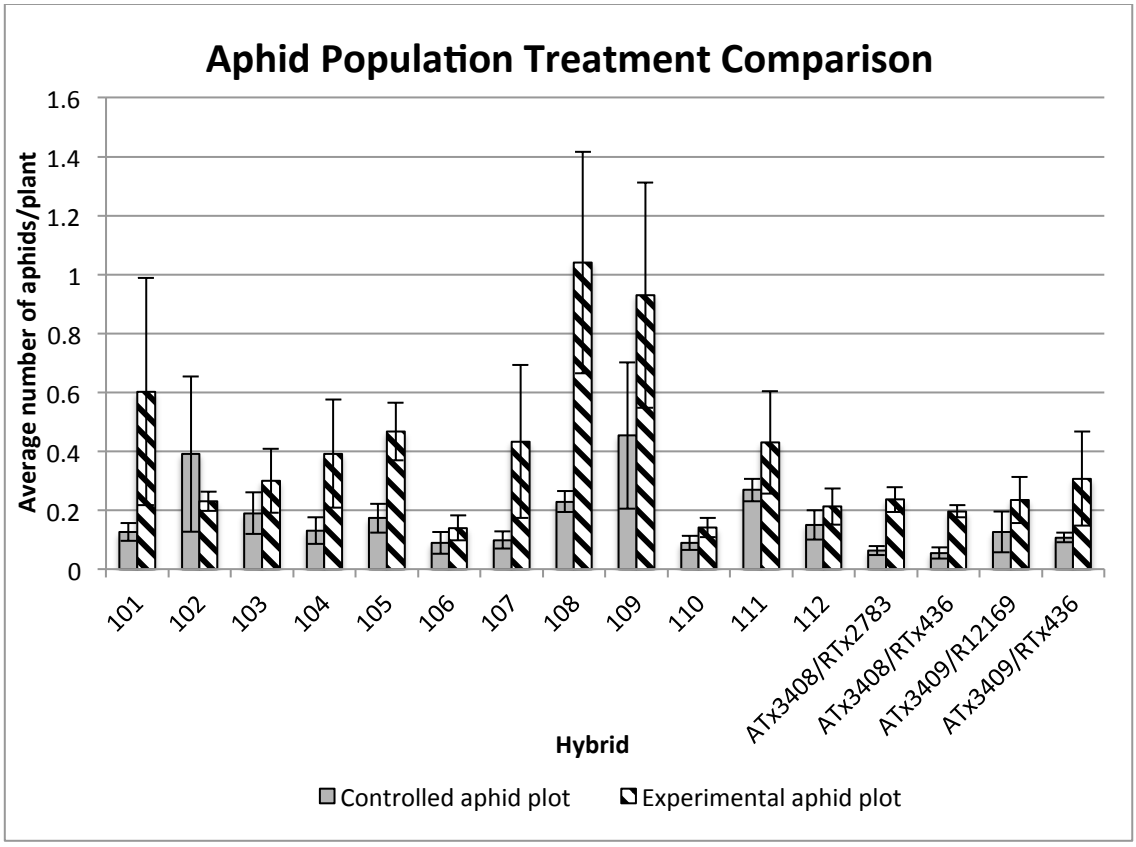

Figure 3 Aphids per plant on controlled and experimental sorghum plots. No significant differences were present among the number of aphids. The number of aphids per plant was averaged across all sorghum hybrids.

The amount of rainfall was averaged across the week of recording data and it ranged between 0.0±0.0 and 3.1±0.4 inches per week, which resulted in undesirable conditions for sorghum. The number of aphids did not begin rising until rainfall fell below 1 inch (Figure 4).

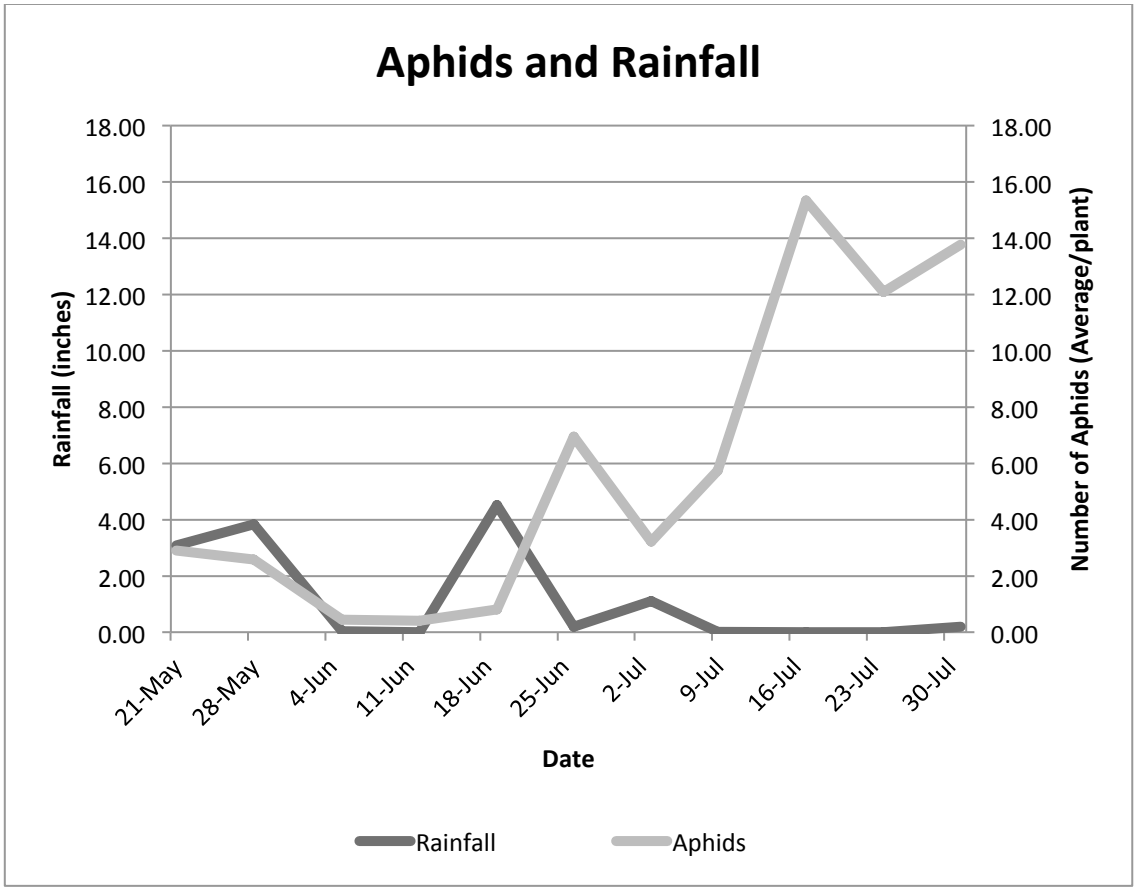

Figure 4 Rainfall in comparison to the average number of aphids per plant. The amount of rainfall measured in inches per week.

Within each hybrid the growth stages of sorghum were compared between the controlled aphid and experimental sorghum plants. The average growth stage per sorghum plots was not significantly different (F = NA; df = NA; P = NA) between treatments (Figure 5).

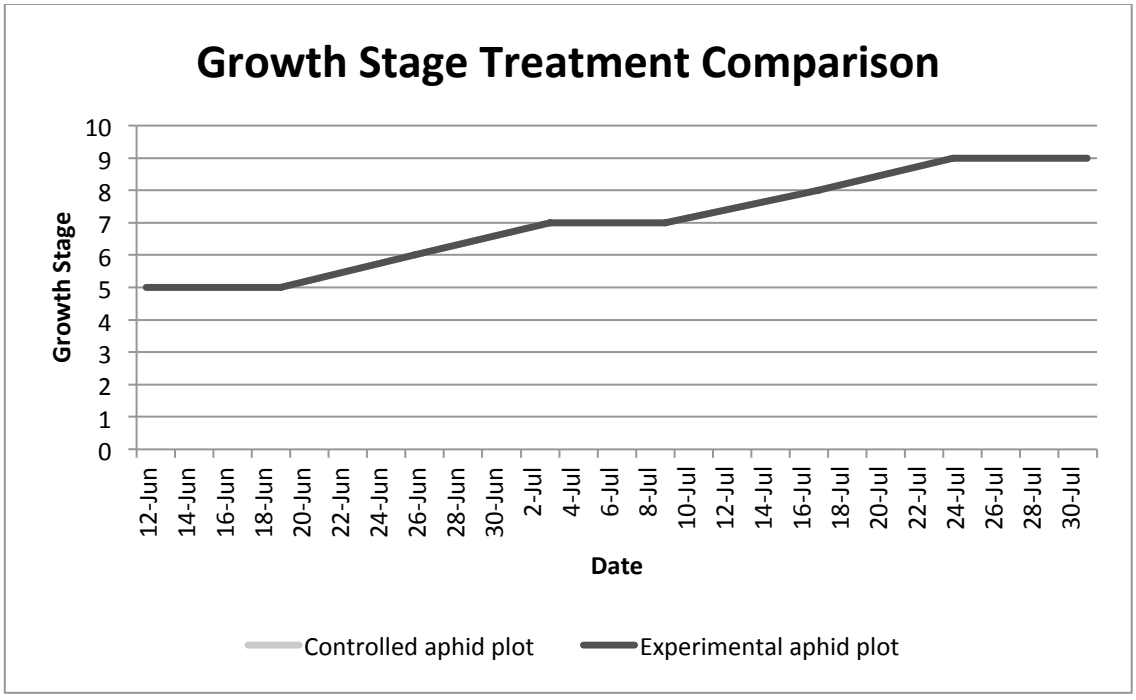

Figure 5 No significant differences present among growth stages. Average sorghum growth stage recorded weekly for both the controlled and experimental aphid plots.

Across all hybrids the NDVI readings were compared between the controlled aphid and experimental sorghum plants. The average NDVI for each sorghum hybrid was not significantly different ($F = 0.1061$; $df = 15$; $P = 0.7491$) between treatments. The average NDVI ranged from 0.70 to 0.75 and an average of 0.73 (Figure 6).

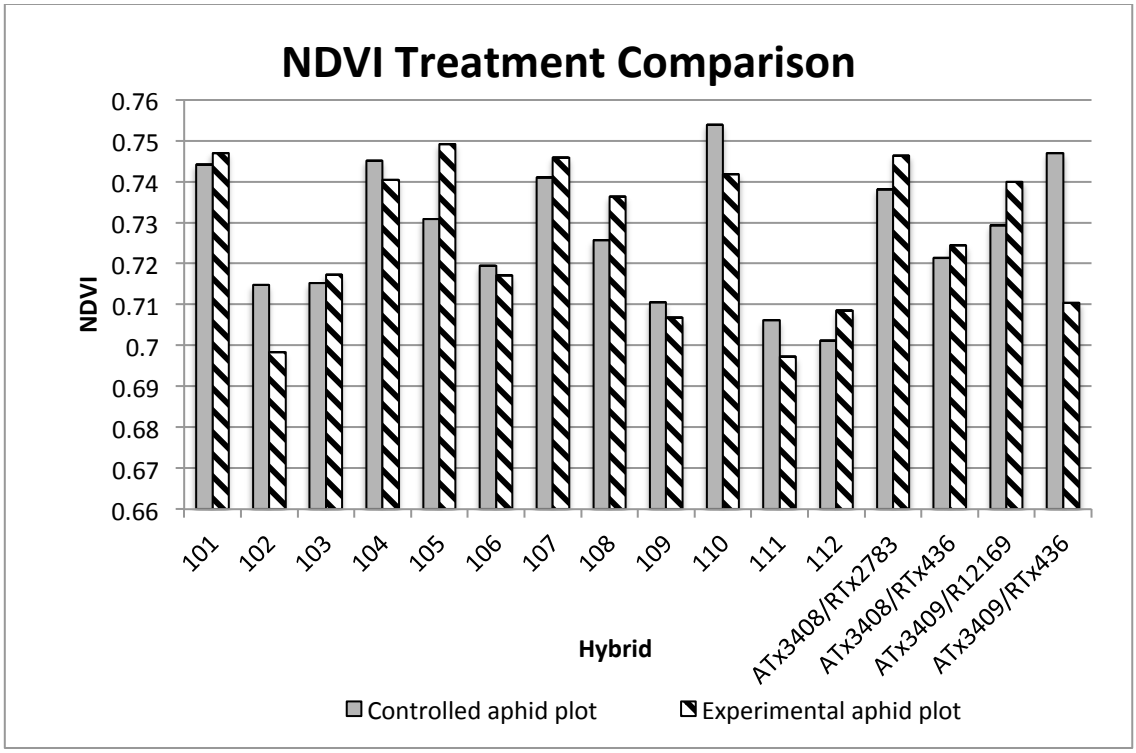

Figure 6 No significant differences present among NDVI readings. NDVI was averaged across each sorghum hybrid for both the controlled and experimental aphid plots.

Within each hybrid the SPAD readings were compared between the controlled aphid and experimental sorghum plants. The average SPAD reading per plant was not significantly different ($F = 4.000$; $df = 15$; $P = 0.0636$) between treatments. The average SPAD reading ranged from 45.88 to 55.34 with an average of 49.89 (Figure 7).

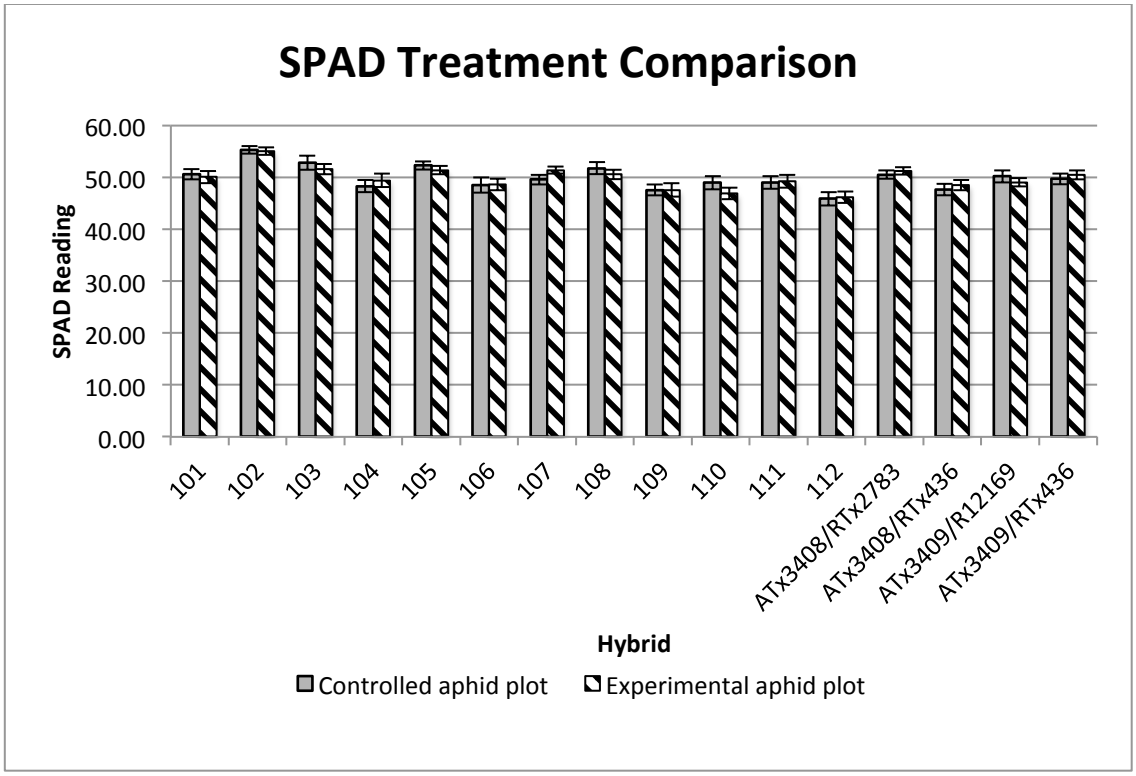

Figure 7 No significant differences present among SPAD readings. SPAD was averaged across each sorghum hybrid for both the controlled and experimental aphid plots.

Within each hybrid the moisture contents were compared between the controlled aphid and experimental sorghum plants. The average number of aphids per plant was not significantly different ($F = 4.000$; $df = 15$; $P = 0.0636$) between treatments. The average moisture content ranged between 14.75% and 19.85% with an average of 16.20% (Figure 8).

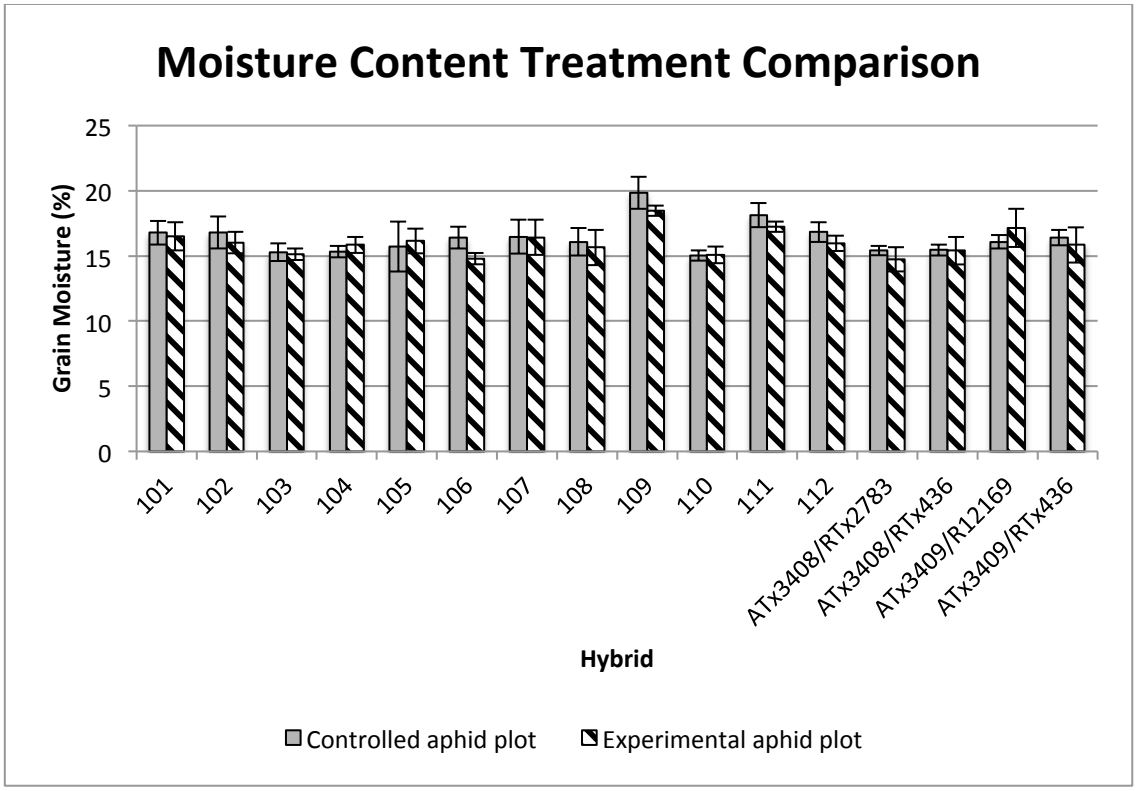

Figure 8 No significant differences present among percent moisture. The percent moisture was averaged across each sorghum hybrid for both the controlled and experimental aphid plots.

Within each hybrid the yields were compared between the controlled aphid and experimental aphid sorghum plants. The average number of aphids per plant was not significantly different ($F = 3.5859$; $df = 1$; $P = 0.0777$) between treatments. The national average for 2015 was 67 bushels per acre of sorghum (Figure 9) (USDA 2015). The yield ranged from 26 to 137 bushels per acre with and average of 86 bushels per acre.

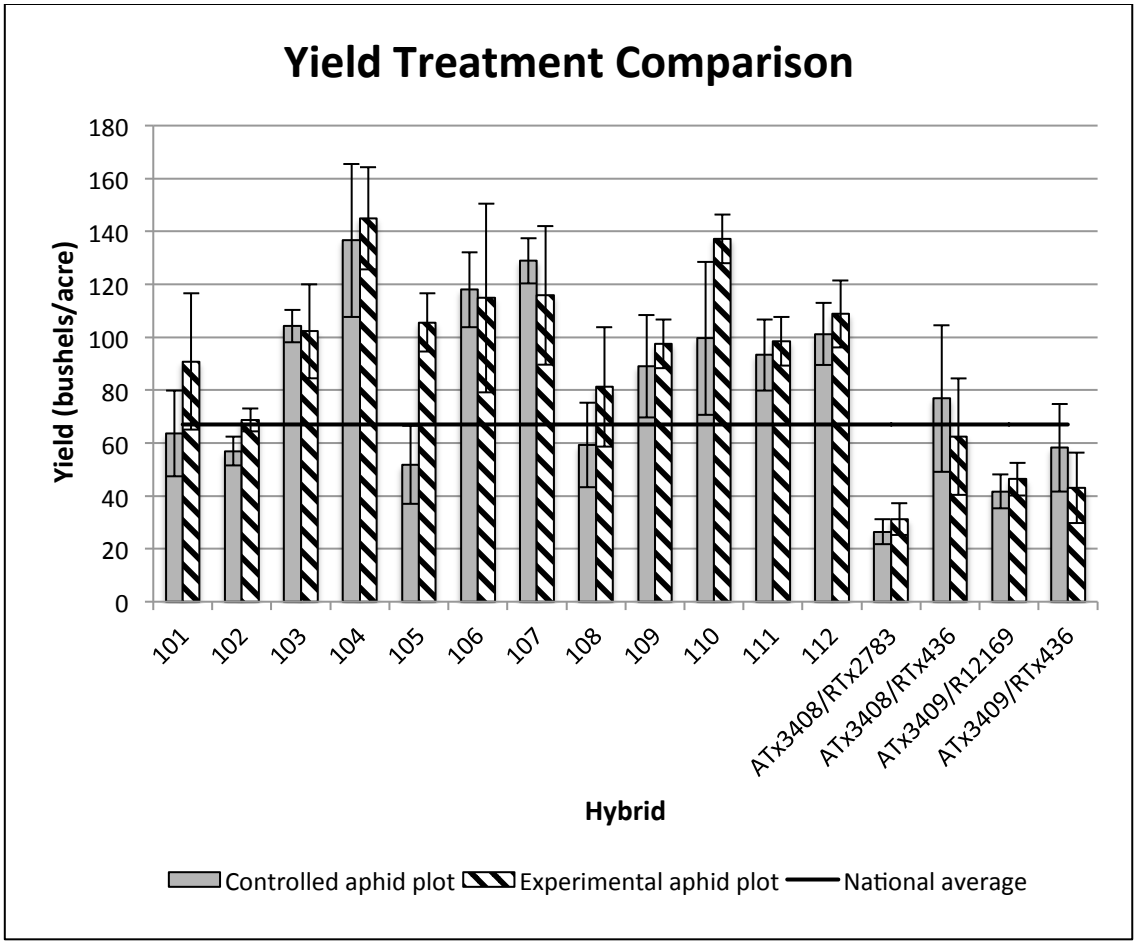

Figure 9 No significant differences present among yield. The yield was averaged across each sorghum hybrid for both the controlled and experimental aphid plots.

Discussion

The goal of this field study was to identify resistant and/or tolerant sorghum hybrids to the sugarcane aphid. This is important because the only control strategy presently available to control this pest is through the application of pesticides. While bioassays in controlled conditions (laboratory) can be used to identify putatively

resistant hybrids, field trials are essential to validate resistance in field conditions and to assess the resistance effect in the yield under high and low insect pressure conditions, since abiotic conditions might affect the expression of resistance. Examples of the abiotic factors evaluated in this study were the amount of rainfall, temperature, and reflectance from the sun.

Characteristics of resistance were evaluated across sixteen hybrids analyzing yield, chlorophyll content (SPAD), reflectance (NDVI), and percent moisture content at harvest, in relation to aphid populations. Insect pressure was very low and no significant differences in the number of aphids or any other characteristics were present between the tolerant and resistant hybrids were observed. This may have been due to the abnormally high amounts of rainfall in Texas and the College Station 2015 growing season. Fifty aphids per plant was the threshold for pesticide application. The average number of aphids across 5 plants for each plot never reached 50. This extreme weather conditions may have had an effect on aphid populations since they were lower than in previous years.

A resistant hybrid could not be determined under the tested conditions though, because the resistant and susceptible hybrids used for checks were not significantly different from one another when analyzing all measurements, or from any of the tested hybrids. By expanding the number of locations and years of testing, resistant hybrids to sugarcane aphids may be determined.

CHAPTER IV

SORGHUM BICOLOR (L.) MOENCH (CYPERALES: POACEAE) DEFENSE AGAINST *MELANAPHIS SACCHARI* (ZHETNER) (HEMIPTERA: APHIDIDAE) EVALUATION

Introduction

Sorghum crop losses related to sugarcane aphids are a result of water loss, necrosis, and loss of combine ability to harvest due to honeydew. Symptoms associated with sugarcane aphid infestations are wilting and chlorosis (Singh et al. 2004).

Defense response genes are induced in the plants through the feeding and ovipositing of the insect (Smith 2005). Several studies have reported that in response to aphids, plants activate genes involved in defenses against bacterial and fungal pathogens (Zhu-Salzman et al. 2004). Jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), and ethylene (ET) are four phytohormones that involved in the activation of these defense genes in response to insect herbivory and other biotic or abiotic stressors. Strategies used by plants to defend against stresses, in particular aphids, have been studied extensively (Zhu-Salzman et al. 2004, Salzman et al. 2005, Walling 2008, Smith et al. 2010, Armstrong et al. 2015).

As plant and herbivore insects co-evolved, they have developed a dynamic competition known as the arms-race (Mello and Silva-Filho 2002). As plants improved their defenses, insects developed strategies to avoid those defenses or to undermine the effect of plant defenses. The outcome of the plant-insect interaction depends on the

ability of the plant to successfully mount defenses that reduce the insect performance and of the insect to counteract the defenses.

The objective of the present study was to identify pathways induced in sorghum in response to sugarcane aphids and to validate if results obtained in other plant-aphid systems could be extrapolated to the study of sugarcane aphid-sorghum. These results could be a stepping-stone towards the deployment of resistant hybrids to this invasive pest.

Methods and materials

***Plant Material*:** Seeds of sorghum hybrid M81E (sweet) were obtained from the Texas A&M Sorghum Breeding program. Seeds were planted individually in cone-tainers (SC7, Greenhouse Megastore, white cells, Danville, IL) in Metro-Mix (900, Sun-Gro Horticulture, Agawam, MA) in the laboratory. The plants were watered every two days after an initial application of 0.09 oz of all-purpose plant food per gallon of water (Miracle Gro, Marysville, OH). Plants were kept under 40 watts T12 plant/aquarium bulbs (General Electric, Houston, TX) set at a long photoperiod of 16:8 (L : D) h by a15-AMP light timer (Utilitech, San Francisco, CA). Once the sorghum was ten days old, a 3.81 x 30.48 centimeters clear plastic tube (Uline, Pleasant Prairie, WI) with high density polyethylene insect mesh on the top opening was placed over each plant and the plants were placed in a Percival Incubator (Percival, Boone, IA) at 16:8 L : D and 23°C for 24 hours prior to aphid infestation

***Aphids*:** An aphid colony was maintained on M81E sweet sorghum in a growth chambers (Percival, Boone, IA) at 16:8 L : D and 23°C.

***Experimental Procedure*:** Thirty adult aphids were placed on each of the six individual sorghum plants each inside a clear plastic tube using a size 4 flat paint brush (Hobby Lobby, Oklahoma City, OK). Three plants without aphids acted as the controls and were brushed to mimic placing the aphids on the plant (Appel et al. 2014). Each plant was caged individually using clear plastic tubes. After 24 hours the sugarcane aphids were removed using a paintbrush and the control plants were brushed as well. The tissues above the sorghum roots were cut off using foil shears (Hobby Lobby, Oklahoma City, Oklahoma), placed immediately in a 3-inch by 3-inch aluminum foil (Kroger, Cincinnati, OH) and flash-frozen by dipping into liquid nitrogen. The samples were kept at -80°C until further processing.

***RNA Extraction And Sequencing*:** RNeasy Mini Kit (Qiagen, Hilden, Germany) was used for RNA extraction and DNA contamination was eliminated with Turbo DNase (Ambion, Life technologies, CA). RNA quality and quantity were tested by agarose gel electrophoresis and Infinite® 200 PRO NanoQuant (Tecan, Männedorf, Switzerland). The RNA was sent for sequencing to Cold Spring Harbor Laboratory. There, four Illumina TruSeq mRNA stranded libraries were synthetized (two from the aphid treatment and two from the control) and were pair-end sequenced on an Illumina NextGen 500.

***Bioinformatics Analysis*:** DNA Subway was used for RNA-sequence analysis using the Tuxedo protocol, deployed by the supercomputer Stampede. Reads were mapped to the sorghum genome (Paterson et al. 2009). Annotation of genes were completed through the use of GO enrichment analysis through the website, Gene

Ontology Consortium and Ensembl Plants. Differentially expressed gene functions were visualized using Mapman (Thimm et al. 2004).

Results and discussion

Transcriptome Sequencing And Alignment: To evaluate sorghum responses to sugarcane aphid herbivory, RNAseq was performed on control plants and 24 h after aphid herbivory. After quality control sequence trimming, a total of 12,310,379 million reads were obtained by 75 bp pair-end sequencing (Table 5). Reads were aligned to the sorghum reference genome (Paterson et al. 2009) using the Tuxedo suite in CyVerse (iPlantcollaborative.org). In all, 87.99% of the reads mapped the sorghum genome (Table 5). Therefore, the quality of the libraries was sufficient for proper transcriptome analysis.

Table 5 Summary of the mapped reads for each individual library

Library Mapped Reads			
Library	Total number of reads	Mapped	Multiple
Control 1	10,866,886	Left: 5,450,822 reads: 87.9% Right: 5,416,064 reads: 87.4%	Left: 6.60% Right: 6.60%
Control 1	44,281,477	Left: 22,129,148 reads: 87.6% Right: 22,152,329 reads: 87.7%	Left: 6.00% Right: 6.00%
Insect 1	24,467,959	Left: 12,209,086 reads: 88.2% Right: 12,258,873 reads: 88.6%	Left: 6.70% Right: 6.70%
Insect 2	12,310,379	Left: 6,120,318 reads: 88.00% Right: 6,190,061 reads: 88.50%	Left: 7.700% Right: 7.60%
Total	91,926,701	87.99%	

Cufflink assemblies were merged using the Cuffmerge script. In total, 38,827 genes and 48,161 transcripts were generated. Of those transcripts, 4,488 (8.7%) were potentially new isoforms and 32,690 (67.9%) completely matched sorghum annotated transcripts. There were 4,520 (9.4%) unknown intergenic transcripts.

GO term enrichment analysis of the 100 most expressed genes from each libraries identified significant enrichment on terms associated with photosynthesis. Among the most enriched terms were, for Biological Process: Photosynthesis (GO:0015979), Photosynthesis, light reaction (GO:0019684), Photosynthesis, light harvesting (GO:0009765), Protein-chromophore linkage (GO:0018298), and Generation of precursor metabolites and energy (GO:0006091); for Molecular Functions: Chlorophyll binding (GO:0016168),and Tetrapyrrole binding (GO:0046906); and for Cellular Component: Photosystem (GO:0009521), Photosynthetic membrane (GO:0034357), Thylakoid part (GO:0044436), Thylakoid (GO:0009579), Photosystem I (GO:0009522), Photosystem II (GO:0009523), and Membrane protein complex (GO:0098796) (Table 6).

Table 6 GO term enrichment analyses in each library. Some of the top100 most expressed genes in each library

GO term enrichment analyses				
Molecular function	Control 1	Control 2	Insect 1	Insect 2
Chlorophyll binding (GO:0016168)	3.68E-20 (>5 enrichment)	1.89E-22 (>5 enrichment)	1.89E-22 (>5 enrichment)	2.57E-20 (>5 enrichment)
Tetrapyrrole binding (GO:0046906)	3.75E-08 (>5 enrichment)	2.69E-09 (>5 enrichment)	3.75E-08 (>5 enrichment)	1.75E-09 (>5 enrichment)
Biological process	Control 1	Control 2	Insect 1	Insect 2
Photosynthesis (GO:0015979)	8.12E-32 (>5 enrichment)	1.71E-37 (>5 enrichment)	1.95E-39 (>5 enrichment)	4.6E-34 (>5 enrichment)
Photosynthesis, light reaction (GO:0019684)	1.63E-18 (>5 enrichment)	7.18E-24 (>5 enrichment)	7.18E-24 (>5 enrichment)	1.7E-20 (>5 enrichment)
Photosynthesis, light harvesting (GO:0009765)	4.62E-17 (>5 enrichment)	2.01E-19 (>5 enrichment)	2.01E-19 (>5 enrichment)	1.45E-19 (>5 enrichment)
Protein-chromophore linkage (GO:0018298)	1.93E-13 (>5 enrichment)	1.31E-15 (>5 enrichment)	1.31E-15 (>5 enrichment)	9.79E-16 (>5 enrichment)
Generation of precursor metabolites and energy (GO:0006091)	2.03E-17 (>5 enrichment)	3.65E-22 (>5 enrichment)	3.65E-22 (>5 enrichment)	3.16E-19 (>5 enrichment)
Cellular compartment	Control 1	Control 2	Insect 1	Insect 2
Photosystem (GO:0009521)	6.69E-33	4.16E-35	1.32E-39	1.18E-37
Photosynthetic membrane (GO:0034357)	2.71E-32	3.83E-36	4.29E-40	1.44E-34
Thylakoid part (GO:0044436)	1.79E-31	2.97E-35	3.90E-39	1.03E-33
Thylakoid (GO:0009579)	3.65E-29	1.41E-34	1.97E-36	2.65E-31
Photosystem I (GO:0009522)	5.92E-27	5.92E-27	5.92E-27	3.86E-27
Membrane protein complex (GO:0098796)	2.72E-25	9.89E-29	1.72E-30	4.50E-29
Photosystem II (GO:0009523)	1.54E-22	1.03E-24	3.62E-29	3.86E-27

Several transcripts encoding proteins involved in photosynthesis were among the most expressed in all libraries. Those included *Sb09g028720.1,* Sb03g027040.1, Sb01g015400.1, Sb04g004770.1 and Sb10g023930.2 encoding photosystem II light harvesting complex proteins, and Sb07g005660.1 encoding Photosystem II subunit R. Since all libraries were constructed from leaf samples, it was important to validate that the obtained transcriptional profile had the characteristic of other leaf transcriptomes, which are characterized, by high expression level of photosynthesis-related genes.

***Differentially Expressed Genes*:** In this study, a total of 416 genes differentially expressed (Q value < 0.05) in response to aphid herbivory were identified. Fold change was determined to be at least 2.66 for each of those genes and at most 37.99. Of those, 111 were down-regulated in response to aphid herbivory, while 306 were up-regulated.

Based on GO term enrichment analysis, among the up-regulated genes there was significant enrichment of terms associated with immunity such as detection of biotic stimulus (GO:0009595), regulation of immune system process (GO:0002682), immune response (GO:0006955), regulation of immune and innate immune response (GO: GO:0050776 and GO:0045088), defense response to bacterium (GO:0042742) and to fungus (GO: GO:0050832), incompatible interaction (GO:0009814), regulation of plant-type hypersensitive response (GO:0010363), programmed cell death (GO:0008219), host programmed cell death induced by symbiont (GO:0034050), regulation of programmed cell death (GO:0043067), negative regulation of cell death (GO:0060548), MAPK cascade (GO:0000165), and Calcium ion binding molecular function (GO:0005509) (Figure 10 and 11).

Among the down-regulated genes there was significant enrichment in Biological process terms associated with cell wall biosynthetic processes such as cellulose biosynthetic process (GO:0030244) and beta-glucan biosynthetic process (GO:0051274), cellulose metabolic process (GO:0030243) amino-acid metabolic processes such as alpha-amino acid metabolic process (GO:1901605) and cellular amino acid catabolic process (GO:0009063), and other metabolic processes such as monocarboxylic acid biosynthetic process (GO:0072330) and porphyrin-containing compound biosynthetic process (GO:0006779); Cellular component terms related to chloroplasts such as chloroplast stroma (GO:0009570) and thylakoid (GO:0009579); there was also enrichment of Molecular function terms associated with cellulose synthesis such as cellulose synthase activity (GO:0016759), cellulose synthase (UDP-forming) activity (GO:0016760), as well as structural constituent of cytoskeleton (GO:0005200) (Figures 10, 12, and 13).

Molecular Function of Differentially Expressed Genes

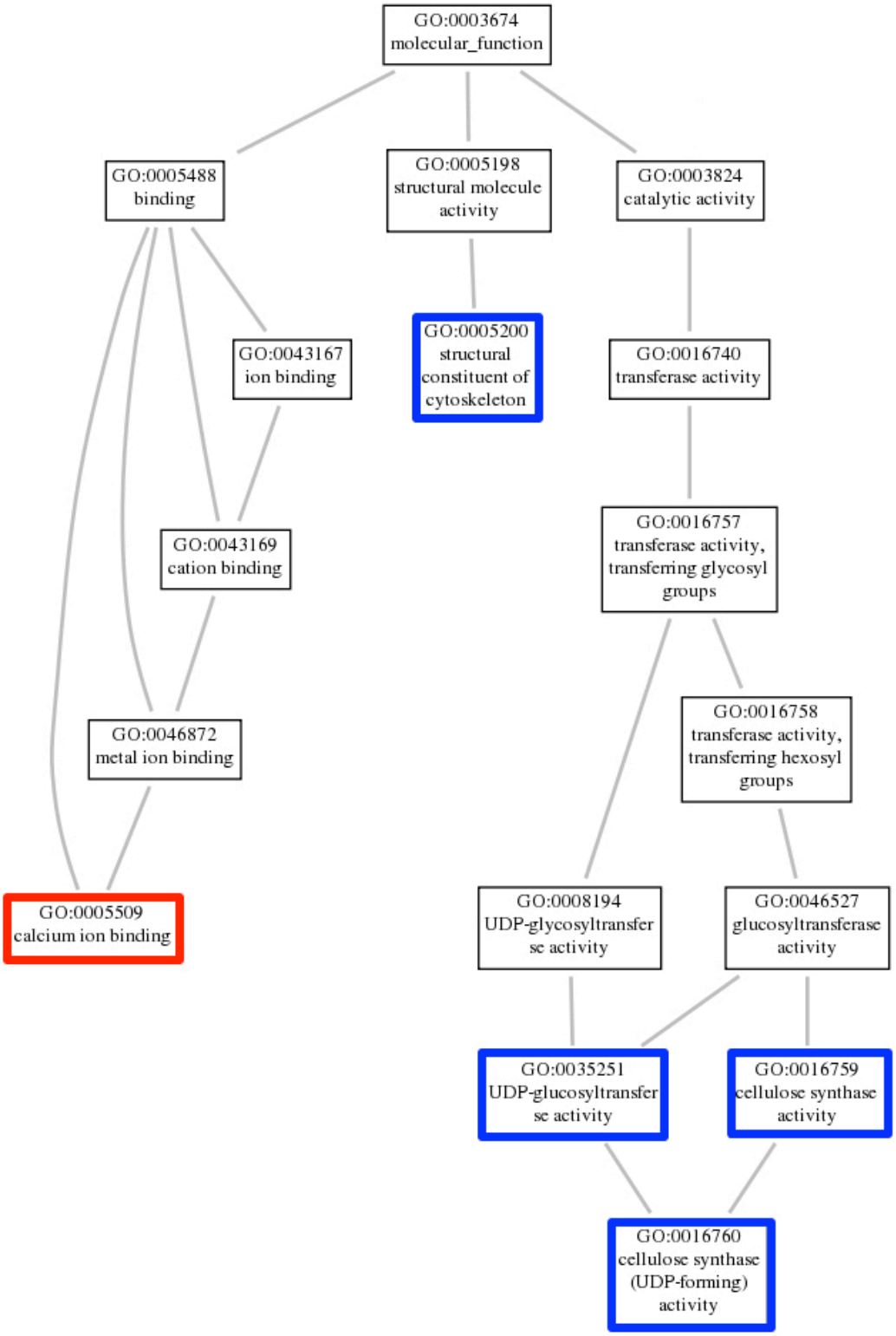

Figure 10 Molecular functions and pathways of differentially expressed genes. Red and blue depict up and down regulated genes accordingly

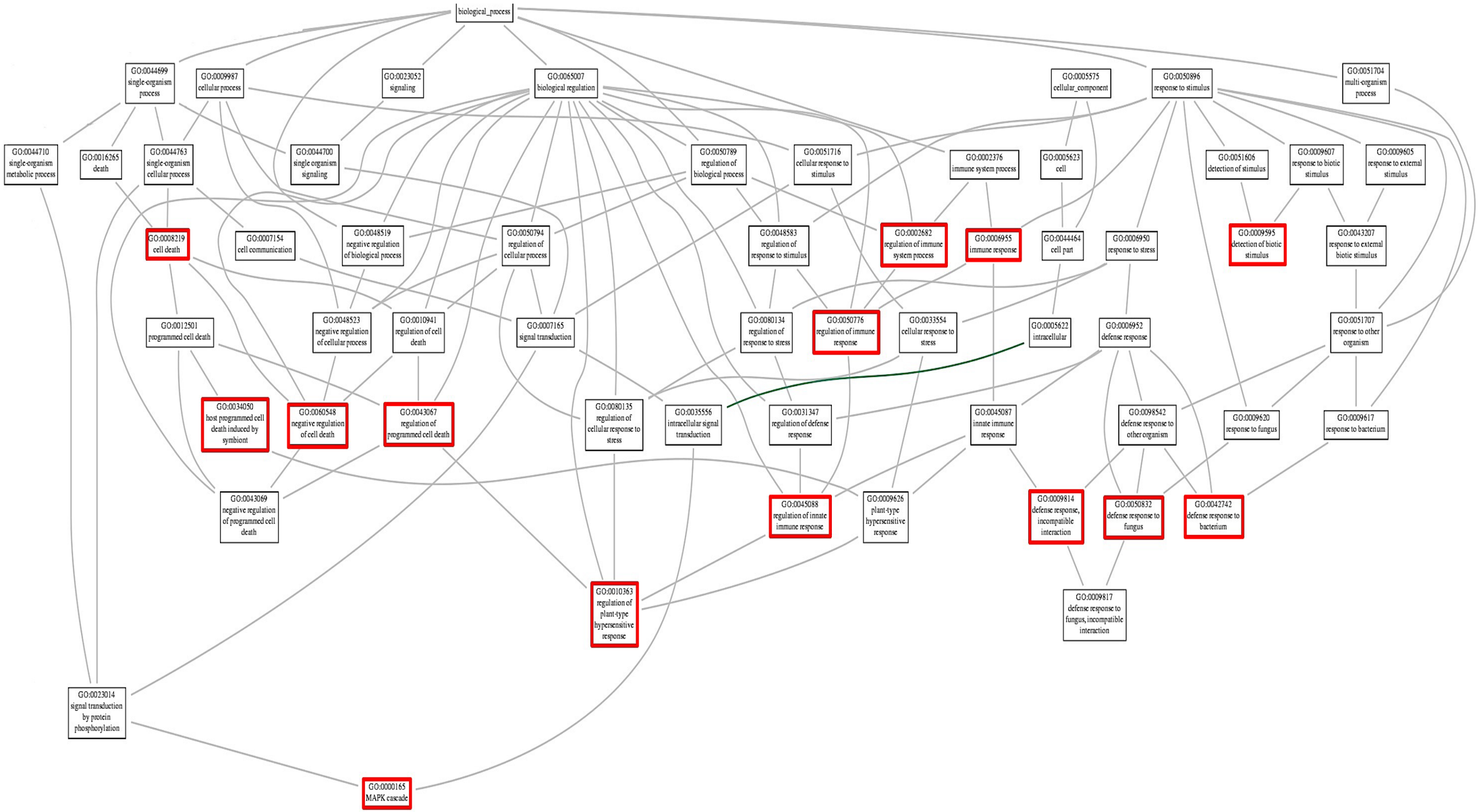

Figure 11 Biological processes and pathways of differentially expressed genes. Up-regulated genes marked with red color.

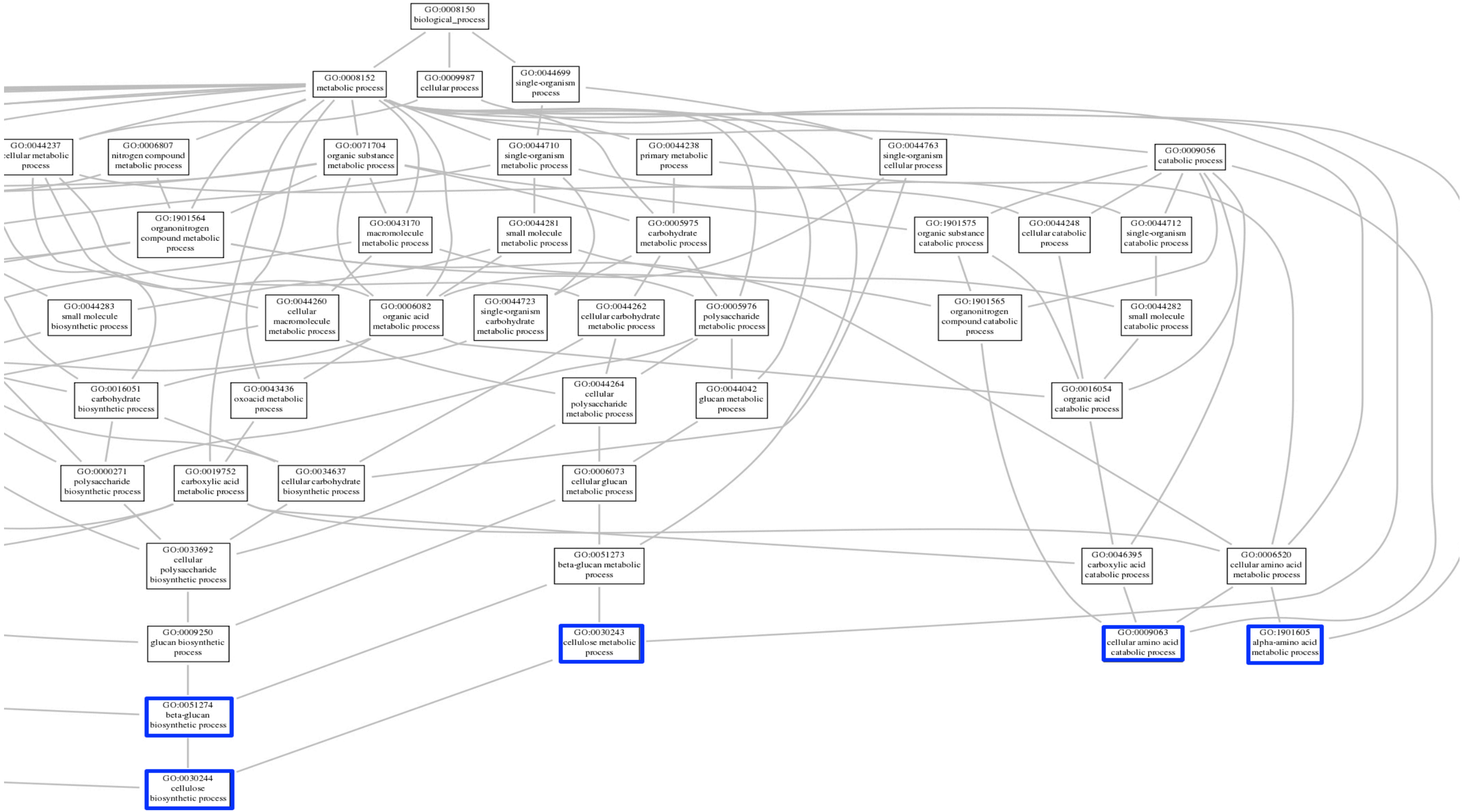

Figure 12 Biological processes and pathways of differentially expressed genes. Down-regulated genes marked with blue color

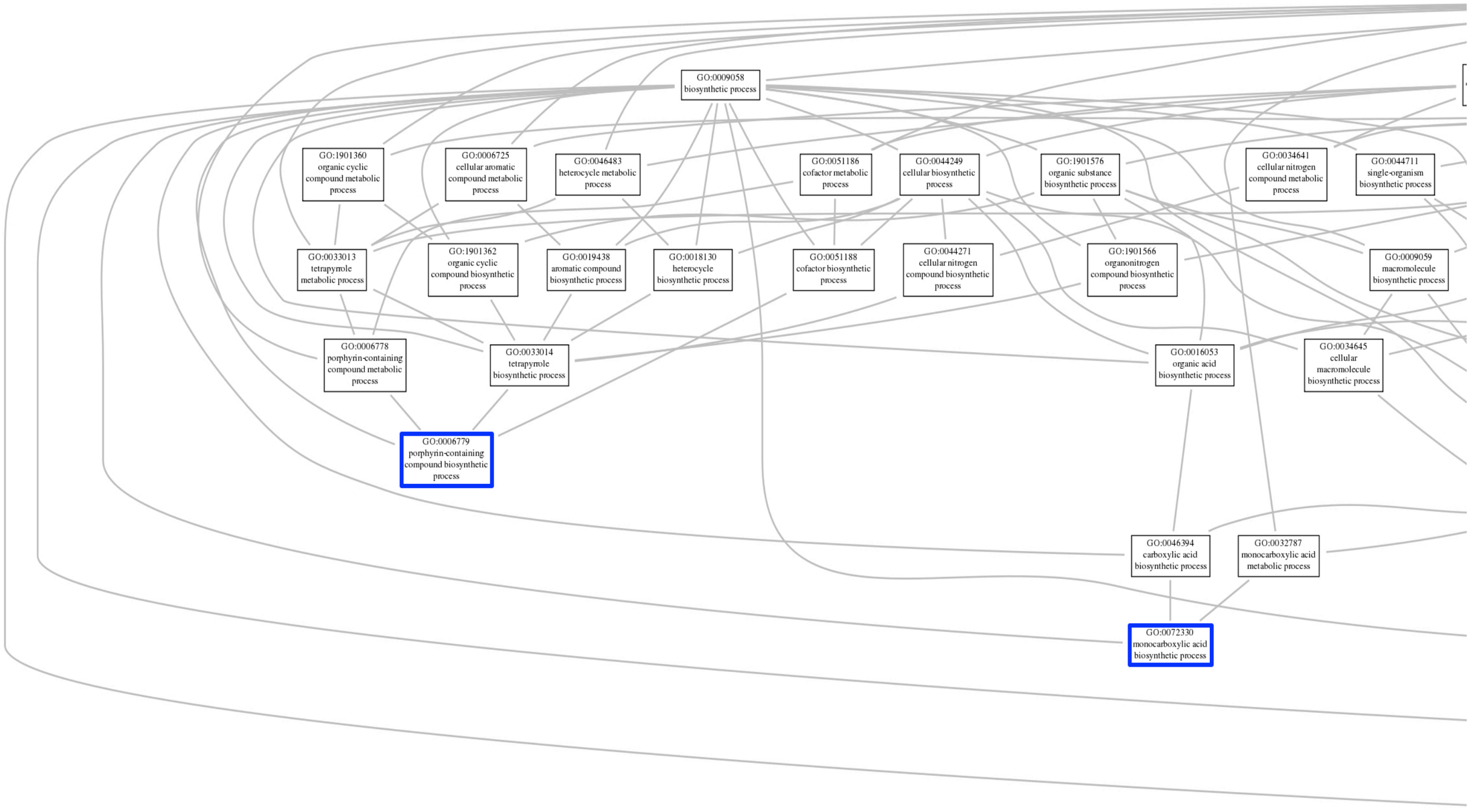

Figure 12 Continued

Cellular Component Pathways of Down-Regulated Genes

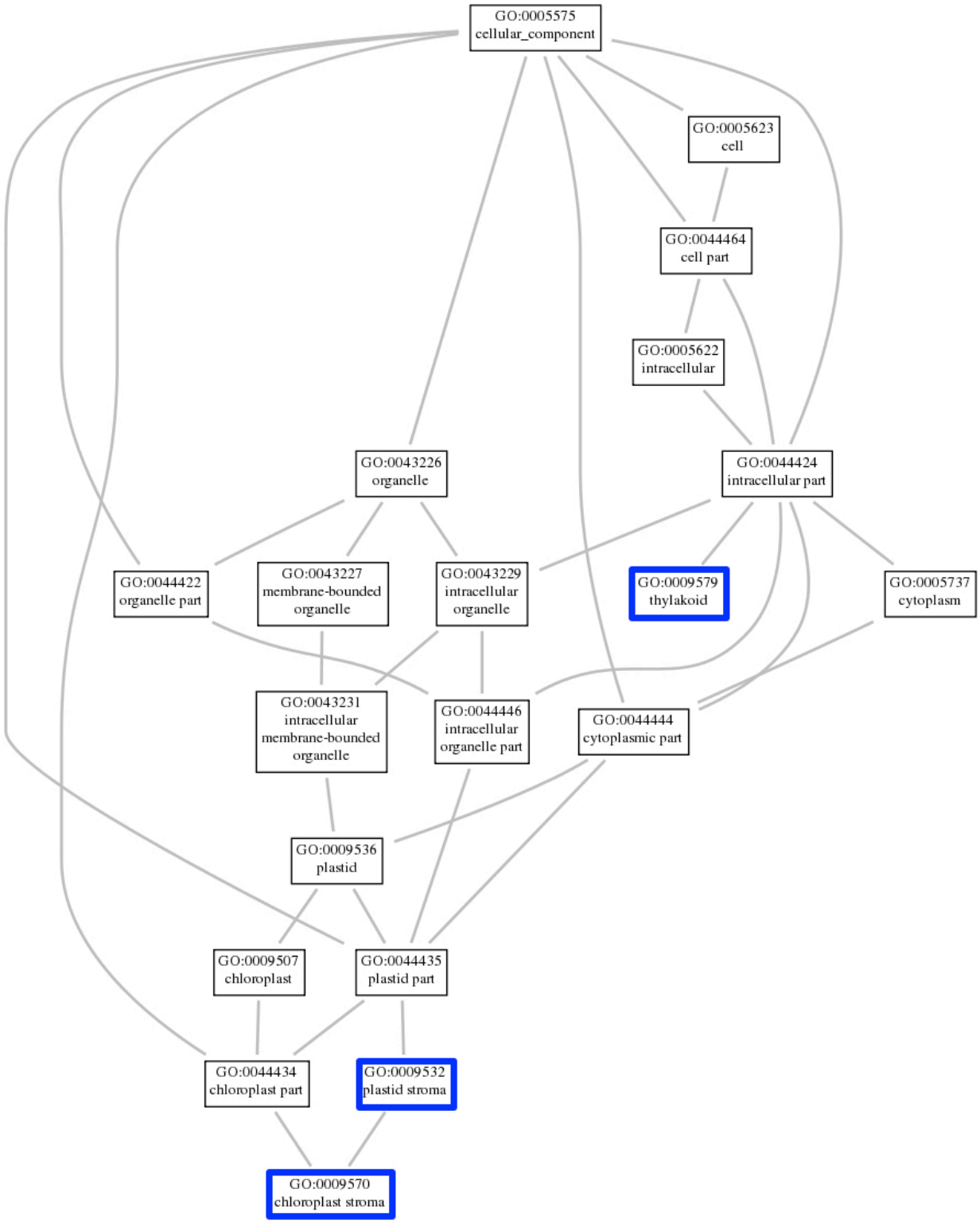

Figure 13 Cellular components and pathways of differentially expressed genes. Blue indicated genes that were down-regulated.

Therefore, sorghum gene expression changes in response to sugarcane aphid were mainly characterized by up-regulation of genes, in particular those involved in signaling in response to stress. It is also interesting to note the down-regulation of genes involved in sugar and amino-acid metabolism.

While aphids are known to cause little damage to plants, several genes with a GO term annotation response to wounding were up-regulated. These genes might be expressed as a result of probing and local penetration of epidermal, mesophyll, and parenchyma cells (Thompson and Goggin 2006). In response to aphids, sorghum plants up-regulated callose synthase and β-1,3-glucanase genes involved in callose deposition and hydrolysis, respectively. Callose is deposited in sieve elements as a defense mechanism against phloem feeding insects. In resistant rice callose remains intact in response to *Nilaparvata lugens* (brown planthopper) while in susceptible plants, activation of β-1,3-glucanases results in unplugging of the sieve tube occlusions (Hao et al. 2008).

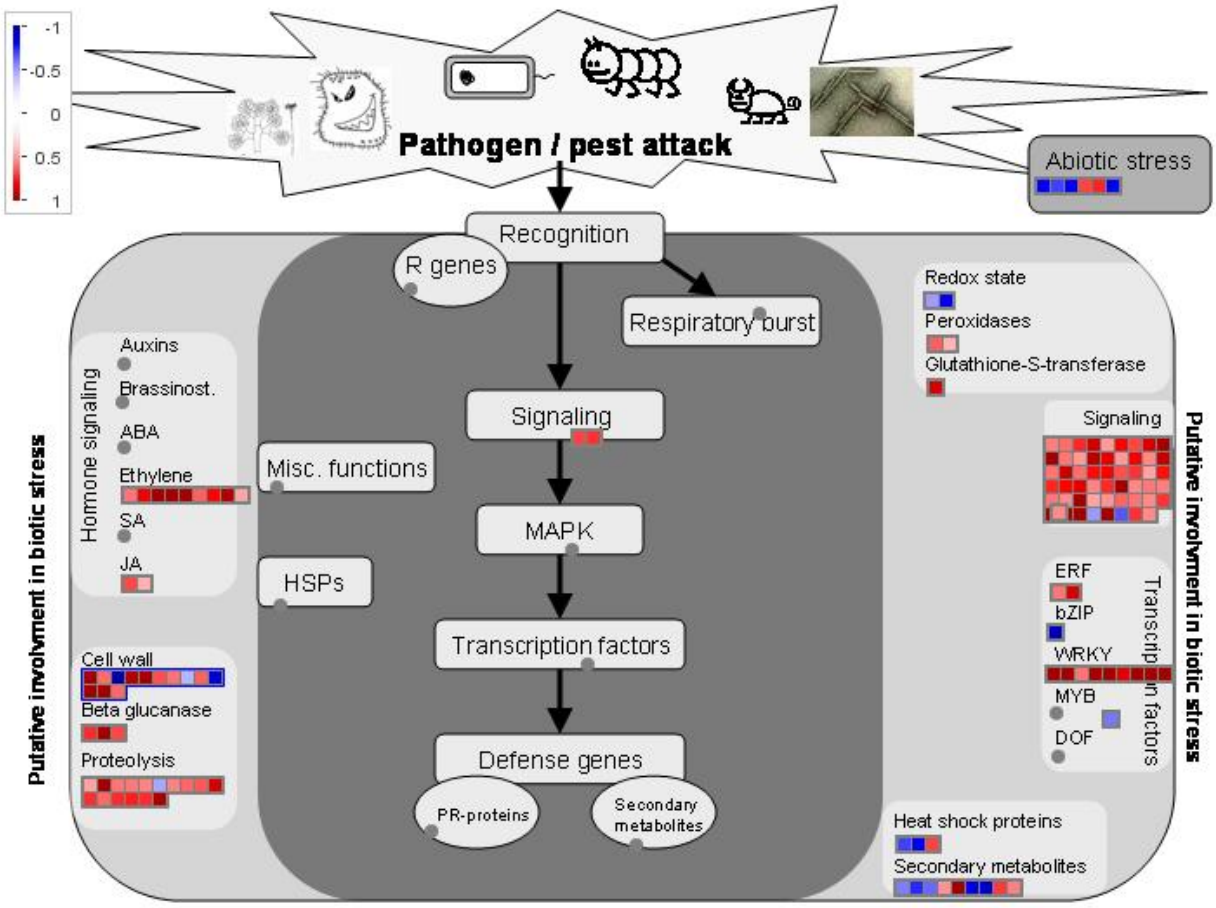

Figure 14 Genes associated with biotic stress pathway. Up-regulated and down-regulated genes are marked by the colors red and blue respectively.

Herbivory by sugarcane aphids resulted in the induction of transcripts involved in plant signaling (Figure 14). Up-regulation of kinase receptors is an early response to aphid herbivory that occurs within the first two days following infestation (Coppola et al. 2013). Thirty-five receptor-like kinases were up-regulated in response to aphids. Similarly, 13 genes involved in Calcium homeostasis, which is a key component of intracellular signal transduction, were also up-regulated and one was down-regulated.

Therefore, the plant perceived the attack by aphids and different signaling cascades were activated in response to this attack.

A majority of genes were up-regulated by aphid feeding in phytohormone-signaling pathways. Genes involved in JA synthesis and metabolism (LOX, allene oxide synthase and CYP94B3) and in JA signaling (jasmonate-zim-domain proteins, JAZ) were also up-regulated by aphids. The LOX and allene oxide synthase are putative genes involved in the JA synthesis (Figure 14). JAZ proteins are repressors of JA-responsive genes, but they are up-regulated in response to JA (Chini et al. 2007, Thines et al. 2007, Yan et al. 2007). In rice, 9 of 15 Jaz genes were responsive to jasmonic acid and wounding treatments (Ye et al. 2009). While JAZ-proteins and other genes in the JA signaling pathway were up-regulated in this dataset, none of the classical JA-response genes (proteinase or cathepsin D inhibitor, leucine aminopeptidase and threonine deaminase (Dammann et al. 1997)) were induced by sugarcane aphids in sorghum. Several studies have shown the local induction of proteinase inhibitors and other wound-responsive transcripts in response to aphid infestation (de Ilarduya et al. 2003, Zhu-Salzman et al. 2004), however, no proteinase inhibitors were up-regulated in this study. It appears that while JA might accumulate in response to aphid herbivory, JA-responsive genes were not activated.

Interestingly, two PAL genes, involved in one of the SA biosynthetic pathway as well as in several secondary metabolites synthesis, were down-regulated. However, a 4-coumarate: CoA ligase, an enzyme downstream PAL was up-regulated in response to aphids. While not obvious in the Mapman figure, several genes involved in SA response

were regulated by aphids. For instance, PR-1, a SA-dependent gene, was up-regulated in response to aphids. However, NPR3-like protein, a negative regulator of the transcriptional SA-mediated defense response, was also up-regulated in response to aphids. The up-regulation of NPR3 could be involved in limiting the spatial induction of SA-dependent genes or in returning the cell to a *at equilibrium* status following SA-dependent defense activation. No other gene in the SA or the JA signaling pathway were down-regulated.

Therefore, sugarcane aphid herbivory activated jasmonate- and salicylate-mediated gene induction, but as in previous studies on sorghum responses to *Schizaphis graminum* (Zhu-Salzman et al., 2004) or other plant-aphid systems (Coppola et al. 2013), there was high induction of SA-regulated defense genes, and only weak induction of JA-regulated defense genes. Several studies have shown the local induction of proteinase inhibitors and other wound-responsive transcripts in response to aphid infestation (de Ilarduya et al. 2003, Zhu-Salzman et al. 2004), however, no proteinase inhibitors were up-regulated in this study.

Nineteen genes involved in ABA transport, signaling or response were up-regulated in response to aphids. There were 15 other up-regulated genes potentially involved in salt stress or water balance. There were 15 down-regulated genes potentially involved in ABA signaling, water or salt response. Therefore, it appears that sugarcane aphids affected sorghum water balance. Eight genes potentially involved in ethylene biosynthesis and signaling were up-regulated by aphids (Figure 14).

Induction of several genes encoding enzymes linked to the oxidative burst in response to aphid herbivory has been found in different studies. Few genes involved in ROS detoxification were up-regulated in sorghum in response to sugarcane aphids: two peroxidases, one glutathione S-transferase and one lipoxygenase (Figure 14). Therefore, in light of the reduced number of regulated genes involved in oxidative stress, it appears that in this response in sorghum was limited when compared to other studies (Argandona et al. 2001, Ni et al. 2001, Park et al. 2006, Gutsche et al. 2009, Smith et al. 2010, Coppola et al. 2013, Prochaska et al. 2015).

In conclusion, sorghum recognized sugarcane aphid attack and mounted defenses. However, those responses were insufficient to deter aphid feeding. For instance, callose deposition blocking phloem flow was induced but apparently not maintained. Very few JA-mediated defenses were activated while those have been found to be efficient against aphids.

CHAPTER V

CONCLUSION

Pesticides are the presently means of sugarcane aphid control on sorghum. A life table analysis of the sugarcane aphid is a first step to better understand the biology of the sugarcane aphid when feeding on various sorghum hybrids without additional environmental factors to influence their life cycle. One requirement for this analysis was that seeds could not be treated with insecticides. Only five hybrids were available that met these requirements. They were either sweet, grain, or photoperiod sensitive hybrid, representing different crop uses. The focus of this first objective was to evaluate patterns of sorghum hybrids resistance to the sugarcane. Differences in aphid overall survivorship were found between hybrids ATx645/R07007 and A05035-C53-6F/UMBRELLA. No differences in aphid development and reproduction across the different hybrids were measured. Therefore, this life table analysis is a good base for the population growth and future studies on sorghum. Since no differences in aphid development time and reproduction were measured among the five hybrids, the values obtained in this study provide a standard life table data of this pest. Future testing needs to be completed at different environmental conditions as well as evaluating sugarcane aphid performance on the five hybrids when planted in the field.

The second objective was a field study testing commercial sorghum hybrids given by a specific company. The goal of this field study was to identify resistant and/or tolerant sorghum hybrids to the sugarcane aphid. Yield, chlorophyll content (SPAD), reflectance (NDVI), and percent moisture content at harvest, in relation to aphid

populations were characteristics evaluated for resistance. Due to low aphid pressure caused by abnormally high amounts of rainfall, no significant differences in the measured parameters were observed. A resistant hybrid could not be determined under the tested conditions. By expanding the number of locations and years of testing, resistant hybrids to sugarcane aphids may be determined.

A transcriptome analysis was performed on sorghum to identify biochemical pathways induced in response to sugarcane aphid herbivory. A majority of the genes were up-regulated in response to aphid feeding, resulting in the induction of transcripts involved in plant signaling. Kinase receptors as well as genes involved in calcium homeostasis, involved in early response to aphid herbivory, were up-regulated (Coppola et al. 2013). Few genes involved in ROS detoxification were up-regulated in sorghum in response to sugarcane aphids. Sorghum plants recognize sugarcane aphid herbivory, mounted defenses, but those were insufficient in deterring aphid feeding.

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