EVALUATING HOST-PLANT RESISTANCE AGAINST SUGARCANE APHID (*Melanaphis sacchari* (Zehntner)) IN SORGHUM (*Sorghum bicolor* (L.) Moench)

A Dissertation

by

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ABSTRACT

The sugarcane aphid (*Melanaphis sacchari* (Zehntner)) is an established and problematic pest on sorghum (*Sorghum bicolor* (L.) Moench) in the United States. The virulent pest on sorghum was initially identified in Southeast Texas and significantly affects production. Heavy infestation will decrease yield and quality of grain and forage sorghum. The aphid's sticky honeydew secretions cause harvest losses and clogging of combines. Using artificial and natural infestations, 500 lines from Texas A&M AgriLife Research were evaluated, including mechanisms of resistance and phenotypic traits useful for breeding.

Resistant lines A/B.Tx3408 and A/B.Tx3409 were identified, and released to the public in 2016. Grain and forage sorghum hybrids produced using resistant lines also exhibited resistance. The resistant lines and hybrids produced from resistant sources were subsequently evaluated for their relative agronomic and breeding value. The performance of resistant hybrids was better than susceptible hybrids under sugarcane aphid infestation. The mechanisms of resistance were identified as antibiosis and antixenosis (non-preference). Some phenotypic traits also influenced aphid damage.

Further investigation into the phenotypic, biochemical and genotypic traits responsible for conditioning sugarcane aphid resistance is planned through heritability and quantitative trait locus (QTL) mapping studies. This will enable more efficient selection of genotypes that maintain grain and/or forage yield and quality when subjected to aphid infestation.

DEDICATION

This dissertation is a dedicated to my loving wife Yordanos Kebede Bogale for her unconditional love and support that has enabled me complete my Doctor of Philosophy Degree (Ph.D.) studies. Her encouragements continue to ignite my enthusiasm in humanistic research. I am also indebted to the love and support I have received from both my family and my wife's family. I therefore, would like to include a special dedication to the following people; my father Mr. Fredrick Chibale Kabosha, my father in law Mr. Kebede Bogale, my mother Mrs. Theresa Chali Kabosha, my mother in law Mrs. Senait Seifu and not forgetting Maureen Kabosha Musukuma, Getrude Kapambwe Kabosha, my late sister Violet Mwila Kabosha, Fredrick Chabala Kabosha also known as "Dangote", Thresa Kabosha Gondwe, Alex Mwila Kabosha, Oscar Kwimwe Kabosha, Octavia Kabosha Choongo, Sibira Ng'andu Kabosha, Justine Chongo also known as "Inshimbi" translated as "the metal" or "Iron Man", Rebecca Kebede Bogale also known as Blacki Dacki, Genet Jenny Sahal Amedamicheal, late Samuel Sahal Amedamicheal, Pastor Biniam Kebede Bogale, Bersabeh Kebede Bogale and last but not the least my son Immanuel Lloyd Fredrick of who I have followed the naming by the Ethiopian custom otherwise he would be called Immanuel Lloyd Kabosha according to the Zambian custom. There are many people I would have loved to mention in this dedication such as my nephews, nieces, my extended family members and my friends who have always given me great support but the page is not large enough for me to fit in all the names.

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NOMENCLATURE

$	United States Dollar
%	Percent
<	Less than
=	Equal to
>	Greater than
µ	Mew
AgriLife	Agriculture and Life Sciences
A-line	Male Sterile Line in the CMS system
ANOVA	Analysis of Variance
APPSA	Agriculture Productivity Program for Southern Africa.
B_1	Beta One
B-line	Male Fertile Line (Maintainer line in the CMS System)
B_o	Beta Zero
CGIAR	Centre for Genetic Resources Institute for Agriculture Research
cm	Centimeters
CMS	Cytoplasmic Male Sterility
CNglcs	Cyanogenic Glucosides
CPPM	Crop Protection and Pest Management Program
DNA	Deoxyribonucleic Acid
EBAFOS	Ecosystems Based Adaptation for Food Security

EPA	Environmental Protection Agency
F_1	First Filial Generation
F_6	Sixth Filial Generation
FAO	Food Agriculture Organization
F-test	F-Statistic (Named after Sir Ronald Fisher)
g	Gram
GART	Golden Valley Agriculture Research Trust
Ha	Hectares
H_0	Null Hypothesis
i	i^{th} term
INTSORMIL	International Sorghum and Millets
IPM	Integrated Pest Management
j	j^{th} term
k	k^{th} term
L	Liters
LS	Least Squares
LSD	Least Significant Difference
m	Meter
M.S.	Master of Science Degree
MAL	Ministry of Agriculture and Livestock
MAS	Marker Assisted Selection
NASS	National Agriculture Services

NDVI	Normalized Differential Vegetative Index
NIFA	National Institute of Food and Agriculture
NIR	Near-Infrared Spectroscopy
PCA	Principal Component Analysis
Ph.D.	Doctor of Philosophy
PI	Plant Introduction Number (National Plant Germplasm System (NPGS-USDA))
P-value	Probability (Smallest level of significance to reject H_0)
QTL	Quantitative Trait Locus
RCBD	Randomized Complete Block Design
RILs	Recombinant Inbreed Lines
R-line	Restorer Line (In the CMS system)
SADC	Southern African Development Cooperation
SAS	Statistical Analysis Institute
SCA	Sugarcane Aphid
SCAB	Sugarcane Aphid Breeding Lines
SCAF	Forage Sorghum Sugarcane Aphid Trial
SCAG	Grain Sorghum Sugarcane Aphid Trial
SCAH	Sugarcane Aphid Hybrid Trial
SCAP	Sugarcane Aphid Preliminary Trial
SMIL	Sorghum and Millet Innovation Laboratory
SMIP	Sorghum and Millets Improvement Program

sp	Standard Deviation (Pooled)
SPD	Split-Plot Design
T	Tau
t	*t*-statistic (Students *t*-test)
TAM	Texas A&M (Old way of naming varieties from Texas A&M University)
TAMU	Texas A&M University
Tx	Texas
UNEP	United Nations Environmental Program
US	United States
USA	United States of America
USD	United States Dollar
USDA	United States Department of Agriculture
USDA-ARS	United States Department of Agriculture-Agriculture Research Service
USDA-NRCS	United States Department of Agriculture-Natural Resources Conservation Services
WG	Water Dispersible Granule
WHO	World Health Organization
WTO	World Trade Organization
$\bar{X}$	Mean
Y	Response Variable

ZARI	Zambia Agriculture Research Institute
Π	Pi
σ	Sigma (Standard deviation)

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CHAPTER I

INTRODUCTION

In 2013, the sugarcane aphid (*Melanaphis sacchari* (Zehntner)) appeared as a virulent pest on sorghum (*Sorghum bicolor* (L.) Moench) in southeast Texas. Farmers reported significant economic losses resulting from aphid infestation (Villanueva et al., 2014). The devastating effects of this pest were also seen in 2014 in most parts of Texas. In 2015, heavy rainfall at the beginning of the season in South Texas kept aphid pressure low but the pest was prolific in the fall season and caused economic damage on the Southern High Plains.

During 2015 the aphid continued to spread throughout the United States (US) sorghum production region and was confirmed in at least 417 counties and 17 states (Bowling et al., 2016a). In Florida, 100% yield loss was reported, input costs worth $8 million (USD) was lost and $20 million (USD) lost substituting stock-feed (Hollis, 2014). In Texas, economic losses per hectare stood at $158.7 (USD) and $89.4 (USD) in 2014 and 2015 respectively. Grain worth $31.6 million (USD) has been lost since 2014 in Texas alone (Samuel et al., 2016). This pest is a threat to the $1.63 billion (USD) sorghum industry in Texas (USDA-NASS, 2013). As a result, the United Sorghum Checkoff Program committed $1 million (USD) to research and educational materials since 2015 (Bean, 2017).

Melanaphis sacchari has been previously reported as a sorghum pest in areas of Africa, the Far East and Australia where it can cause grain yield loss as high as 73%

(Van den berg, 2002) and reduce the quality of grain for malting by as much as 16.5% (Van den berg, 2000). The pest may have a significant effect on the yield and quality of forage as well (Sharma, 1993). Yield loss due to aphid activity occurs in four ways: (i) infestation on susceptible seedlings leading to plant death; (ii) infestation on mature plants reducing photosynthetic activity; (iii) infestations on panicles before flowering affecting seed set (Singh et al., 2004) and (iv) honeydew reducing the efficiency of machinery at harvest resulting in grain yield loss. The pest can cause significant grain yield loss by as much as 73% (Van den berg, 2002).

Options for the control of sugarcane aphid (SCA) fall into one of four categories: (i) chemical control, (ii) cultural control, (iii) biological control and (iv) host-plant resistance. Each of these methods has relative strengths and weaknesses and eventual management of the pest will likely involve all four or some combination thereof.

Chemical control is highly effective but broad spectrum insecticides have indiscriminate effects on non-target beneficial insects. Highly efficacious insecticides are also known to cause development of resistance in arthropods because they exert a selection pressure (Daly et al., 1998). Because aphids are highly fecund with a complicated life cycle they can easily develop resistance and become progressively virulent in subsequent generations (Dixon, 1973). Other disadvantages of chemicals are toxic effects on the health of humans, animals, plants and the environment (FAO, 2001; Lorenz, 2009). Cultural and biological control is challenging to implement and execute on a large scale. In the past, among the reasons given for modest success with these two methods of control is that research funds that went into cultural and biological control

were rather limited (Kring and Gilstrap, 1984). Host-plant resistance is an economical method of pest management in sorghum (Sharma and Ortiz, 2002), but host plant resistance must be bred into elite genotypes and is subject to loss due to changes in the pest ecotype or unusual insect pressure.

Other considerations for aphid management should take into account factors such as weather and edaphic conditions. Environmental conditions are known to affect the dynamics of ecosystems and aphid outbreaks and must be considered when developing strategies to manage aphids (Harvell et al., 2002; Van Emden and Harrington, 2007; Beyene et al., 2014).

Justification

Developing methods to mitigate SCA damage is important because aphids can easily evolve and occurrences tend to be unpredictable. There are numerous reports of defeated pest resistance in plants, and resurging pests are usually extra virulent (Daly et al., 1998). Hence, continued research to develop durable resistance should be an ongoing process. To do so, generating relevant information on plant resistance to aphids is of great importance (Esquinas-Alcázar, 2005). To maintain and preserve resistance, it is appropriate to identify additional sources of resistance to SCA. Wild sorghums and landraces have a natural capacity to endure biotic stress and are potential sources of resistance (Hajjar and Hodgkin, 2007). Ultimately, breeding is made more efficient if molecular tools identifying genes associated with plant resistance to aphids are available to compliment the current efforts in conventional breeding (Chang et al., 2012). Introducing aphid resistant traits from wild sorghums into cultivated sorghums takes

time and marker-assisted selection (MAS) may help to expedite the process (Sharma et al., 2014).

Objectives

Within this context, the objectives in this project were to: (i) evaluate sorghum germplasm for resistance to SCA; (ii) determine the effect of SCA infestation on yield and quality of resistant and susceptible forage hybrids; (iii) determine performance of grain sorghum lines and hybrids under SCA pressure (iv) determine categories of resistance and correlation between phenotype and resistance.

CHAPTER II

LITERATURE REVIEW

Biology of the Sugarcane Aphid (*Melanaphis sacchari* (Zehntner))

Figure 1 Characteristics of the sugarcane aphid (*Melanaphis sacchari* (Zehntner)) in North America. A = newly born nymph, B = alate, and C = adult nymph.

The SCA is one of over 4000 aphid species currently identified (Wijerathna and Edirisinghe, 1995; Dixon, 1998). The SCA is a soft bodied green insect with a piercing-sucking mouthpart that allows it to feed on sap in phloem vessels of sorghum. On average an adult nymph measures 0.85mm long and 0.64mm wide (Figure 2) and can weigh as much as 0.6mg. The newly born nymphs measure approximately one-tenth the size of adults. They can be distinguished by dark cornicles, tarsi and antennae tips. Dark cross marks on the backs and dark veins on wings are characteristic of winged SCA

(Figure 1). Sugarcane aphid populations in North America almost always consist of females that reproduce by parthenogenesis and are clonal; sexual reproduction and oviposition has not been observed in North America (White and Grisham, 2004; Knutson, et al., 2016). An adult viviparous aphid can produce 96 offspring under optimal conditions while a winged form, an alate, produce fewer offspring on average five (Bowling et al., 2016a). There are reports of sexual reproduction and oviparity on sorghum in Asia (Wang et al., 1961; Yadava, 1966; Setokuchi, 1975, David and Sandhu, 1976). More recently, there were reports of sexual forms in Mexico (Pena-Martinez et al., 2016) but these findings need further validation.

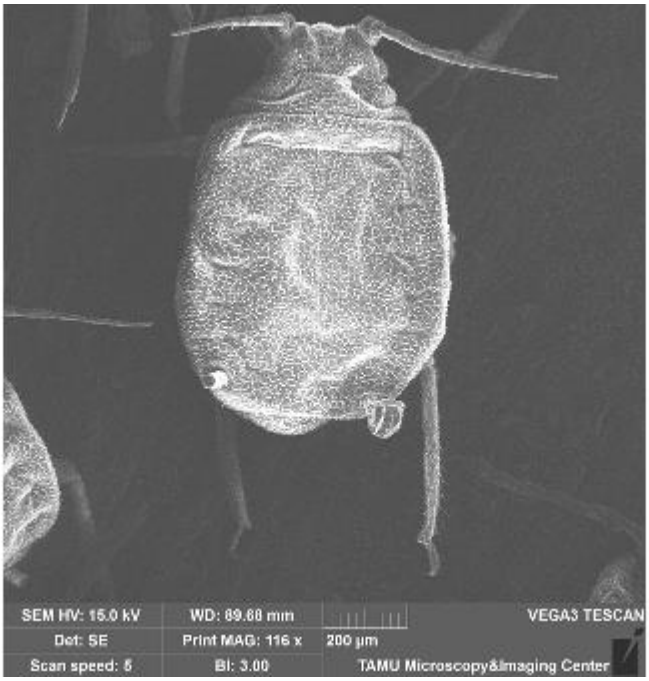

Figure 2 Dimensions of an adult 10 day old sugarcane aphid nymph (*Melanaphis sacchari* (Zehntner)) under an electron microscope (FEI Quanta 600 FE-SEM).

The aphid matures to adulthood in five days and has a lifespan of up to 30 days. Because of their rapid cycling and lifespan, exponential growth may take place in a favorable environment and result in overcrowding. In these situations, winged aphids (alates) develop and facilitate migration of the pest (Sharma et al., 2014). Colonized plants may have both wingless nymphs and winged adult aphids under the leaf (abaxial leaf surface). But under heavy pressure, feeding on the upper leaf surface (adaxial leaf surface) and the panicle (grain) has been observed (Villanueva et al., 2014).

The SCA can cause devastating effects on sorghum production (Buntin, 2012). Infestations can occur any time after plant emergence, but if natural enemies are present and rainfall abundant the aphid populations are suppressed (Singh et al., 2004). In South Texas, heavy infestations usually occur in fall and greater than 10,000 aphids can colonize a single plant (Brewer et al., 2016). While the aphid is not toxic to the plant, such colonies can suppress plant growth in susceptible sorghum. In the fall alates become more abundant and they overwinter on ratoon sorghum and Johnson grass (*Sorghum halepense* (Linnaeus)) (Armstrong et al., 2015).

In the spring, some of the overwintering aphids (i.e., the foundress) give rise to new alates and more non-winged aphids. The offspring of the two morphs will produce many more non-winged aphids that increase in numbers allowed by environmental factors and host availability. If the host-plants are limited or in poor condition, alates are produced and migration begins to colonize other plants. (Figure 3).

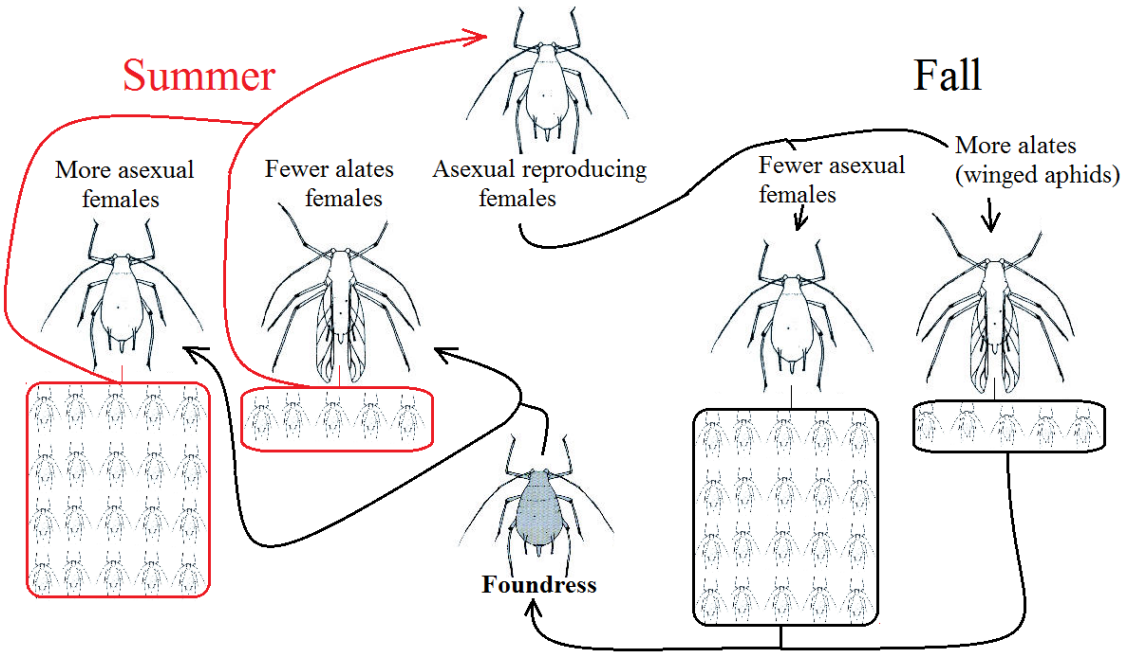

Figure 3 Schematic flow diagram of the sugarcane aphid life cycle in North America as is currently known. Rectangular boxes represent offspring of winged (alates) and non-winged aphids. Adapted from Shingleton et al., 2003.

Geography and Differentiation of Sugarcane Aphid

The SCA outbreak in North America may have been caused by a recent introduction from Hawaii. Perhaps due to increased trade between Hawaii and the USA (Mondor et al., 2007). SCA was first reported in Hawaii on sugarcane in 1896 (Zimmerman, 1948) then in Florida USA, on hairy crabgrass (*Paspalum sanguinale* (Lamarck)) in 1922 (Wilbrink, 1922). However, this pest only became problematic after 2013. Genetic relationships were determined using 10 microsatellite markers that support the introduction hypothesis. SCA in Hawaii shared the same multilocus lineages with SCA in Florida USA (Nibouche et al., 2014). The genetic study was not designed to

detect SCA host-plant association differentiation (HAD) or host-plant specialization, but rather geographic genetic diversity worldwide.

Genetic diversity of SCA worldwide is low with no clear distinction of SCA into species of sorghum (*Sorghum bicolor* (L. Moench)) and sugarcane (*Saccharum officinarum* (L. (Poaceae)) (Nibouche et al., 2014). The distinction was geographical distribution rather than speciation. Only five multilocus lineages were found worldwide and genetic divergence among these was low (Nibouche et al., 2014). Nevertheless, in the late 1990s SCA on sorghum and sugarcane were classified as *Melanaphis sorgi* (Theobald) and *Melanaphis sacchari* (Zehntner) respectively (Remaudière and Remaudière, 1997). More recently on Reunion Island in France, host-plant specialization was observed on sugarcane and sorghum using host transfer experiments (Nibouche et al., 2015). *Sorghum bicolor* (L. Moench), *Sorghum halepense* (L. Moench) and *Sorghum verticilliflorum* (L. Moench) were collectively called sorghum.

16 multilocus genotypes were identified but three of these (Ms11, Ms15 and Ms16) were more common and exhibited host-plant specialization. Ms11 and Ms16 were more specialized on sugarcane and Ms15 on sorghum (Nibouche et al., 2015). Another independent study of SCA on Sorghum (*Sorghum bicolor* (L. Moench)), Johnsongrass (*Sorghum halepense* (L. Pers)) and sugarcane (*Saccharum officinarum* (L. (Poaceae)) was done using amplified fragment length polymorphisms (AFLPs). SCA in USA was grouped into three distinct clusters, but HAD or geographic relationship was not observed (Medina et al., 2016).

Despite low genetic diversity on a wide scale specialization can exist. Specialization of Ms15 on sorghum in France could be recent since it only differed by one allele from Ms11. The same may be true for USA. Only one genetically distinct cluster of SCA was observed from Louisiana, USA (Nibouche et al., 2015) and recently three genetically distinct clusters were observed despite luck of HAD (Medina, 2016). This may signify that even though SCA is highly parthenogenetic, evolution can occur quickly and may explain the host-plant shifts. However, evolutionary shifts of insect populations can also be driven by host-availability and a high adaptation capacity (Facon et al., 2006; Ward et al., 2008).

An asexual insect species such as the SCA with a global ecological range possess a threat to crop production because evasive biotypes can easily evolve. As a result, biological and genetic characterization of SCA is important for plant breeders when developing resistant lines. This is because genetically distinct clones may be highly specific and require a different strategy relevant to their management and control (Medina, 2012).

Economic Importance of Sugarcane Aphid

SCA is a destructive pest of sorghum (*Sorghum bicolor* (L. Moench)) in tropical and subtropical regions of the world; it is known to occur in Africa, Asia, Australia, South and North America (Singh et al., 2004). Outbreaks of SCA almost always follow cultivation of sugarcane (*Saccharum officinarum* (L. (Poaceae)) and sorghum (Manthe, 1992). An ecologically wide spread parthenogenetic species such as SCA is considered a "superclone" (Gilabert et al., 2015).

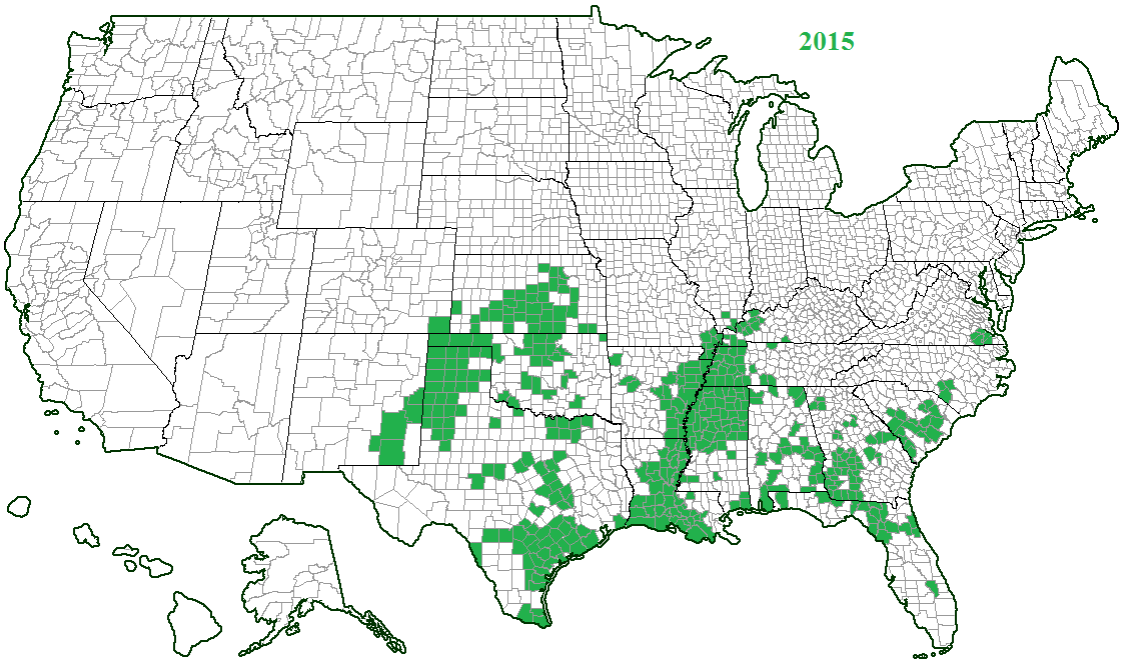

Figure 4 States and counties in the United States of America where sugarcane aphid was reported in 2015. 17 States (417 counties) were affected by the aphid, compared to only four States and 38 counties in 2013. Adapted from Bowling et al., 2016a and USCP, 2017.

Sugarcane aphid was of minimal economic importance in North America until 2013 when a rapid increase in SCA numbers affected sorghum in Texas and Louisiana (Villanueva et al., 2014). Incidences prior to this were typically on sugarcane (Summers, 1978; White et al., 2001). Since 2013, SCA has moved across sorghum growing regions in the USA and is now an established and highly problematic pest (Figure 4).

SCA can cause economic grain yield losses in cultivated sorghums (Sharma et al., 2014). A heavy infestation at germination can kill sorghum seedlings or reduce yield significantly. At boot stage a heavy infestation of aphids may result in reduced grain yield and quality (Knutson et al., 2016). Poor grain yield and quality reduces the value of

sorghum and lowers incomes for farmers, hence the importance of addressing this problem.

Effects of Sugarcane Aphid on Sorghum

Biotic stress caused SCA can be very significantly in susceptible grain and forage sorghums. The effect of SCA can be very devastating and reduce yields considerably. To avoid yield losses due to SCA, keeping pest populations below economic threshold levels is essential (Pedigo et al., 1986; Villanueva et al., 2014; Gordy et al., 2015; Knutson et al., 2016).

The degree of damage caused by SCA depends on the type of germplasm (resistant versus susceptible), the plant growth stage when infestation occurs and the aphid population pressure. While resistance to the pest is documented, no sorghum genotype is immune to the pest. In terms of growth stage (Vanderlip and Reeves, 1972), sorghum is susceptible in all three phases of development (seedling; vegetative; and reproductive), but due to environmental conditions and seed treatments, SCA infestation is rare in the seedling stage (Mbulwe et al., 2016). Infestations in the vegetative and reproductive phases are more common.

Sorghum plants affected by SCA exhibit many or some of the following symptoms: presence of honeydew on leaves, stems and panicles; reduced plant growth rate; mottled or browning of leaves; and reduced yields and premature plant death (USAID-ARCC, 2014). While the SCA does not inject toxins in the plant during feeding (Knutson et al., 2016) it depletes nutrients from the plant and is notorious for production

of honeydew that encourages growth of grain mold. The honeydew can reduce the effectiveness of fungicides (Buntin, 2012).

In South Africa, grain yield reductions between 46-78% have been reported (Matthee, 1962; Van Rensburg and Van Hamburg, 1975; Van den Berg et al., 2003). In the USA, and Mexico, SCA has been implicated in losses of up to 50% of expected grain yield (Villanueva et al., 2014). SCA also affects the quality of grain for malt (Van den Berg et al., 2003). Reductions in grain quality are influenced by reduced nutrient supply to the developing grain and honeydew that triggers secondary infections and grain molding. The mold affects the quality of grain carbohydrates and proteins (Leung, 2002). Eventually the ability of the grain to germinate and malt is drastically reduced (Van den Berg et al., 2003).

Aphids feeding on leaves deplete the cell sap (nutrients), disrupt photosynthesis and cause damage (chlorosis). This may affect the nutrient composition of forage sorghums (forage, hay and silage) and reduce plant vigor and plant health in susceptible plants. If the infestation is severe plant development may be disrupted. Disruption of plant development during dry matter accumulation of cereals and grasses changes plant nutrient composition (Torrecillas et al., 2011). It follows that aphid infestations may possibly affect forage quality in sorghums but this remains to be verified.

Finally, besides the negative biological effects, sugarcane aphids affect yield by reducing harvesting efficiency. Sorghum infected with aphids and honeydew are a nuisance to machinery used for harvesting. Sticky leaves and grain tends to clump together and block moving parts of machinery. The end result is work stoppages and lost

time (Villanueva et al., 2014). Clumpy grain easily falls to the ground and a considerable percentage of the harvest is lost.

Attempts to use early detection systems are being made using aerial multispectral remote sensing cameras that use Normalized Difference Vegetation Index (NDVI). NDVI uses visible near-infrared light. The idea behind this approach is to have a quick and inexpensive way of detecting SCA in sorghum fields (Elliott et al., 2015). Remote sensing techniques are made possible because NDVI readings and plant injury (chlorosis) are negatively correlated and significant. NDVI readings essentially measure chlorophyll, an indicator of photosynthetic activity in plants. Photosynthetic activity is disrupted by aphid feeding and can be detected using multispectral sensors (Goggin et al., 2015). This method is useful for crops grown on a large scale such as sorghum.

Control of Aphids

Integrated pest management

Utilizing one method of pest control is often futile. Combining several strategies is more sustainable (Horber, 1980; Brewer et al., 2016), hence the philosophical concept of Integrated Pest Management (IPM). The IPM approach consists of combining and utilizing different pest control methods in an economical and environmentally sensitive approach to manage pests (Akbar et al., 2011). IPM may comprise using resistant varieties, crop rotations, alternate planting to allow some plants to escape aphid attacks, and selective use of insecticides. Overall, IPM is designed to minimize risks to the environment, human, animal and plant health. Thus, development of new varieties resistant varieties to SCA is an integral component of the IPM concept.

Chemical control

Currently, chemical control is the most effective option for managing SCA and is the only method available to contain SCA infestations especially once pest levels reach economic thresholds (Gordy et al., 2015). The use of broad spectrum chemicals may kill or disrupt non-targeted beneficial insects that help in the biological control of aphids. Highly effective chemicals put a greater selection pressure on aphids and cause the evolution of exceptionally virulent bio-types. In the end, aphids can easily develop resistance against chemical insecticides (Balikai, 1993). Nonetheless, the harmful effects can be minimized by using selective insecticides. Seed dressing insecticide is another way of minimizing deleterious effects on beneficial insects.

In the U.S. chemical control options for SCA are limited by label restrictions. In March 2014, Tranform WG® was approved for emergency exception (USEPA, 2014) and remains an option for control of SCA. More recently, Sivanto has been approved for use and is an important option given regulatory challenges related to using Transform WG®. Malathion, Dimethoate and Chlorpyrifos have labels for SCA control as well.

Biological control

Research on the use of biological control to manage aphids has produced modest success in the past partly because few farmers, if any, have adopted this method of control, and inadequate policies on biological control (Kring and Gilstrap, 1984; Van Lenteren, 1988). Regardless, biological control occurs naturally (Waage, 2007). Biological control of aphids makes use of parasitoids and predatory insects like wasps, lady bugs, lacewings and syrphid flies (Van Lenteren, 1988; Knutson et al., 2016).

Biological control is easily influenced by weather. For instance, hot and dry weather tends to favor SCA outbreaks rather than natural enemies (Sharma, 1993; Sharma et al., 2014). For biological control to be effective, natural enemies of aphids need to be conserved either in natural vegetation, border crops or in surrounding crops (Van Rensburg and Van Hamburg, 1975).

Cultural control

There are a number of highly recommended management practices to reduce aphid infestations, termed, "good cultural practices" (Edwards, 1989). Crop rotation has proven to be a good method of managing insect pest outbreaks (Buttel and Shulman, 1997). Burying crop residues and eliminating volunteer crop also assists in reducing aphid populations (Abawi and Widmer, 2000). Planting aphid resistant varieties with good vigor is a recommended practice to manage aphids (Buntin, 2009) and can be considered a part of cultural control.

Host plant resistance

Host-plant resistance compliments chemical, biological and cultural control (Peters and Starks, 1990; Knutson et al., 2016) and will increase the effectiveness of these control methods. Resistance as used in this dissertation refers to the trichotomous "categories of resistance" scheme (antibiosis, antixenosis or tolerance) in a host-plant (Painter, 1951). Resistance in this research was not equal to immunity. It was defined as heritable characteristics of a plant that influence the amount of damage done by the insect. However, Stout proposed a dichotomous scheme of "plant defense mechanisms"

(Stout, 2013). He only identified 2 types of “plant defense mechanisms”, resistance and tolerance, of which resistance encompasses antibiosis and antixenosis.

Sorghum germplasm exhibits genetic variation for aphid damage and durable resistance should be possible. Durable resistance is when a host-plant possess both vertical and horizontal resistance (Palloix et al., 2009). Expression of variation to aphid damage is found in elite lines, landraces and wild sorghums (Sharma et al., 2014). Nevertheless, most sorghum hybrids currently grown in the USA are susceptible and have low levels of tolerance to SCA. Evidence reveals that wild sorghums or grassy sorghums tolerate heat, drought, nutritional stress, pests and diseases better than cultivated sorghums (Li et al., 2008). And so, wild sorghums, though challenging to work with in conventional breeding programs, are a potential source of SCA resistance. Even if resistant hybrids of sorghum are available, they might not be the preferred varieties by farmers or sorghum processing industries. As a result, crop improvement is required in existing cultivated sorghums to breed in durable resistance to SCA. Durable resistance is effective resistance in a widely cultivated crop (Scott et al., 1980).

Resistance to SCA in sorghum may be the result of structural or biochemical traits. Sorghums with a higher nutritional content were reported to attract higher aphid populations than those with lower nutrition (Akbar et al., 2011). Additionally, plants with higher nitrogen levels were reported to be more susceptible to aphid outbreaks (Hsieh, 1988). Alternatively, the presence of cyanogenic glucosides (CNglcs) and phenolic compounds found in plants such as sorghum have been implicated in induced defense against insect pests (Kahn et al., 1997; Fürstenberg-Hägg et al., 2013). Whether

these compounds are involved in defense against SCA is a research area that requires further investigation.

Physical barriers such as presence of wax and trichomes are among traits also associated with antixenosis (non-preference) (Singh et al., 2004). Other traits in sorghums that have been implicated in pest resistance include small leaves, narrow leaves, fewer leaves, erect leaves at seedling stage and vigorous growth after plant germination (Mote and Shahane, 1994). Expression of resistance influenced by the environment (Kogan, 1994), is also called ecological resistance (Painter, 1951). For example, early flowering plants can evade aphid infestation and so breeding for earliness by manipulating maturity genes may be essential.

Identifying additional sources of resistance is key for durable resistance. In addition, bioinformatics and genomic resources available online such as the sequenced pea aphid (*Acyrthosiphon pisum*) genome (Legeai et al., 2010) are sources of information for aphid management. The genetic database for sorghum (Paterson, 2008) is another useful resource critical for understanding the genetics behind host-plant resistance to SCA in sorghum germplasm.

Variation for SCA resistance exists in sorghum germplasm. In India, the sorghum germplasm lines: ICSB215; ICSB323; ICSB724; ICSR165; ICSV12001; ICSV12004 and IS40615 manifested resistance to aphid outbreaks under natural and artificial infestation. A list of some of the resistant germplasm lines from all over the world is available (Singh et al., 2004). The mechanisms of resistance in these lines have been mainly identified as antixenosis and antibiosis. Antixenosis has been observed in the

following germplasm lines: R.TAM428; IS1144C; IS1366C; IS1598C; IS6416C; IS6426C; IS12661C and IS12664C. R.TAM428, IS12609C, and IS12664C also exhibited antibiosis (Singh et al., 2004).

Sorghum breeders are actively developing varieties resistant to SCA and are determining the genetics of resistance. In a study of a population created from a susceptible line (B.Tx623) and a tolerant line (HN16), SCA resistance was controlled by a single dominant gene (Pi and Hsieh, 1982; Singh et al., 2004; Chang et al., 2012; Wang et al., 2013). It is believed that the genetic region on chromosome 6 that harbors the gene for resistance to SCA resides in a chromosomal segment of about 126 kilobase (kb) containing only five predicted genes (Chang et al., 2012; Wang et al., 2013).

Previous heritability studies on resistant F_1 hybrids and F_2 progenies based on crosses between susceptible (A.Tx3048/B.Tx378) and resistant (R.TAM428/SC170) lines disclosed that the resistance trait for SCA is dominant (Manthe, 1992). Resistance was reported as monogenetic because the segregation pattern; resistance vs susceptible, showed Mendelian inheritance pattern (3:1). Additive and complementary gene action was also observed in sorghum lines.

CHAPTER III

EVALUATING SORGHUM GERMPLASM FOR RESISTANCE TO SUGARCANE APHID*

Introduction and Objectives

The sugarcane aphid *Melanaphis sacchari*, (Zehntner) was first reported on sorghum in the United States in 1922 (Wilbrink, 1922). The sugarcane aphid (SCA) has long been a pest of sorghum in regions of Asia and Southern Africa but until recently this insect had no significant effect on sorghum productivity in the U.S. and was rarely noted to even occur in sorghum fields. Late in the 2013 production season, the SCA suddenly expanded its regional presence by infesting grain sorghum in the Upper Gulf Coast of Texas, Louisiana and Mississippi and then moved into sorghum production regions throughout South Texas. In 2014, the SCA continued to disperse through 18 southern and southeastern grain sorghum producing states representing several million acres of grain sorghum production. The presence of SCA in sorghum fields has also been reported as far north as Kansas and in Arkansas, Tennessee, Alabama, Georgia, Florida, South Carolina, North Carolina, Arizona and California.

[1]The SCA initially infests sorghum on the underside of the lower leaves with populations increasing rapidly from flowering to grain-fill (Van Rensburg et al., 1975). While SCA feeding does not appear to introduce toxins into the leaves, the sheer

* Reprinted with permission from "Journal of Plant Registrations" by Mbulwe, L., G.C. Peterson, J. Scott-Armstrong, and W.L. Rooney. 2016. Registration of Sorghum Germplasm Tx3408 and Tx3409 with Tolerance to Sugarcane Aphid (*Melanaphis sacchari* ((Zehntner)). Journal of Plant Registrations, 10:51-56.

numbers of aphids that accumulate results in the leaves and inflorescences being covered by aphids or sticky honeydew followed by sooty mold (due to fungal growth from the sugar). This can result in yield loss and harvesting problems associated with sticky plants that make combines and harvesters less efficient. In drought stress situations, combining post-flowering drought stress with aphid infestation may enhance the occurrence of charcoal rot (Van Rensburg et al., 1975; Sharma et al., 2014). If infestation occurs prior to reproductive growth, the diversion of energy caused by severe infestation inhibits panicle development and/or grain development resulting in yield reduction.

Given that sorghum is negatively affected in various ways by high SCA infestations control methods are required. Seed-based systemic insecticides offer seedling protection but gradually dissipate, providing protection for up to one month past planting. Post-emergence application of labelled insecticides is effective for short-term control of the aphid, but fields must be continually monitored and insecticide applications add to production expense and potential development of insecticidal resistance. In 2014, Section 18 emergency exemptions were approved in several states for Transform WG® which is effective but were restricted to two applications. In 2015, Sivanto® was approved under section 3 federal registrations with restrictions (Bowling et al., 2016a). For these reasons, genetic resistance is the best long-term option for SCA control.

The history of sorghum and greenbug (*Schizaphis graminum* (Rondani)) provide an example of effective deployment of genetic resistance (Young and Teetes, 1977). The

greenbug became a significant pest of sorghum production in the U.S. in the late 1960's and early 1970's. Much like the SCA, initial control of the greenbug was based on chemical application but sorghum breeding programs were able to identify sources of genetic resistance to the greenbug. Resistance from these sources was successfully bred into commercial sorghum hybrids which reduced the need for chemical control. Many sources of resistance to SCA have been identified in Asia and Southern Africa (Manthe, 1992). Preliminary results from the evaluation of sorghum germplasm for SCA resistance suggest that resistance sources from Southern Africa are effective against the SCA in Texas (L. Mbulwe, unpublished data).

Herein, we describe the evaluation of sorghum germplasm for tolerance to SCA and the identification of two seed parent lines (B.Tx3408 and B.Tx3409) that possess high levels of tolerance to the SCA in both greenhouse and field trials that is stable across environments. Tolerance in these lines is dominant, meaning that tolerance need to be present in only one parent to produce hybrids that are tolerant as well. A/B.Tx3408 and A/B.Tx3409 should have application as both seed parents in hybrid production as well as breeding new lines with tolerance to SCA.

Materials and Methods

A/B.Tx3408 and A/B.Tx3409 were developed from intentional breeding crosses using the pedigree method of plant improvement. Breeding crosses for B.Tx3408 and B.Tx3409 were made in College Station, Texas. Selection in subsequent generations was completed in nurseries throughout Texas and Puerto Rico over several years. The progenitors of B.Tx3408 and B.Tx3409 were individually selected from a single panicle

in the F6 generation and then bulk pollinated as an inbred line since that time. The male sterile version (A.Tx3408 and A.Tx3409) of each line was developed using A.Tx623 as the A1 cytoplasm source followed by five generations of backcrossing to the respective recurrent parent (B.Tx3408 and B.Tx3409). A.Tx623 is the sterile counterpart to B.Tx623 and both were released by the Texas Agricultural Experiment Station in 1977. The pedigree of B.Tx623 is B.Tx3197*SC170-6. As SCA was not a significant problem while A.Tx3408 and A.Tx3409 were developed, there was no selection for tolerance until these lines reached more advanced generations.

Table 1 Summary of sugarcane aphid (SCA) evaluation environments in Texas, 2014, with test planting date, date of SCA infestation, growth stage at initial SCA infestation and relative SCA infestation pressure. Ratings were based on a scale proposed by Sharma (Sharma et al., 2014).

Type of Information	Weslaco Spring	Corpus Christi	College Station	Weslaco-Fall
Planting Date	Feb. 20	Mar. 10	Apr. 6	Aug. 15
Date of Natural SCA Infestation	~May 18	~June 8	~July 10	~Sep 20
Crop Growth Stage at Infestation	Grain Fill - Milk to Soft Dough	Grain Fill - Soft to Hard Dough	Grain Fill - Hard Dough	Vegetative to Pre-Boot
Relative SCA pressure at peak (approximate no. SCA plant^{-1})	High (>500)	Moderate (>350)	High (>500)	High (>500)
SCA Rating Date	Jun. 4	Jun. 3	Aug. 10	Oct. 11
Ratings were based on	- Leaf chlorosis - Honeydew	- Leaf chlorosis - Honeydew	- Leaf chlorosis - Honeydew	- Panicle Emergence - Seed Set - Leaf chlorosis - Plant death

B.Tx3408 has a pedigree of B.Tx631/08PR047. B.Tx631 was released by the Texas Agricultural Experiment Station in 1985 (Miller, 1986). 08PR047 is an AgriLife Research breeding line selected for agronomic desirability and greenbug resistance with the pedigree GB102A/B.Tx631. GB102A is a line originally developed for resistance to biotype C greenbug, and subsequently biotype E greenbug. The GB102A pedigree is (((4dwf BTx378*(4 dwf B.Tx378*Capbam der))-1-1-6-1)*B.Tx3042). Capbam is a greenbug biotype C and E resistant line originally introduced from Russia. The other lines in the 'Capbam der' are not known.

B.Tx3409 has a pedigree of DLON357/08PR047. DLON357 is a seed parent with the pedigree of (B.Tx643*(B7904*(SC748*SC630))). B7904 is an unreleased sister line of B.Tx629 (Miller, 1986). DLON357 was developed in the AgriLife Research program and selected for agronomic performance and post-flowering drought tolerance.

To identify lines with SCA tolerance, 500 Texas A&M AgriLife Research lines were evaluated in Weslaco, Texas in the fall of 2013. The trial was planted in mid-August and heavy infestations (> 1000 SCA $leaf^{-1}$ $plant^{-1}$) occurred in these plots in mid- to late-September. Infestations were severe enough to restrict or stop growth; susceptible genotypes never headed while tolerant lines flowered and set grain (Figure 5).

Figure 5 Effect of sugarcane aphid (*Melanaphis sacchari* (Zehntner) on sorghum growth and development of B.Tx3408 (left) and a susceptible breeding line (right) in a fall planted nursery in Weslaco, Texas, 2013.

All lines that flowered in that preliminary screening as well as selected checks were evaluated in controlled greenhouse infestations by the USDA-ARS, in Stillwater OK using methodology described by Armstrong et al. (2015). Lines identified and used as tolerant checks included R.Tx2783 (Peterson, et al., 1984), R.TAM428 and susceptible checks included R.Tx2737 (Johnson et al., 1982) and R.Tx7000. Lines that demonstrated consistent tolerance in both field and greenhouse trials were advanced for testing in replicated trials in four environments in 2014 (College Station, Corpus Christi and Weslaco (Spring and Fall), Texas) under natural infestations of SCA. In addition to

those lines, hybrids produced using these lines were also evaluated. Across these environments, SCA pressure varied and reached a peak at different growth stages in sorghum development therefore the ratings for tolerance were based on different criteria in each environment (Table 1).

The experimental design in the field was a randomized complete block design (RCBD) with four replications. Standard crop management practices for sorghum production in each region were used with the exception that no insecticides (seed-based or foliar) were applied during the evaluation. Upon aphid infestation, the SCA population (levels of infestations) was measured using methods described by Armstrong (Armstrong et al., 2015). Damage caused by SCA was rated using the scale proposed by Sharma (Sharma et al., 2014) where 1 = few aphids present on lower one to two leaves, no apparent leaf damage; 2 = lower one to two leaves showing aphid infestation, 1 - 20% of the infested leaves/area showing damage symptoms; 3 = lower two to three leaves showing aphid infestation, 20 - 30% of the infested leaves/area showing damage symptoms, moderate levels of honeydew/black molds on the leaves/soil; 4 = lower three to four leaves showing aphid infestation, 30 - 40% of the infested leaves/area showing damage symptoms, moderate levels of honeydew/black molds on the leaves/soil; 5 = lower four to five leaves showing aphid infestation, 40 - 50% of the infested leaves/area showing damage symptoms, moderate levels of honeydew/black molds on the leaves/soil; 6 = aphid infestation up to five to six leaves, 50 - 60% of the infested leaves/area showing damage symptoms, heavy honeydew and black mold on the leaves and on the soil below; 7 = aphid infestation up to six to seven leaves, 60 - 70% of the

infested leaves/area showing damage symptoms, heavy honeydew/black molds on the leaves and on the soil below; 8 = aphid infestation up to seven to eight leaves, 70 - 80% of the infested leaves/area showing damage symptoms, heavy honeydew/black molds on the leaves and on the soil below; 9 = heavy aphid infestation up to the flag leaf, 80% of the leaves showing aphid damage (drying-up symptoms), heavy honeydew/black molds on the leaves and on the soil. Analyses were completed by comparing experimental entries with control susceptible lines.

For agronomic performance, experimental hybrids of A.Tx3408 were evaluated in field trials grown at Monte Alto and College Station, Texas in 2014. Hybrid seed of A.Tx3409 was not available for evaluation. Agronomic production practices standard for the region were used in both locations including fertilization at recommended rates. One post-plant irrigation was applied in Monte Alto while the College Station test was rain-fed. SCA were present at both locations; at Monte Alto, the SCA were controlled at the hard dough stage with an aerial application of Transform WG® at labeled rates. In College Station, the SCA infestation was too late to have any effect on the hybrids. At both locations, standard agronomic data (plant height, days-to-anthesis, grain yield, test weight and moisture content) were measured. All statistical comparisons were completed using PROC GLM in SAS (v 9.3) and means were compared with test trial L.S.D values ($P < 0.05$).

Results

Characteristics of germplasm

Inbred lines

B.Tx3408 and B.Tx3409 are maintainers of sterility in the A1 CMS system. While their reaction in other cytoplasmic genetic male sterility systems (A2 and A3) has not been tested, based on pedigree it is likely a maintainer of sterility in both of these systems. Both lines are genetically three-dwarf (dw1Dw2dw3dw4) with some variation in height (Table 2). Both lines are photoperiod insensitive and medium (B.Tx3409) to medium late (B.Tx3408) maturity (Table 2). B.Tx3408 has white grain and tan plant color while B.Tx3409 has red grain and purple plant color. The endosperm of both lines is normal (non-waxy).

Table 2 Agronomic data for B.Tx3408, B.Tx3409 and standard seed parents grown in three environments (Weslaco, College Station and Lubbock) in Texas, 2014.

Trait	B.Tx3408	B.Tx3409	B.Tx2928	B.Tx631	B.Tx645	LSD
Days-to-anthesis						
Weslaco	83	80	70	84	79	2.1
College Station	77	77	74	75	72	2.2
Lubbock	63	60	63	63	63	2.0
Plant height (cm)						
Weslaco	135	117	102	150	104	6.1
College Station	142	117	112	137	117	5.6
Lubbock	93	90	90	93	90	4.3
Panicle exertion						
Weslaco	5.1	10.2	10.2	10.2	10.2	1.0
College Station	7.6	7.6	10.2	10.2	5.1	3.1
Lubbock	7.6	7.6	5.2	10.2	3.5	1.8

Days-to-anthesis (d), Panicle exertion (cm), LSD (P>0.05).

Sugarcane aphid tolerance

While the numbers of SCA in the trials varied (Table 1), each trial was uniformly infested with SCA (they were found in all genotypes). The reaction of B.Tx3408 and B.Tx3409 to SCA varied in each environment, dependent primarily on the timing and pressure of infestation, but B.Tx3408 and B.Tx3409 consistently had the lowest damage ratings of any genotypes and were significantly better than the susceptible genotypes R.Tx2737 and R.Tx7000 (Tables 3 and 4). Compared to the tolerant checks, B.Tx3408 and B.Tx3409 were comparable to R.Tx2783 and slightly more tolerant than R.TAM428 in greenhouse assays (Table 3) and with similar, albeit less consistent trends observed in the field trials (Table 4).

Table 3 Aphid damage, seedling height (cm), and number of leaves for sorghum entries subjected to sugarcane aphid infestations under no-choice greenhouse evaluation in Stillwater, Oklahoma, 2014.

Entry	Aphid Damage[a]	Seedling Height[b]	Leaves[c]
		cm	no.
R.Tx2783	1.69a	32.06a	4.50a
B.Tx3408	1.71a	30.82a-b	4.47a
B.Tx3409	3.64a-c	23.68a-c	4.22a-b
R.TAM428	5.01b-d	20.05b-d	3.93a-d
R.Tx2737	8.55d-e	17.01c-d	3.25b-d
R.Tx7000	8.76d-e	10.88d	3.09d
R.Tx436	8.88e	8.11d	3.14c-d
R.Tx430	8.99e	10.13d	3.16c-d

a = Aphid damage measured by chlorosis ratings (1 - 9); column means followed by the same lowercase letters are not significantly different, P > 0.05; LSD.
b = Mean difference in seedling height, (controls - Infested); column means followed by the same lowercase letters are not significantly different, P > 0.05; LSD.
c = Mean difference in leaf numbers; column means followed by the same lowercase letters are not significantly different, P > 0.05; LSD.

Agronomic performance of A.Tx3408 hybrids

A.Tx3408 hybrids had tolerance to SCA similar to that observed in B.Tx3408 (Table 4). The same trend was observed for a single observation of A.Tx3409 hybrid in the 2014 Weslaco Fall environment. The consistent presence of tolerance in the hybrids demonstrates that the SCA tolerance in these lines is sufficiently dominant and therefore tolerance is not required in both parents. This was especially noticeable in the hybrids using R.Tx436 as a pollinator. From the greenhouse studies, R.Tx436 is susceptible to SCA (Table 3) but A.Tx3408/R.Tx436 and A.Tx3409/R.Tx436 hybrids are tolerant (Table 4).

Table 4 Aphid damage, for sorghum entries subjected to sugarcane aphid infestations under natural infestation in four field environments (Weslaco, Corpus Christi and College Station), Texas, 2014.

	Weslaco Spring	Corpus Christi	College Station	Weslaco-Fall	Combined
Lines					
B.Tx3408	2.5g-h	2.2g	4.7e-g	1d-e	2.6e
B.Tx3409	4.0d-g	2.1g	5.3d-f	1e	3.1d-e
R.TAM428 (Tol. check)	5.0c-e	3.9e-f	6.0b-e	6a-b	5.3a-c
R.Tx2737 (Sus. check)	8.7a	6.5b-c	8.3a	4.7a-c	7.1a-b
R.Tx7000 (Sus. check)	7.5a-b	7.2a	8.0a-b	7a	7.4a
Hybrids					
A.Tx642/R.Tx2783	5.5c	3.7e-f	5.8c-e	1e	4.0c-e
A.Tx2752/R.Tx2783	6.1b-c	3.9e-f	5.0e-g	2c-e	4.3b-d
A.Tx2752/R.Tx437	7.5a-b	5.6d	5.1e-g	3.7b-e	5.5a-c
A.Tx642/R.Tx436	4.5c-f	4.2e-f	5.7c-e	2.3c-e	4.2b-d
A.Tx3408/R.Tx436	3.3f-h	1.9g	5.6d-e	1.3b-e	3.0d-e
A.Tx3408/R.Tx437	2.7g-h	2.1g	5.4d-e	1e	2.8e
A.Tx3409/R.Tx436				1e	

Aphid damage = chlorosis ratings on a scale of 1 = healthy; 2 = 1-5% chlorotic; 3 = 5-20%; 4 = 21-35%; 5 = 36-50%; 6 = 51-65%; 7 = 66-80%; 8 = 81-95% and 9 = 95-100% or dead , df = 15, 96, F = 37.2, P > F = 0.0001; means followed by the same lowercase letters are not significantly different, P > 0.05; LSD. Tol. = tolerant and sus. = susceptible.

In terms of agronomic performance, A.Tx3408 hybrids were comparable in performance with public check hybrids in yield trials in South and Central Texas (Table 5). In these trials, no statistical differences in maturity, height, grain test weight, harvest moisture content or grain yield were detected between A.Tx3408 and A.Tx631 hybrids with the same pollinators (Table 5). A.Tx3408 hybrids were comparable in agronomic performance with A.Tx631 hybrids because SCA were either controlled or infestation occurred too late, it was not possible to assess the relative value of SCA tolerance in these hybrids.

Table 5 Agronomic trait means of hybrids of A.Tx3408 compared with two public check hybrids grown in Weslaco and College Station, Texas, 2014. Hybrids in Weslaco were irrigated once and insecticide applied once to control the sugarcane aphid during the production season; while the College Station trial was rain-fed and no insecticide was applied. Aphid pressure in both locations was similar to that present in the SCA trials.

Trait	A.Tx3408 /R.Tx436	A.Tx3408 /R.Tx437	A.Tx631 /R.Tx436	A.Tx631 /R.Tx437	L.S.D (P<.05)
Days to Anthesis (d)					
College Station	78	75	78	74	2
Weslaco	78	78	78	79	1
Panicle exertion (cm)					
College Station	18	15	18	18	6
Weslaco	15	15	12	8	8
Moisture content (%)					
College Station	11	11	11	11	1
Weslaco	15	15	14	15	1
Test weight (kg hl-1)					
College Station	75	74	75	73	2
Weslaco	74	72	74	72	1
Plant height (cm)					
College Station	152	152	152	152	8
Weslaco	140	143	140	140	10

Table 5 Continued

Trait	A.Tx3408 /R.Tx436	A.Tx3408 /R.Tx437	A.Tx631 /R.Tx436	A.Tx631 /R.Tx437	L.S.D (P<.05)
Grain yield (kg ha-1)					
College Station	10,000	9,235	9,220	8,967	1,510
Weslaco	6,700	6,868	6,203	6,750	1,350

Breeding consideration for sugarcane aphid evaluation

The differences in SCA ratings in the field environments reflect the difficulty and challenge of rating SCA tolerance in sorghum. First, the definition of tolerance changes depending on the timing of SCA infestation. For infestations that occur post-anthesis, the visual rating is based primarily on leaf chlorosis and honeydew deposit. However, it is still not known exactly what effect post-anthesis infestation of SCA has on yield (these studies are underway) and assessment of yield to make early generation selections in a breeding environment are not possible. For infestations that occur prior to anthesis, the visual and agronomic effects are much more obvious. In these infestations, damage is manifested as delayed flowering or no panicle emergence at all and in severe cases, plant death. In this context, B.Tx3408 and B.Tx3409 are exceptional in that they consistently showed minimal effect of the SCA on panicle development when infested prior to anthesis (Weslaco Fall 2014, Table 4). This, in fact, was the type of environment in which they were first identified (Weslaco, fall 2013).

Neither B.Tx3408 nor B.Tx3409 are immune to SCA and the type of resistance depends on the growth stage of the plant. This germplasm appears to escape or avoid excessively high numbers of SCA and/or it can maintain growth and development

despite the presence of SCA. Regardless of the type of resistance, it does seem valuable in mitigating the effect of SCA on sorghum productivity. The specific type of resistance and how it relates to protecting yield potential must still be determined.

The resistance in both B.Tx3408 and B.Tx3409 is very likely derived from their common parent. 08PR047 was originally selected for greenbug biotype C and E resistance, this line is also resistant to SCA. It is unknown if resistance to greenbug biotype C and E has functionality for SCA as well. Not all sources of resistance to greenbug are effective against SCA (Armstrong et al., 2015). For example, while R.Tx2783 demonstrates tolerance to both pests, SC110-9 (a parent in R.Tx2783) is resistant to SCA but susceptible to all greenbug biotypes. What causes this difference is unidentified at this time and further research will be necessary to determine if tolerance to SCA is associated in any way with resistance to greenbug biotypes.

Discussion

A/B.Tx3408 and A/B.Tx3409 were released to provide the sorghum breeding industry with new sources of tolerance to SCA, which is a new and devastating insect pest of sorghum. This germplasm is sufficiently developed to be used as either a seed parent in hybrid combination or as a breeding line for the development of new seed parents with SCA tolerance.

Availability

Seed of A/B.Tx3408 and A/B.Tx3409 will be maintained by personnel in the Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474. Requests for this germplasm can be directed to W.L. Rooney, AgriLife

Research Sorghum Breeding or to Texas A&M Technology Commercialization, Texas A&M University, College Station, Texas 77843-3369.

CHAPTER IV

DETERMINING EFFECT OF SUGARCANE APHID ON YIELD AND QUALITY OF FORAGE HYBRIDS

Introduction and Objectives

The SCA presents problems in managing sorghum for hay, forage and silage (collectively called forage sorghum). The greater canopy of forage sorghums makes it more difficult for insecticides applied on foliage to go through to lower lying leaves. On the other hand, concerns about chemical residues in farm products (animals and crops) and insecticide resistance has prompted research on reducing insecticide rates (Pardío et al., 2012; Handford, 2014; Knutson, 2016; Bean, 2017). Because of pesticide residue restrictions, managing SCA becomes even more complex (Armstrong et al., 2016). Grazing, feeding or haying crops sprayed with pesticides (insecticides or herbicides) are restricted from immediate use for a specified time window which may be as much as eight weeks in some cases.

Evaluating resistance to SCA or the effect of reduced application rates is of paramount importance. Thus, there was need to make efforts to determine the effects of SCA on the yield performance and quality of forage sorghum hybrids. The idea was to investigate if the response to heavy SCA infestation varied between controlled (insecticide) and uncontrolled (no-insecticide) crops in the field. The response of resistant and susceptible sorghum hybrids to heavy SCA pressure under natural conditions also needed to be investigated. Thirdly, photoperiod sensitive and

photoperiod insensitive hybrids needed to be evaluated in terms of how they react to SCA under heavy aphid pressure in the field.

Forage sorghum hybrids have natural variation for many qualitative and quantitative traits. It is this variation that was explored to determine the effect of SCA on the performance of forage sorghums. This was done in summer (Lubbock, College Station, and Corpus Christi) and fall (Weslaco) in Texas 2016. The germplasm A.Tx3408 and A.Tx3409 developed by the Sorghum Breeding Program at Texas A&M AgriLife Research (Mbulwe et al., 2016) was used as the source of resistance. The goal was to determine the effect of SCA on the yield performance and quality of forage sorghum hybrids under natural SCA infestations and assess the viability of resistant hybrids in addressing this problem.

Materials and Methods

Plant germplasm

Twelve forage sorghum hybrids were selected for evaluation, and these hybrids were composed of seed parents that varied in SCA tolerance levels. All of the pollinator parents were rated as susceptible to SCA. Four hybrids were produced using the SCA tolerant seed parents (A.Tx3408/R.Tx2910, A.Tx3408/R.Tx2909, A.Tx3409/R.Tx2785 and A.Tx3408/R.Tx2785 (Mbulwe et al., 2016). The hybrids rated as susceptible included A.Tx645/R.Tx2909, A.Tx631/R.Tx2910, A.Tx645/R.Tx2910, A.Tx631/RTx2909, A.Tx631/R.Tx2785 and A.Tx645/R.Tx2785. A.Tx645 and A.Tx631 are commonly used to produce forage hybrids (Rosenow et al., 2002; Miller, 1986). R.Tx2785 is a downy mildew resistant forage pollinator line that produces a photoperiod

insensitive hybrid (Frederiksen et al., 1983). A.Tx2909 and A.Tx2910 produce photoperiod sensitive forage hybrids (Rooney et al., 1998; Rooney and Aydin, 1999). Hybrid seed was produced in College Station, 2015. Due to seed limitations, not all hybrids were included in every location.

Experimental design and locations

To determine the effect of SCA on the yield and quality of forage hybrids, a split-plot design was used (Appendix II) with two whole plot treatments of insecticide (control) and no-insecticide, sub-plots in the study were hybrids. In the control, aphids were controlled to minimize infestation by spraying with Transform WG® at the rate of 0.11L/ha. Aphids were not controlled in the other whole plot treatment. Differences in yield and quality of forage hybrids between whole plots was assumed to indication the effect due to SCA.

Table 6 Dates of planting, SCA infestation, insecticide application and harvest for evaluating the effect of sugarcane aphid (SCA) on yield and quality of forage sorghum hybrids in four environments in summer and fall, Texas, 2016.

Location	Planting Date	Date of SCA Infestation	Date of Insecticide Application	Date of Harvest
Lubbock (insecticide) ****	25 May	30 Jun.	2 Aug.	26 Sep.
Lubbock (no-insecticide)***	6 Jun.	30 Jun.	No-insect.	No-insect.
College Station-1 *	23 Mar.	26 Aug.	29 Aug.	18 Sep.
College Station-2 ****	Cut: 19 Sep.	31 Oct.	3 Nov.	21 Nov.
Corpus Christi *	14 Apr.	23 May	27 May	6 Jul.
Weslaco-fall***	15 Aug.	19 Oct.	23 Oct.	29 Nov.

Weslaco evaluated in fall. In Lubbock one half of the split-plot was each planted on different dates and locations. Aphid pressure according to location: ****Heavy (> 1000 SCA leaf^{-1} plant^{-1}), ***high (500-1000 SCA leaf^{-1} plant^{-1}), *low (< 350 SCA leaf^{-1} plant^{-1}). The population in College Station was initially high (500-1000 SCA leaf^{-1} plant^{-1}) for a month then crashed. No-insect. = no-insecticide.

Each of the 12 hybrids planted in the split-plot trial was replicated four times in each whole plot planted in the four Texas locations (Table 6). In most locations, the two whole plot treatments were adjacent with a buffer to mitigate insecticide efficacy. However, in Lubbock, the whole plot effects were planted in separate fields on different days (Table 6). In College Station, the main crop (CS-1) and a second ratoon crop (CS-2) were harvested. Plot sizes, spacing, agronomy and management of the crop was done according to agronomic practices standard for each location.

Agronomic traits

Regardless of SCA pressure, forage hybrids were harvested to measure yield potential. Because of the differences in photoperiod response, trials were purposely harvested later than normal for a photoperiod insensitive hybrid. In Weslaco and Lubbock, yield was estimated by hand harvesting 1.0 contiguous meter of the row and weighing the biomass using a portable electronic balance (Ohaus Defender® 5000 Deluxe Bench Scale-250 lbs (113.4kgs). x .02 lb (0.01kgs)). In College Station and Corpus Christi harvesting was done using a tractor mounted 1-row forage harvester with an inbuilt forage collection and weighing system. In all locations, fresh biomass samples were pulled and weighed, followed by drying to stable weight in an electric oven drier (Three Phase Large Cabinet Oven, 400°F Max Temperature (230V) Model: BO-60EB). After dried, samples were reweighed to calculate moisture content at harvest, and samples were saved for composition analysis.

Dried samples were ground in a Wiley Mill (Model No. 3, Serial number 43102H) to pass through a 2 mm screen and used for compositional analysis. Forage

quality (percent nutrient composition) was determined using Near-Infrared Spectroscopy (NIR) (FOSS XDS Rapid Content™ Analyzer) using calibration curves developed in the Texas A&M AgriLife Research Sorghum Breeding Program (Hoffmann et al., 2012). Percent composition estimates were obtained for protein, cellulose, hemicellulose, lignin and ash. In addition, plant height (m), days to 50% anthesis, seed color, plant color and desirability were also measured (Appendix III).

Statistical analysis

The statistical model for the split-plot design with two treatments (insecticide and no-insecticide) is given by the formula: $\mathbf{Y_{ijk} = \mu + T_i + d_{jk} + Y_j + TY_{ij} + \varepsilon_{ijk}}$; were $\mathbf{Y_{ijk}}$ = observed damage due to the ith level of aphids (insecticide), $\boldsymbol{\mu}$ = average damage resulting from aphids, T_i = fixed effect due to the ith level of aphids (insecticide), $\mathbf{d_{jk}}$ = random effect due to the kth plot (block) receiving the ith level of aphids (insecticide), $\mathbf{Y_j}$ = fixed effect on different forage hybrids, $\mathbf{TY_{ij}}$ = fixed effect for the ith level of aphids on forage hybrids and $\boldsymbol{\varepsilon_{ijk}}$ = experimental (random) error. The null hypothesis is denoted by: $\mathbf{H_0: ØT = 0}$ (H_0: All $T_i = 0$) (Ott and Longnecker, 2015). Statistical comparisons were computed using the statistical software PROC GLM in SAS v 9.3 (SAS Institute, 2011), and means were compared with test trial LSD values ($P < 0.05$). Included in the analysis was a single degree of freedom contrast of resistant versus susceptible hybrids.

Results

Agronomic yield

Aphid infestation levels varied depending on time and location. For example, the SCA infestation was non-existent to low in College Station-first harvest (CS-1) but heavy in the second harvest (CS-2) and in Lubbock (>1000 SCA leaf^{-1} plant^{-1}). Differences in biomass yield were not detected among insecticide treatments in CS-1, but they were highly significant in CS-2, Lubbock and Weslaco (Table 7).

In all locations, genotypes were different except in Lubbock where genotypes were not significant primarily because the insecticide and no-insecticide whole plots were planted on different dates. The environment, management and irrigation regime of whole plots was also different for insecticide and no-insecticide treatments in Lubbock.

Some of the differences among genotypes were the effect of photoperiod sensitivity; photoperiod sensitive hybrids were higher yielding than photoperiod insensitive hybrids. Within these hybrids, no genotypic differences were detected. Single degree of freedom contrasts detected differences in yield response between resistant and susceptible hybrids in all environments in which SCA were present.

Table 7 Analysis of variance for effect of sugarcane aphid (SCA) (insecticide and no-insecticide) on forage sorghum hybrids (biomass yield per hectare (yield ha^{-1}), Lubbock (LB) and College Station (CS-1 and CS-2) in summer and Weslaco in fall (WE-fall), Texas, 2016.

Source of Variance	Combined Locations	CS1	CS2	LB	WE Fall
Genotype	<0.0001*	<0.0001*	<0.0001*	<0.3404	0.0001*
Genotype Error	<0.0001*	<0.0001*	<0.0001*	0.5826	0.0012*
Insecticide	<0.0001*	0.1436	0.0009*	<0.0001*	0.0001*
Insecticide Error	0.0048*	0.4451	<0.0420*	<0.0001*	0.0003*
Genotype x Insecticide	0.7958	0.3153	0.4982	0.3481	0.6819
Replication(Insecticide)	0.5015	0.1371	0.1576	0.7981	0.1510
Location	<0.0001*				
Location x Genotype	<0.0001*				
Location x Insecticide	0.0002*				
Locati. x Genoty. x Insect.	0.7961				
Corrected Total	0.0001*	<0.0001*	<0.0001*	0.0107*	<0.0001*
LS Means Contrast: R vs S	<0.0001*	<0.5629	<0.0001	<0.6540	0.0021*
Aphid pressure		None	Heavy	Heavy	Moder.

Split-plot design. Twelve entries by four replications by two whole plots by four environments. R = resistant hybrids, vs = versus and S = susceptible hybrids. Moderate is abbreviated as moder. The asterisk (*) indicates significance. CS-1 = College Station, summer, first harvest, SCA pressure none; CS-2 = College Station, summer, second harvest, SCA pressure heavy (> 1000 SCA leaf^{-1} plant^{-1}); Weslaco, fall, SCA pressure moderate (350-500 SCA leaf^{-1} plant^{-1}).

Table 8 Means for yield (tons ha^{-1}) of resistant versus susceptible hybrids across four locations in Texas, 2016 showing effect of sugarcane aphid (insecticide and no-insecticide) on the yield of forage sorghum hybrids in Lubbock and College Station in summer and Weslaco in fall, Texas, 2016. Means were grouped by location, insecticide treatment and type of germplasm i.e. photoperiod sensitive (PS) and photoperiod insensitive (PI).

Combined	Insecticide	No-insecticide	Difference
Resistant	61.7	53.2	-8.6
Susceptible	61.7	51.4	-10.2
Difference	0.0	-1.7	-1.7

Location	SCA	Type	PS Insect.	PS No-insect.	PI Insect.	PI No-insect.
College Station-1	None	Resistant	130.2	134.4	58.3	54.5
		Susceptible	128.7	126.5	56.0	59.4
College Station-2	Heavy	Resistant	23.9	19.7	24.4	22.5
		Susceptible	12.5	7.6	20.6	17.8
Lubbock	Heavy	Resistant	72.8	42.7	69.6	49.2
		Susceptible	77.1	48.3	76.0	57.3
Weslaco	Moderate	Resistant	59.7	57.4	54.8	45.3
		Susceptible	65.6	47.1	56.9	47.6

Split-plot design. Twelve entries by four replications by two whole plots by four environments. Sugarcane aphid (SCA) pressure was present except for College Station, summer, first harvest. The effect of SCA on yield (tons ha^{-1}) between resistant and susceptible germplasm in the insecticide and no-insecticide treatments is further analyzed in Appendix IV. Insect. = insecticide, No-insect. = no-insecticide.

In forage hybrids, it appears that resistance provides some protection from yield loss. Overall, no differences in yield performance were detected between susceptible (61.7 tons) and resistant (61.7 tons) forage hybrids when they were treated with insecticide (Table 8). When aphids were not controlled, the average yield for resistant

hybrids (53.2 tons ha^{-1}) was higher than susceptible hybrids (51.4 tons ha^{-1}). Both susceptible and resistant hybrids incurred yield losses when not treated with insecticide. But, the yield loss was higher in susceptible hybrids (10.2 tons ha^{-1}) than in resistant hybrids (8.6 tons ha^{-1}) (Table 8). Interestingly, in College Station first harvest the increase in yield from insecticide to no-insecticide in the resistant photoperiod sensitive hybrids was not significant. There was no SCA pressure, implying that the differences were due to factors that appeared prior to chemical control. In the Weslaco environment, with the exception of photoperiod insensitive hybrids (PI), resistance definitively provided value to forage yield. In Lubbock, no differences in performance between resistant and susceptible hybrids was seen because the insecticide and no-insecticide trials were managed differently (Table 8).

Percent biomass yield loss in susceptible forage sorghum hybrids was linearly related to aphid damage. Using percent biomass yield loss as a dependent variable (Y-axis) and SCA damage as a predictor variable (X-axis) percent biomass yield loss could be predicted by the linear relationship: **Percent biomass yield loss (%) = 31.6 - 7.8 x Aphid damage**. As SCA damage approached nine on Sharma's chlorosis scale of 1-9 percent biomass yield loss due to aphids was nearly 50%. Percent biomass yield loss was negatively linearly related to SCA damage and was significant (Figure 6). For every one unit increment due to SCA damage 7.8% of expected biomass yield was lost as a result of aphids.

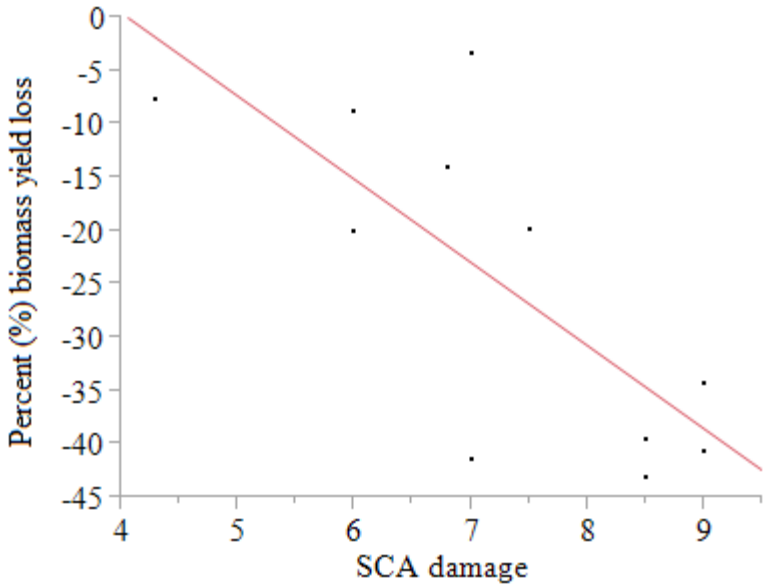

Figure 6 Negative linear relationship of percent biomass yield loss versus sugarcane aphid (SCA) damage in susceptible forage sorghum hybrids under heavy (> 1000 SCA leaf^{-1} plant^{-1}). In Lubbock, College Station and Weslaco, Texas, 2016. SCA damage measured using Sharma's chlorosis scale of 1-9. Percent yield loss (%) = 31.6 - 7.8 x Aphid damage. R-square = 0.56, F. Ratio = 11, Probability > F = 0.00085*.

Like biomass yield, dry matter accumulation was also affected. Susceptible genotypes suffered a higher loss. Using linear regression, and using biomass yield loss (tons ha^{-1}) as a predictor variable against dry matter loss (accumulation) in tons per hectare as a response variable, dry matter yield loss (tons ha^{-1}) due to aphids could be predicted from biomass yield loss (tons ha^{-1}) by linear a relationship: **DMLha^{-1} = 154.9 + 0.184 x BYLha^{-1}**. This was significant at 0.0088*. Where **DMLha^{-1}** = Dry matter yield loss (tons ha^{-1}) and **BYL** = Biomass yield loss (tons ha^{-1}). Spearman's correlation for biomass versus dry matter yield was **0.96 r** and significant at < 0.0001* (Figure 7).

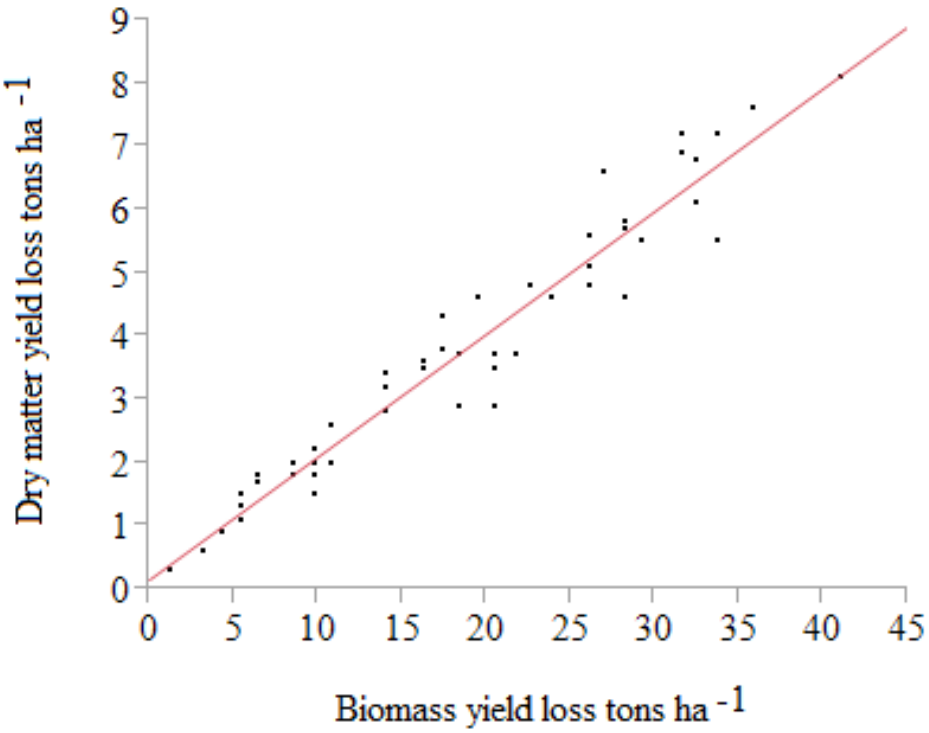

Figure 7 Positive linear relationship of dry matter yield loss (tons ha^{-1}) versus biomass yield loss (tons ha^{-1}) due to sugarcane aphid (SCA). Heavy (> 1000 SCA leaf^{-1} plant^{-1}) in Lubbock, College Station (second harvest) and Weslaco, Texas, 2016. DMLha^{-1} = 154.9 + 0.184 x BYLha^{-1}. Significant at 0.0088*. Where DMLha^{-1} = Dry matter yield loss (tons ha^{-1}) and BYL = Biomass yield loss (tons ha^{-1}). Spearman's correlation for biomass versus dry matter yield was 0.96 r and significant at < 0.0001* and R-square = 0.94.

Relative to susceptible hybrids, resistant forage hybrids generally remained green and healthy looking with no obvious disruption in chlorophyll accumulation and photosynthesis under heavy aphid pressure (> 1000 SCA leaf^{-1} plant^{-1}) (Figure 8). This may explain why resistant hybrids yielded higher than susceptible hybrids under heavy aphid pressure.

Figure 8 Effect of sugarcane aphid (*Melanaphis sacchari* (Zehntner)) on forage sorghum growth and development of resistant (left) and susceptible (right) genotypes. A = Photoperiod insensitive forage hybrid A.Tx3408/R.Tx2785 (left) and a photoperiod sensitive hybrid A.Tx3408/R.Tx2909 (right). B = photoperiod sensitive forage hybrids A.Tx645/R.Tx2910 (left) and A.Tx631/R.Tx2910 (right), Trial grown in College Station, Texas, 2016 (Second cutting 19th September 2016). Sugarcane aphid (SCA) heavy (> 1000 SCA leaf^{-1} plant^{-1}).

Forage quality

In terms of forage quality, there were significant differences among genotypes for protein, starch, sucrose, cellulose and lignin. SCA infestation affected protein content but had no effect on starch, sucrose, cellulose and lignin. Environment (location) as well as genotype by environmental interactions were significant for protein, starch, sucrose, cellulose and lignin. Environment by insecticide interactions were only significant for protein. Genotype by environment by insecticide interactions were not significant but for

starch and cellulose. The least squares means contrast was an indication of differences between resistant and susceptible hybrids and were all significant except for lignin (Table 9).

Table 9 Analysis of variance for forage quality for resistant versus susceptible hybrids across four locations showing effect of sugarcane aphid (insecticide and no-insecticide) on the quality of forage sorghum hybrids in Lubbock and College Station (first and second harvest) in summer and Weslaco in fall, Texas, 2016.

Source of Variance	Protein	Starch	Sucrose	Cellulos.	Lignin
Genotype	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Genotype Error	0.0001*	0.0005	0.0643	0.0006*	0.0013*
Insecticide	<0.0001*	0.6142	0.0954	0.8711	0.3677
Insecticide Error	0.0416*	0.5215	0.5729	0.0241*	0.4077
Genotype x Insecticide	0.5835	0.8870	0.8027	0.9404	0.6738
Replication (Insecticide)	0.0693	0.8777	0.9573	0.2969	0.7055
Location	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Location x Genotype	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Location x Insecticide	0.0264*	0.4078	0.5040	0.5817	0.8659
Locati. x Genoty. x Insect.	0.0731	<0.0001*	0.0366	0.0072*	0.1127
Corrected Total	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
LS Means Contrast: R vs S	0.0445*	<0.0001*	0.0475*	<0.0001*	0.2418
Aphid pressure		None	Heavy	Heavy	Moder.

Split-plot design. Twelve entries by four replications by two whole plots by four environments Insect. = insecticide, R = resistant hybrids, vs = versus and S = susceptible hybrids, Moder. = Moderate and Cellulos. = Cellulose. CS-1 =College Station, summer, first harvest, SCA pressure none; CS-2 = College Station, summer, second harvest, SCA pressure heavy (> 1000 SCA leaf^{-1} plant^{-1}); Weslaco, fall, SCA pressure moderate (350-500 SCA leaf^{-1} plant^{-1}).

SCA influenced protein quality and further evaluation revealed trends. The effect due to aphids was the difference between insecticide and no-insecticide. Overall, without insecticide treatment for SCA, both resistant and susceptible germplasm had a reduction in protein by 0.1 and 0.2 respectively (Table 10). With the exception of Lubbock, the

general trend was a reduction in protein values between the insecticide and no-insecticide regardless of whether the germplasm was photoperiod sensitive or photoperiod insensitive (Table 10). Surprising, regardless of the type of photoperiodic response by the germplasm, resistant hybrids had a lower protein content than susceptible hybrids except for the College Station difference between photoperiod sensitive insecticide and no-insecticide treatments (Table 10).

Table 10 Means for percent protein quality in forage sorghum for resistant versus susceptible hybrids across four locations showing effect of sugarcane aphid (insecticide and no-insecticide) on the quality of forage sorghum hybrids in Lubbock and College Station (first and second harvest) in summer and Weslaco in fall, Texas, 2016. Means were grouped by location, insecticide treatment and type of germplasm i.e. photoperiod sensitive (PS) and photoperiod insensitive (PI).

Combined	Insecticide	No-insecticide	Difference
Resistant	4.6	4.5	-0.1
Susceptible	5.2	5.0	-0.2
Difference	0.6	0.5	-0.1

Location	SCA	Type	PS Insect.	PS No-insect.	PI Insect.	PI No-insect.
College Station-1	None	Resistant	3.6	3.5	4.1	4.0
		Susceptible	3.2	3.2	4.4	4.3
College Station-2	Heavy	Resistant	6.8	6.4	6.0	5.5
		Susceptible	8.0	7.4	7.0	6.8
Lubbock	Heavy	Resistant	4.4	5.3	4.9	4.6
		Susceptible	5.7	6.2	6.0	5.2
Weslaco	Moderate	Resistant	3.4	3.3	3.6	3.1
		Susceptible	4.1	3.4	3.6	3.6

Split-plot design. Twelve entries by four replications by two whole plots by four environments. Sugarcane aphid (SCA) pressure was present except for College Station, summer, first harvest. The effect of SCA on protein quality between resistant and susceptible germplasm in the insecticide and no-insecticide treatments is further analyzed in Appendix V. Insect. = insecticide, No-insect. = no-insecticide.

Discussion

Heavy SCA infestations reduced biomass yield in both resistant and susceptible forage hybrids when trials were not treated with insecticide for SCA. It must also be noted that heavy SCA infestation resulted in yield loss in resistant genotypes at rates similar to that observed in the susceptible genotypes. But the effect was more pronounced in susceptible hybrids. In the four environments, much of the yield differences among resistant and susceptible hybrids were noted where insecticide was applied. This implies that the effect of SCA was occurring prior to chemical control and that resistance in these hybrids is not immunity. Under more moderate infestations (350-500 SCA $leaf^{-1}$ $plant^{-1}$), resistance appeared even more effective. In all environments, the resistant hybrids did not have as high an infestation rate as susceptible hybrids. Further, plant vigor and health were better in the resistant hybrids than in the susceptible hybrids. This supports findings by Armstrong (Armstrong et al., 2016).

Without controlling SCA, yield loss in susceptible forage sorghum hybrids were substantial. This has important implications for farmers growing sorghum for forage, silage or hay. This research support reports that SCA cause biomass yield loss (Knutson et al., 2016). SCA also reduce forage quality, for example without aphid control, loss in percent protein was nearly 9.0% in Weslaco Texas (Appendix V). Loss in protein quality resulting from aphids feeding on sorghum was identical between resistant and susceptible genotypes but resistant genotypes had a better yield advantage. Nonetheless, forage quality is severely affected when plants are infested at an early stage (Sharma et al., 2014) and the end result is a plant with a lower quality of silage. In the field, heavily

infested susceptible plants developed mold more easily. But the effect of mold on the quality of silage requires further investigation. Mold can affect forage quality and reduce the effectiveness of fungicides and cause secondary fungal infections (Jessica, 2002).

Dry matter accumulation is equally affected by SCA. Biomass yield loss was linearly related to dry matter yield loss. The amount of dry matter lost could be directly calculated from biomass yield loss. Dry matter yield evaluation is costly and time consuming. Calculating dry matter accumulation involves taking samples from biomass harvests, weighing, preventing moisture loss from samples, and drying in an oven for several days. The linear equation provided a quick way to access dry matter loss directly from biomass yield loss.

Consumer concerns about detrimental effects of improper use of agricultural chemicals (Maloni and Brown, 2006; Weber and Matthews, 2008; Zweig, 2013), attracts regulatory agencies. EPA has label restrictions on chemical insecticides used to control SCA, including re-entry period and application rates per hectare (USEPA, 2014; Bowling et al., 2016a). Under the international trade agreements of the World Trade Organization (WTO) there are regulations on the amount of allowed chemicals residues on agriculture produce (Pardío et al., 2012; Handford et al., 2014). Fortunately, a number of studies are looking at reducing applications rates i.e. liters/hectare and number of applications per season (Bean, 2017). This research suggests that resistant forage hybrids are valuable in averting excessive use of pesticides. Resistant hybrids would also work synergistically with reduced application rates in terms of number of applications or the application rates per hectare.

Since aphids are notorious for breaking resistance further studies to find additional sources of resistance in forage sorghums is an important consideration. The advantages of additive gene action for resistance to SCA can also be further investigated since this has been reported before (Manthe, 1992). Additive gene action has been demonstrated in many cases to have advantages in breeding for durable resistance against aphid pests in a number of crops (Reddy and Patil, 2015) and this should also be possible in sorghum considering that variation for SCA resistance exists.

CHAPTER V

PERFORMANCE OF GRAIN SORGHUM LINES AND HYBRIDS UNDER SUGARCANE APHID PRESSURE

Introduction and Objectives

Grain sorghum is an important crop in Texas and many other states in the United States of America (USA). The outbreak of SCA has caused concern on how to effectively manage this devastating pest. The pest causes yield and quality losses in a number of ways. The feeding activity of this insect affects yield and quality of grain sorghum. At grain maturity, the sticky honeydew secretions disturb harvesting of grain, and also causes problems with grain storage due to mold (Knutson et al., 2016). Ultimately, this necessitated evaluation of the effect of this pest on grain sorghums.

Evaluation of the relative value of resistance in grain sorghum hybrids was done using a Grain Sorghum Hybrid Trial (SCAG). The trial was grown in four environments, Lubbock, College Station, Corpus Christi and Weslaco. The approach was the same as the one used for forage hybrids SCA evaluation except the main interest was grain. Because SCA either did not infest the SCAG trial or where they did (Lubbock) bird damage was severe, yield data could not be not collected.

Aphids did not establish on the SCAG trial in College Station summer. The experiment in Weslaco at Rio Farms, and Weslaco Hiler Farms had a low SCA pressure (< 350 SCA $leaf^{-1}$ $plant^{-1}$), and minimal effect on the germplasm was observed. In Corpus Christi, aphids began infesting the fields on 6th June 2016, but after two weeks

the aphid population collapsed. The only trial that had SCA was in Lubbock but this test had heavy bird damage and yield data could not be collected. Yield data was not collected in Corpus Christi as well.

Despite these challenges, data was available from four trials grown in 2014, 2015 and 2016. The four trials were thus used for evaluating the effect of SCA on grain sorghums. The data consisted of aphid damage ratings due to SCA and yield under SCA pressure. The damage due to aphids was recorded on a chlorosis rating of 1-9 (Sharma et al., 2014). Some trials were under aphid pressure and some were not. To make inferences on the yield performance of resistant and susceptible germplasm the data was cross-examined.

The major objective of this study was to evaluate the performance of resistant and susceptible germplasm under aphid pressure, and determine whether resistant hybrids were advantageous in the event of a heavy SCA infestation.

Materials and Methods

Data from four trials was evaluated to determine the effect of SCA on grain sorghums. The four trials were: (i) screening of grain sorghum against SCA based on aphid damage to the plant in Weslaco-fall in 2014 designated as SCAP (I), (ii) screening of grain sorghum, based on panicle yield performance (plus yield per hectare in Halfway only), under SCA pressure in College Station, Corpus Christi and Weslaco in summer 2014 designated as SCAP (II), (iii) evaluation of developed grain hybrids for SCA resistance under SCA pressure in Halfway, College Station and Weslaco in summer 2015 designated as SCAH and (iv) evaluation of the effect of SCA on grain yield of

developed sorghum hybrids, in Lubbock, College Station, Corpus Christi and Weslaco in 2016 designated as SCAG.

Table 11 Dates of planting, sugarcane aphid (SCA) infestation, insecticide application and harvest for grain sorghum lines and hybrid trials, used to evaluate aphid damage (SCAP (I)), panicle yield (SCAP (II)), yield per hectare (yield ha^{-1}) and panicle yield (yield p^{-1}) in hybrids (SCAH), and yield per hectare (yield ha^{-1})) in hybrids (SCAG) in Texas, 2014 to 2016.

Location	Planting Date	Date of SCA Infestation	Date of Insecticide Application	Date of Harvest
SCAP (I): aphid damage (2014)				
Weslaco (fall)***	15 Aug.	8 Sep.	12 Sep.	N/A
SCAP (II): panicle yield (2014)				
College Station (summer)****	31 Mar.	28 May	No-insecticide	Aug.
Corpus Christi (Summer)****	18 Feb.	28 May	No-insecticide	2 Sep.
Weslaco (summer)****	18 Feb.	28 May	No-insecticide	Aug.
SCAH: yield (2015)				
Halfway (summer)*	10 Jun.	13 Jul.	No-insecticide	12 Oct.
College Station (summer)**	22 May	17 Aug.	No-insecticide	27 Aug.
Weslaco (summer)***	30 Mar.	05 May	No-insecticide	5 Oct.
SCAG: yield (2016)				
Lubbock-I (summer)***	25 May	30 Jun.	2 Aug.	26 Sep.
Lubbock-II (summer)***	6 Jun.	30 Jun.	No-insecticide	27 Sep.
College Station-I (summer)*	23 Mar.	26 Aug.	29 Aug.	5 Sep.
College Station-II (fall)***	26 Jul.	28 Aug.	2 Sep.	Nov.
Corpus Christi (summer)*	14 Apr.	23 May	27 May	6 Jul.
Weslaco-I, Rio Farms (summer)*	14 Aug.	Aug.	No-insecticide	Aug.
Weslaco-II, Hiler Farms (summer)*	15 Aug.	Aug.	No-insecticide	Aug.

Aphid pressure according to location: ****Heavy (> 1000 SCA leaf^{-1} plant^{-1}), ***high (500-1000 SCA leaf^{-1} plant^{-1}), **moderate (350-500 SCA leaf^{-1} plant^{-1}), *low (< 350 SCA leaf^{-1} plant^{-1}). The population in College Station was initially high (500-1000 SCA leaf^{-1} plant^{-1}) for three weeks then crashed. In Corpus Christi the population was low (< 350 SCA leaf^{-1} plant^{-1}) and crashed after two weeks. Yield data not collected in Lubbock due to bird damage. Corpus Christi yield data was not collected as well.

Details of the trials are discussed under their respective subheadings below. In the four trials; dates of planting, SCA infestations, insecticide application and harvest was recorded (Table 11). Agronomic management of the crop was done according to standard practices at the AgriLife research station in each location. The summary of dates of planting, SCA infestation and where insecticide was applicable are presented (Table 11).

Aphid damage (SCAP (I))

SCAP (I) consisted of evaluating aphid damage to grain sorghum using the chlorosis rating of 1-9 (Sharma et al., 2014). This was evaluated in Weslaco fall 2014. In the SCAP (I) aphid damage trial, the experimental design was a split-plot, consisting of whole plot treatments of insecticide and no-insecticide and sub-plot treatments of genotypes. The trial was planted on 15th August 2014. The test was composed of 20 entries of 12 lines and 8 hybrids. Each of the 20 entries was replicated nine times. The germplasm consisted of resistant and susceptible sorghums (breeding lines and hybrids). The resistant lines were Ent62/SADC, SC170, SC110, B.Tx3408, B.Tx3409, R.Tx2783, R.TAM428 (Macia/R.TAM428)-LL9 and (SV1*Sima/IS23250)-LG15. Susceptible lines were JS222, R.Tx2737, M 627, AF7301 and R.Tx7000. Two resistant hybrids (A.Tx642/R.Tx2783 and A.Tx2752/R.Tx2783) and four susceptible hybrids (A3.Tx436/R.Tx437, A3.Tx436/R.Tx437, A.Tx642/R.Tx436, and A.Tx2752/R.Tx437) were used. R.Tx2783 was developed for greenbug resistance (Peterson et al., 1984; Peterson et al., 2009) and R.TAM428 was previously evaluated for SCA resistance (Manthe, 1992; Singh et al., 2004) and were used as resistant checks. There was a high

SCA pressure (500-1000 SCA leaf^{-1} plant^{-1}) when the crop was at seedling stage (Stage 2) (Decimal code for plant stages of development stage 0-9 (Zadoks et al., 1974). When susceptible plants were 90% damaged on Sharma's scale, the SCA damage rating were recorded.

Panicle yield (SCAP (II))

SCAP (II) was used to evaluate the performance of grain sorghum (panicle yield) under SCA pressure. The trial was evaluated in summer 2014 in College Station, Corpus Christi and Weslaco, using a randomized complete block design. The evaluation was done in College Station, Corpus Christi and Weslaco in summer 2014. Dates of planting and SCA infestation were recorded. The germplasm used was identical to SCAP (I) above. Heavy aphid pressure (> 1000 SCA leaf^{-1} plant^{-1}) occurred at anthesis stage (stage 6) in all the locations. When susceptible genotypes were at least 90% damaged (Sharma et al., 2014), panicle yield were collected. Panicle yield was measured by harvesting ten random panicles by hand from the middle of the plot. Panicles were threshed using a single panicle thresher and grain was weighed using an electronic balance (Ohaus Adventurer™, model AV4101 x 0.1g). The weights were adjusted to an average of panicle yield for each experimental unit. But, it must be pointed out that panicle yield is not a direct reflection of yield per hectare.

Yield (SCAH)

SCAH consisted of evaluating the yield performance of grain hybrids under aphid infestation. The trial was planted in Halfway, College Station and Weslaco in summer 2015. In the SCAH, the primary trait of emphasis was yield under aphid

pressure, but SCA pressure was mostly very light and very late in the season. The SCAH yield trial, consisted of 15 entries (nine resistant and six susceptible hybrids) and three replications organized in a randomized complete block design. The nine resistant hybrids were A.Tx3409/R12169, A.Tx3408/R.Tx2783, A.Tx3409/R.Tx437, A.Tx2928/R.Tx2783, A.Tx3409/R.Tx436, A.Tx645/R.Tx2783, A.Tx3408/R.Tx436, A.Tx2752/R.Tx2783 and A.Tx3408/R.Tx437. Six susceptible hybrids were A.Tx645/R12169, A.Tx2752/R.Tx437, A.Tx2928/R.Tx436, A.Tx645/R.Tx436, A.Tx2752/R.Tx436 and A.Tx2928/R.Tx437. A.Tx2928 is a 3-dwarf line used for grain (Rooney, 2003). B.Tx2752 was released as a greenbug resistant line in 1976 (Johnson et al., 1982). B.Tx3408 and B.Tx3409 were released for SCA resistance in 2016 (Mbulwe et al., 2016). R.Tx436 and R.Tx437 are grain lines (Rooney, 2003). This trial was grown in Halfway, College Station and Weslaco in summer 2015.

Across these locations during the trial, the SCA pressure varied with a low infestation (< 350 SCA leaf^{-1} plant^{-1}) in Halfway but moderate in College Station and Weslaco (350-500 SCA leaf^{-1} plant^{-1}). In Halfway, aphids appeared when grain on the plants was at hard dough stage (V8 stage). In Weslaco and College Station aphids appeared at anthesis (stage 6) and soft dough (stage 7), respectively. The primary traits measured were the same as described in the SCAP (II) yield, with the exception of grain yield on a per hectare basis in Halfway because full plots were harvested and threshed.

Yield (SCAG)

Similar to SCAH in 2015, SCAG was used to further evaluate the effect of SCA on the yield of developed grain hybrids. The trial was planted in Lubbock, College

Station (summer and fall), Corpus Christi and Weslaco in summer 2016. The experiment was composed of 12 grain sorghum hybrids that included eight resistant and four susceptible sorghums. Eight hybrids were produced using SCA resistant lines namely; A.Tx631/R.Tx2783, A.Tx3408/R.Tx437, A.Tx3409/R.Tx437, A.Tx3409/R.Tx2783, A.Tx3408/R.Tx2783, A.Tx3408/R.Tx436, A.Tx3409/R.Tx436 and A.Tx645/RTx2783 (Mbulwe et al., 2016). The susceptible hybrids were A.Tx631/R.Tx437, A.Tx645/R.Tx436, A.Tx645/R.Tx437 and A.Tx631/R.Tx436. A.Tx631 released in 1985 and A.Tx645 released in 2002 are common seed parents used to produce forage hybrids (Miller, 1986; Rosenow et al., 2002) while R.Tx436 and R.Tx437 are used as male parents in grain hybrids (Rooney et al., 2003). The SCAG trial was grown in six locations; Lubbock, College Station, Corpus Christi, Weslaco Rio Farms and Hiler Farms in summer 2016. The College Station trial planted in fall was the sixth location. The SCAG trial was laid out in a split-plot design with whole plot treatments for chemical mitigation and a control. The subplots were the hybrid genotypes with each hybrid designated as resistant or susceptible based on parents in the hybrid. Each whole plot treatment had four replications.

Whole plots were harvested by hand and threshed using a belt thresher (Almaco single plant thresher, Model BT14). Yield was reported as tons per hectare. Plant height and exertion were recorded using a calibrated height stick (Barcode readable). Plant height was the perpendicular length in meters from the apex of the sorghum head (panicle) to the ground. While exertion (panicle exertion) was the length between the bottom of the panicle and the flag leaf (final top most leaf). Days to 50% anthesis (the

time it takes for half of the sorghum panicle to flower) was recorded using the Julian calendar and grain moisture was recorded using a moisture meter (Dickey John, Model MINI GAC1).

Statistical analysis

The ANOVA model for the split-plot design with two treatments (insecticide and no-insecticide), used to analyze the forage sorghum hybrid trials in chapter four, was used in the SCAP (I) trial of Weslaco fall 2014, and also in the SCAG yield trials of 2016. One way ANOVA was used to analyze germplasm differences in panicle yield in the SCAP (II) and SCAH trials and means compared with test trial LSD values ($P < 0.05$). Statistical comparisons were calculated using the statistical software PROC GLM in SAS v 9.3 (SAS Institute, 2011).

Results

Analysis of variance for aphid damage (SCAP (I))

Table 12 Analysis of variance for aphid damage (SCAP (I)). Plant damage evaluated under high sugarcane aphid (SCA) pressure (500-1000 SCA leaf^{-1} plant^{-1}), for a month in fall, Weslaco, Texas, 2014. Aphid damage was done using chlorosis rating of 1-9 under insecticide and no-insecticide conditions.

Source of Variance	Weslaco Fall
Genotype	<0.0001*
Genotype Error	0.0002*
Insecticide	<0.0001*
Insecticide Error	<0.0001*
Genotype x Insecticide	0.0003*
Replication (Insecticide)	0.2233
Corrected Total	<0.0001*
LS Means Contrast: R vs S	<0.0001*

Randomized complete block design. Twenty entries by nine replications by one environment. R = resistant hybrids, vs = versus and S = susceptible hybrids. Asterisk (*) Indicates significance.

Analysis of variance showed a significant difference between genotypes and insecticide treatments (Table 12). Genotype by insecticide interaction was also significant. The Least Squares (LS) means contrast also showed significant differences. However, replication effects could not be detected by the statistical model.

Mean comparison for aphid damage (SCAP (I))

The mean for aphid damage (chlorosis rating) of resistant lines with insecticide treatment was 1.0 and without insecticide was 2.3 while the mean for susceptible lines was 5.7 with insecticide and 7.7 without insecticide (Table 13). The effect due to SCA damage, which is the difference between insecticide and no-insecticide, was less for resistant lines (1.3) and greater for susceptible lines (2.0) (Table 13). In the hybrids, the mean for resistant germplasm under insecticide was 2.0 and susceptible 2.2 while without any insecticide treatment the mean for resistant was 4.8 and susceptible 8.1. The effect as a result of SCA damage was lower for resistant hybrids 2.8 and higher for susceptible hybrids 5.9 (Table 13).

Table 13 Mean separation of aphid damage (SCAP (I)). Plant damage evaluated under high sugarcane aphid (SCA) pressure (500-1000 SCA leaf^{-1} plant^{-1}), for a month in fall, Weslaco, Texas, 2014. Aphid damage was done using chlorosis rating of 1-9 under insecticide and no-insecticide conditions.

Pedigree	Type	Genotype	Insect.	No-insect.	Difference
Ent62/SADC	Line	Resistant	1.0c	1.0b	0.0c
SC170-14	Line	Resistant	1.0c	1.0b	0.0c
SC110-14	Line	Resistant	1.0c	1.7b	0.7c
B.Tx3408	Line	Resistant	1.0c	3.3a-b	2.3b-c
B.Tx3409	Line	Resistant	1.0c	4.7a-b	3.7a-c
R.Tx2783*	Line	Resistant	1.0c	9.0a	8.0a
R.TAM428*	Line	Resistant	6.0a-b	9.0a	3.0a-c
(Macia/R.TAM428)-LL9*	Line	Resistant	1.0c	9.0a	8.0a
(SV1*Sima/IS23250)-LG*	Line	Resistant	4.0a-c	6.3a-b	2.3b-c
Mean	**Line**	**Resistant**	**1.0**	**2.3**	**1.3**
JS222	Line	Susceptible	4.0a-c	7.3a-b	3.3a-c
R.Tx2737	Line	Susceptible	5.0a-c	9.0a	4.0a-c
M 627	Line	Susceptible	6.0a-b	6.0a-b	0.0c
AF7301	Line	Susceptible	6.0a-b	6.7a-b	0.7c
R.Tx7000	Line	Susceptible	7.0a	8.3a	1.3c
Mean	**Line**	**Susceptible**	**5.6**	**7.5**	**1.9**
A.Tx642/R.Tx2783	Hybrid	Resistant	2.0b-c	4.3a-b	2.3b-c
A.Tx2752/R.Tx2783	Hybrid	Resistant	2.0b-c	5.3a-b	3.3a-c
Mean	**Hybrid**	**Resistant**	**2.0**	**4.8**	**2.8**
A3.Tx436/R.Tx437	Hybrid	Susceptible	1.0c	8.7a	7.7a
A3.Tx436/R.Tx437	Hybrid	Susceptible	1.0c	9.0a	8.0a
A.Tx642/R.Tx436	Hybrid	Susceptible	2.0b-c	8.3a	6.3a-b
A.Tx2752/R.Tx437	Hybrid	Susceptible	4.0a-c	7.0a-b	3.0a-c
Mean	**Hybrid**	**Susceptible**	**2.0**	**8.3**	**6.3**

Randomized complete block design. Twenty entries by nine replications by one environment. Column means followed by the same lowercase letters are not significantly different. Insect. = insecticide, No-insect. = no-insecticide. * R.Tx2783, R.TAM428, (Macia/R.TAM428)-LL9) and (SV1*Sima/IS23250)-LG) appeared susceptible but are in fact resistant. Hard to control factors (random errors) associated with environmental conditions, spatial distribution of aphids, edaphic conditions or plant physiology may have contributed to the germplasm appearing susceptible in this one incidence.

Analysis of variance for panicle yield (SCAP (II))

Significant differences for grain weight per panicle in the SCAP (II) trial were found for genotype, location, genotype by location and resistant versus susceptible. In

the combined analysis, the main effects, interaction and contrast were all significant (Table 14).

Table 14 Analysis of variance for panicle yield (SCAP (II)) of resistant versus susceptible germplasm under moderate SCA pressure (350-500 leaf^{-1} plant^{-1}), in College Station and Corpus Christi, and at high SCA pressure (500-1000 SCA leaf^{-1} plant^{-1}) in summer, Weslaco, Texas, 2014.

Sources of Variance	Combined Locations	College Station	Corpus Christi	Weslaco
Genotype	<0.0001*	<0.0001*	0.0022	<0.0001*
Replication	0.1352	0.0942	0.0018	0.5704
Location	<0.0001*			
Genotype*Location	<0.0001*			
LS Mean Contrast: R vs S	<0.0001*	<0.0001*	0.0010*	<0.0001*
Aphid Pressure		Moderate	Moderate	High

Randomized complete block design. Twenty entries by nine replications by three environments. S = susceptible hybrids, vs = versus, and R = resistant hybrids. Asterisk (*) indicates significance.

Mean comparison for panicle yield (SCAP (II))

Under high aphid pressure (500-1000 SCA leaf^{-1} plant^{-1}), the mean panicle yield (Table 15) for resistant lines was 55.4, 33.1 and 56.3 in College Station, Corpus Christi and Weslaco respectively while the susceptible lines in the same locations averaged 28.4, 17.8 and 18.6 respectively (Table 15). Likewise, in respective order of location; College Station, Corpus Christi and Weslaco, the resistant hybrids had panicle yield of 52.8, 31.5 and 55.8. The susceptible hybrids panicle yield averaged 46.0, 34.5 and 35.0 respectively (Table 15). The implication is that the differences in panicle yield were due to susceptibility to SCA. However, there was no control to look at relative loss. Therefore, the differences could be genotypic as well.

Table 15 Mean separation for panicle yield (SCAP (II)) of resistant versus susceptible germplasm under moderate SCA pressure (350-500 leaf^{-1} plant^{-1}), in College Station and Corpus Christi, and high SCA pressure (500-1000 SCA leaf^{-1} plant^{-1}) in summer, Weslaco, Texas, 2014.

Pedigree	Type	Genotype	CS Yield p^{-1}	CC Yield p^{-1}	WE Yield p^{-1}
Ent62/SADC	Line	Resistant	61a-c	26a-b	91a
SC170	Line	Resistant	52b-e	32a-b	54b-d
SC110	Line	Resistant	41d-g	30a-b	50b-d
B.Tx3408	Line	Resistant	57a-d	26a-b	42b-f
B.Tx3409	Line	Resistant	50b-e	31a-b	48b-e
R.Tx2783	Line	Resistant	49b-e	30a-b	53b-d
R.TAM428	Line	Resistant	69a	52a	68a-b
A.Tx2752/R.Tx2783	Line	Resistant	63a-b	30a-b	42b-f
(SV1*Sima/IS23250)-LG15	Line	Resistant	57a-d	41a-b	59b-c
Mean	**Line**	**Resistant**	**55.4**	**33.1**	**56.3**
JS222	Line	Susceptible	23g-h	11b	16f-g
R.Tx2737	Line	Susceptible	27g-h	9b	1g
M 627	Line	Susceptible	39e-h	23a-b	27d-g
AF7301	Line	Susceptible	25g-h	18a-b	28d-g
R.Tx7000	Line	Susceptible	28f-h	28a-b	21e-g
Mean	**Line**	**Susceptible**	**28.4**	**17.8**	**18.6**
A.Tx3408/R.Tx437	Hybrid	Resistant	49b-e	32a-b	68a-b
A.Tx3408/R.Tx436	Hybrid	Resistant	44c-f	42a-b	65a-b
A.Tx642/R.Tx2783	Hybrid	Resistant	55a-e	22a-b	48b-e
A.Tx2752/R.Tx2783	Hybrid	Resistant	63a-b	30a-b	42b-f
Mean	**Hybrid**	**Resistant**	**52.8**	**31.5**	**55.8**
A.Tx642/R.Tx436	Hybrid	Susceptible	48b-e	43a-b	49b-e
A.Tx2752/R.Tx437	Hybrid	Susceptible	44c-f	26a-b	21e-g
Mean	**Hybrid**	**Susceptible**	**46.0**	**34.5**	**35.0**

Randomized complete block design. Twenty entries by nine replications by three environments. Column means followed by the same lowercase letters are not significantly different.

Analysis of variance for yield (SCAH)

From the combined (College Station and Weslaco) and individual (Halfway, College Station and Weslaco) analysis of grain yield, genotypic differences were observed (Table 16). In the combined analysis, location and genotype by location

differences were not detected. Where SCA pressure was low in Halfway, the LS means contrast showed that there were no differences in yield per hectare between resistant and susceptible hybrids. In College Station and Weslaco where aphids pressure was moderate (350-500 SCA leaf^{-1} plant^{-1}) the LS means contrast for panicle yield showed significant differences between susceptible and resistant hybrids.

Table 16 Analysis of variance for yield per hectare (yield ha^{-1}), and panicle yield (yield p^{-1}) (SCAH), in Halfway with low sugarcane aphid (SCA) pressure (< 350 SCA leaf^{-1} plant^{-1}). Panicle yield in College Station and Weslaco under moderate aphid pressure (350-500 SCA leaf^{-1} plant^{-1}), summer, Texas, 2015.

Sources of Variance	Combined CS and WE	Halfway Yield ha^{-1}	Halfway Yield p^{-1}	College Station Yield p^{-1}	Weslaco Yield p^{-1}
Genotype	<0.0001*	0.0014*	0.2288	<0.0001*	0.0002*
Replication	0.8096	0.7186	0.9515	0.9468	0.7860
Location	0.0280				
Genotype x Location	0.6626				
LS Mean Contrast: R vs S	<0.0001*	0.058	0.5341	<0.0001*	0.0001*
Aphid Pressure		Low	Low	Moderate	Moderate

Randomized complete block design. Fifteen entries by three replications by four environments. S = susceptible hybrids, vs = versus, and R = resistant hybrids. Asterisk (*) indicates significance.

Mean comparison for yield (SCAH)

In Halfway under low aphid pressure (< 350 SCA Leaf^{-1} panicle^{-1}), resistant and susceptible hybrids performed similarly in terms of panicle yield with a mean of 62.0 grams for resistant and 66.3 grams for susceptible hybrids. The same was true for yield per hectare in Halfway where resistant hybrids had an average of 6.2 tons per hectare and susceptible hybrids had 6.5 tons per hectare. In College Station and Weslaco, where

SCA pressure was moderate (350-500 SCA leaf^{-1} plant^{-1}), resistant hybrids had a higher panicle yield than susceptible hybrids (Table 17). Resistant hybrids had 71 and 77 grams per panicle in College Station and Weslaco respectively, compared to 55 and 61 grams in susceptible hybrids.

Table 17 Mean separation for yield per hectare (yield ha^{-1}), and panicle yield (yield p^{-1}) (SCAH), in Halfway with low sugarcane aphid (SCA) pressure (< 350 SCA leaf^{-1} plant^{-1}). Panicle yield in College Station and Weslaco under moderate aphid pressure (350-500 SCA leaf^{-1} plant^{-1}), summer, Texas, 2015.

Pedigree	Genotype	HW Yield ha^{-1}	HW Yield p^{-1}	CS Yield p^{-1}	WE Yield p^{-1}
A.Tx3409/R.12169	Resistant	5.03b	53.5a	89a	88a-b
A.Tx3408/R.Tx2783	Resistant	4.7b	49.5a	84a-b	96a
A.Tx3409/R.Tx437	Resistant	8.3a	88.5a	80a-c	77a-c
A.Tx2928/R.Tx2783	Resistant	7.0a-b	57.6a	77a-d	77a-c
A.Tx3409/R.Tx436	Resistant	5.0a-b	53.3a	77a-d	71a-c
A.Tx645/R.Tx2783	Resistant	6.2a-b	66.2a	63a-d	74a-c
A.Tx3408/R.Tx436	Resistant	4.6b	48.9a	59a-d	69a-c
A.Tx2752/R.Tx2783	Resistant	7.3a-b	77.7a	55b-d	62b-c
A.Tx3408/R.Tx437	Resistant	7.7a-b	62.8a	54b-d	77a-c
Mean	**Resistant**	**6.2**	**62.0**	**71**	**77**
A.Tx645/R.12169	Susceptible	5.8a-b	61.6a	80a-c	79a-c
A.Tx2752/R.Tx437	Susceptible	7.9a-b	84.0a	57b-d	55c
A.Tx2928/R.Tx436	Susceptible	7.0a-b	52.8a	52b-d	55c
A.Tx645/R.Tx436	Susceptible	6.9a-b	73.8a	48c-d	54c
A.Tx2752/R.Tx436	Susceptible	4.8b	51.4a	47d	61b-c
A.Tx2928/R.Tx437	Susceptible	7.0a-b	74.3a	46d	48c
Mean	**Susceptible**	**6.5**	**66.3**	**55**	**61**

Randomized complete block design. Fifteen entries by three replications by three environments. Column means followed by the same lowercase letters are not significantly different.

Analysis of variance for yield (SCAG)

In the SCAG trial of 2016, there was no aphid pressure and under these conditions, neither insecticide treatment nor contrast were significant. Differences in genotypes were detected as was expected (Table 18). Because the aphid pressure was low (< 350 SCA leaf^{-1} plant^{-1}) the variance due to insecticide treatments was not detected and neither were the interaction effects.

Table 18 Analysis of variance for yield per hectare (yield ha^{-1}) (SCAG) under low sugarcane aphid (SCA) pressure (< 350 SCA leaf^{-1} plant^{-1}), in tons per hectare (tons ha^{-1}) in College Station, Weslaco-Hiler Farms and Weslaco-Rio Farms in Texas, 2016.

Sources of Variance	Combined	College Station (Summer)	College Station (Fall)	Weslaco Hiler (Summer)	Weslaco Rio (Summer)
Insecticide	0.5347	0.5605	0.5905		
Rep(Insecticide)	0.9193	0.8644	0.9572	<0.0001*	0.9201
Genotype	0.0166*	0.0129*	0.0005*	0.0399*	0.0081*
Genotype x Insecticide	0.7621	0.6363	0.5096		
Location	<0.0001*				
Genotype x Location	0.1497				
Geno. x Loca. x Insect.	0.0366*				
LS Mean Cont. R vs S	0.0969	0.1001	0.2976	0.3055	0.7977
Aphid Pressure	Low	Low	Low	Low	Low

Randomized complete block design. Twelve entries by eight replications by four environments. Abbreviations: Geno. = genotype, Loca. = location, Insect. = Insecticide, R = resistant hybrids, vs = versus and S = susceptible hybrids. Asterisk (*) indicates significance.

Mean comparison for yield (SCAG)

Since insecticide treatments effects and LS means contrasts were not significant for yield, the yield data was combined (Table 19). It appears that without SCA pressure resistant and susceptible germplasm yield the same (4.1 tons ha^{-1}). Both resistant and

susceptible germplasm had a similar plant height, grain moisture, and days to 50% anthesis with only a few exceptions. Means followed by the same lowercase letters were not significantly different.

Table 19 Agronomic characteristics (yield per hectare (tons ha^{-1}) (SCAG), height, grain moisture, days to 50% anthesis and leaf exertion) of resistant and susceptible sorghum hybrids under low aphid pressure (< 350 SCA leaf^{-1} plant^{-1}) in summer, College Station, Weslaco-Hiler Farms and Weslaco-Rio Farms in Texas, 2016.

Trait	Yield (tons ha^{-1})	Height (m)	Grain Moisture (%)	Day to Anthesis	Exertion (cm)
A.Tx3408/R.Tx437 (R)	5.0a	1.3b-c	9.4a	68a-b	0.16a
A.Tx3408/R.Tx436 (R)	4.7a	1.3b-c	9.7a	69a-b	0.18a
A.Tx645/R.Tx2783 (R)	4.5a	1.3b-c	9.9a	70a-b	0.19a
A.Tx631/R.Tx2783 (R)	4.3a	1.4a-b	10.1a	74a	0.17a
A.Tx3409/R.Tx2783 (R)	4.0a	1.3b-c	9.5a	67a-b	0.21a
A.Tx3409/R.Tx437 (R)	3.7a	1.3c	9.7a	70a-b	0.18a
A.Tx3408/R.Tx2783 (R)	3.6a	1.5a	10.5a	73a	0.18a
A.Tx3409/R.Tx436 (R)	3.3a	1.2c	9.7a	70a-b	0.21a
Mean	**4.1**	**1.3**	**9.8**	**70**	**0.19**
A.Tx645/R.Tx437 (S)	4.3a	1.3b-c	9.8a	65b	0.22a
A.Tx631/R.Tx437 (S)	4.2a	1.3b-c	9.4a	67a-b	0.20a
A.Tx631/R.Tx436 (S)	4.0a	1.3b-c	9.6a	69a-b	0.21a
A.Tx645/R.Tx436 (S)	4.0a	1.2c	10.1a	67a-b	0.23a
Mean	**4.1**	**1.3**	**9.7**	**67**	**0.21**
LSD (P < 0.05)	**0.62**	**0.03**	**0.22**	**2**	**0.02**

Randomized complete block design. Twelve entries by four replications by two whole plots by four environments. Column means followed by the same lowercase letters are not significantly different R = resistant hybrids. S = susceptible hybrids. Since there were no significant differences yield from the four locations were combined and analyzed.

Discussion

At seedling stage (stage 2), in the whole plot without insecticide treatment, grain sorghum germplasm resistant to SCA (B.Tx3408 and B.Tx3409) exhibited less aphid

damage under high SCA pressure. Conversely, susceptible germplasm (JS222, R.Tx2737, M627, AF301 and R.Tx7000) was more severely affected. This was observed in a controlled split-plot trial called SCAP (I) in Weslaco fall 2014 where one whole plot was treated with insecticide and the other whole plot was not treated with insecticide. This suggested that even at an early stage resistance to SCA is present and valuable.

R.TAM428, a line reported to have SCA resistance (Manthe, 1992; Singh et al., 2004), and other lines (R.Tx2783, R.TAM428, (Macia/R.TAM428)-LL9 and (SV1*Sima/IS23250)-LG), appeared susceptible to SCA only in SCAP (I) but are resistant. Other hard to control factors (experimental error) associated with environmental conditions, spatial distribution of aphids or edaphic conditions could have contributed to the germplasm appearing susceptible in this one particular situation. The said germplasm had performed relatively well in earlier evaluations (Armstrong et al., 2015; Mbulwe et al., 2016).

It should be noted that only one application of Transform WG® (0.11L ha^{-1}) was applied; some aphid pressure was present even in the section sprayed with insecticide. As a result, this caused significant aphid damage or death in completely susceptible lines. Some resistant lines did not have aphid damage regardless of chemical control. Other lines and hybrids were effectively protected by the chemical treatment but were obviously susceptible without chemical protection. These results, roughly align with previous evaluation of these materials in greenhouse conditions (Armstrong et al., 2015). Ultimately, this implies that resistant germplasm would probably survive with fewer insecticide applications than susceptible germplasm under integrated pest management.

The SCA infestations in the summer grain trials occurred during the grain filling stages (stages 7-8). Yield differences (yield ha^{-1}) were not significant when SCA is not present (SCAH) in Halfway. Likewise, in the SCAG trials when SCA were not present, yield differences (yield ha^{-1}) were not seen. Differences in panicle yield were observed between resistant and susceptible lines and hybrids where SCA pressure was present. This was seen in the uncontrolled treatment of SCAP (II) yield trial but this could be due to genotypic differences per se. Equally, in the SCAH yield trial, in College Station and Weslaco summer 2014, where aphid pressure was moderate (350-500 SCA $leaf^{-1}$ $plant^{-1}$), resistant genotypes had higher yield per panicle.

The observed differences in panicle yield may be likely due to SCA. The concomitant drops in panicle yield in both the resistant and susceptible groups implies that either aphid pressure was persistent in each group or that resistance in these hybrids is overcome after a specific SCA load is attained. These findings support a report from Kansas State University that susceptible panicles not protected from SCA damage weigh 70% less (Michaud and Zukoff, 2016). Additionally, the higher panicle yield (all things equal) of resistant germplasm in itself is an advantage. Nevertheless, it appears resistance helps the sorghum plant to continue growing under SCA pressure relative to the susceptible germplasm. In general, sorghum lines and hybrids with resistance under high SCA pressure (500-1000 SCA $leaf^{-1}$ $plant^{-1}$) produced higher panicle yield and less aphid damage than did susceptible germplasm. On that account, there is some benefit in utilizing resistant grain hybrids. Additional evaluation of resistant grain hybrids will continue to confirm this observation and to identify additional sources of resistance.

Since there was no control (insecticide and no-insecticide) to partition variation due to SCA infestations, and since panicle yield does not reflect yield per hectare, conclusions presented herein need additional evaluation. Many additional variables influence panicle yield such as grain weight, size and number as well as panicle size and plant density. Nonetheless, results do point out inherent traits that may be advantageous to resistant grain hybrid sorghums as opposed to susceptible hybrids. These factors need further scrutiny in future investigations.

To consolidate findings of the advantages of resistant grain hybrids, the originally planned sorghum hybrid trial (SCAG) could be repeated using insecticide and no-insecticide treatments in a split-plot design or randomized complete bock design using resistant and susceptible lines with similar maturity and yield in at least three environments to make a better comparison. However, one must take into account position of the field, timing (planting latter), natural energies, spatial distribution of aphids, and weather in order to have a successful experiment (Sharma et al., 2014). However, aphid occurrences are difficult to predict under natural conditions (Bowling et al., 2016b).

It was relatively easy to manipulate planting dates and position of the field to synchronize peak aphid infestations. But natural enemies and aphid occurrences were more difficult to predict in time and space even when aphids were abundant. Generally, late maturing photoperiod sensitive forage hybrid sorghums, influenced by late maturity genes (Rooney and Aydin, 1999; Bhosale et al., 2012), had a higher incidence of SCA because of the longer exposure in the field. On the other hand the majority of grain

sorghums escaped aphid infestations. This was particularly so in some fields in Halfway, College Station and Corpus Christi in summer 2015 and 2016.

CHAPTER VI

DETERMINING CATEGORIES OF RESISTANCE AND CORRELATION BETWEEN PHENOTYPE AND RESISTANCE

Introduction and Objectives

Variation in response to SCA infestation is present in cultivated and wild sorghum genotypes. SCA reproduces at a higher rate and causes more damage to susceptible sorghum genotypes than to resistant sorghum genotypes in both grain and forage types (Armstrong et al., 2016). In this study, B.Tx3408, B.Tx3409 and R.Tx2783 were used as sources of resistance to SCA. The line R.Tx2783 resistant to greenbug biotypes C and E (Peterson et al. 1984) is also resistant to the sugarcane aphid (Armstrong et al., 2015; Bayoumay et al., 2016; Mbulwe et al., 2016).

The earlier studies of SCA resistance mechanisms were accomplished in a greenhouse using standard screening techniques that determined the types of resistance. The mechanisms underlying resistance were identified as antibiosis, antixenosis and tolerance (Armstrong et al., 2016). This study contributed to early efforts by further evaluating resistance mechanisms under natural field conditions using additional approaches. The studies were done in College Station in fall 2016. Because of circumstances pertaining to time and presence of aphids only antibiosis and antixenosis (non-preference resistance) were possible to evaluate.

Traits associated with host-plant resistance against arthropods have been traditionally categorized in three ways: antibiosis, antixenosis and tolerance (Painter,

1951; Stout 2013). Resistance (Painter, 1951) was defined as “the relative amount of heritable qualities possessed by the plant which influence the ultimate degree of damage done by the insect”. Research on host-plant resistance has been largely influenced by Reginald Painter’s definition of resistance to insect arthropods (Stout, 2013).

Antibiosis resistance is a biochemical or morphological defense mechanism used by the host plant to reduce insect damage. Antibiosis may be induced by feeding insects or may be constitutive. Antibiosis effects on insect arthropods range from minimal to deadly. Examples of adverse effects on insect arthropods are poor growth (weight gain), higher mortality, decreased longevity and reduced reproduction capacity (Dixon, 1998). Ultimately, the overall effect of antibiosis is reduced fecundity (Smith, 2005). Antibiosis is usually measured using no-choice insect feeding experiments. Alternative methods are available to screen trials for antibiosis under field conditions. These include assessing amount of damage, arthropod populations, arthropod growth and mortality of insects on the plants.

Antixenosis or non-preference resistance is when the presence of any morphological or biochemical factor of a plant adversely modifies insect arthropod behavior. For example, thick epidermis, wax or trichomes may force insects to abandon their efforts to colonize, feed or oviposit on a host-plant. The lack of phytochemicals may also affect the ability of an insect to recognize a host-plant. Alternatively, resistant plants may possess phytochemicals that fend off or prevent insects from colonizing, feeding or ovipositing (Singh et al., 2004). Antixenosis is normally evaluated by choice insect feeding experiments. Under field conditions, antixenosis can be detected by

looking at feeding activities, population densities and preference of insects for certain plants.

Tolerance is the ability of a host-plant to withstand or recover from insect damage resulting in yield equal to or above the yield of susceptible plants not infested with the insect. Biologically, tolerance is the ability of a host-plant to recover from arthropod injury. From an agronomic perspective, tolerance is the inherent genetic ability of resistant cultivars to produce a greater amount of biomass than susceptible cultivars (Smith, 2005). Tolerance in field crops is measured by comparing yield of resistant cultivars infested with insects to yield of susceptible cultivars without insects. For SCA, tolerance was considered to be the ability of resistant sorghums infested with SCA to yield equal to susceptible sorghums without infestation.

The major objective of this study was to determine the categories of resistance in grain and forage sorghum hybrids. As a result, systematic tests were conducted to identify if antibiosis or antixenosis (non-preference resistance) were contributing to SCA resistance in sorghum. Additionally correlation between phenotypic traits and aphid damage was done to identify traits that contribute to resistance.

Materials and Methods

To measure if antibiosis existed in sorghum germplasm against SCA three methods were used: (i) average weight per aphid and number of aphids per leaf (Method I), (ii) average number of aphids per leaf and field rate of increase (reproductive capacity) (Method II), and (iii) nymphal mortality rate (Method III). To determine antixenosis (non-preference) resistance, only one method was possible under field

conditions, i.e. the average number of alates per leaf (Method IV). Additionally, phenotypic traits were measured and a correlation done between the traits and SCA damage ratings (Method V). These five methods of detecting resistance mechanisms were done in summer and fall 2016 (Table 20). These methods are further described under their respective subheadings.

The experiments were conducted at the Texas A&M University (College Station) research facility located in Burleson County. The research facility lies between latitude (N30º33'11.52" and N30º32'19.68") and longitude (W96º26'51.36" and W96º25'7.68"), elevation (67-68 m) above sea-level, soil type 39 (Clay-loam) (USDA-NRCS, 2008; Google Earth Pro 7.1.8.3036, 2014). The trials were managed according to standard agronomic management practices at this research facility. The list of methods used, trials, evaluation dates and type of morphs on which data was collected in College Station summer and fall 2016 are listed (Table 20).

Table 20 List of methods used, trials, evaluation date and type of morphs on which data was collected in College Station, Texas, summer and fall 2016.

List of Methods	Trial	Evaluation Date	Data Collected on
Method I	8 Lines	8 July.	Aphid colonies
Method II	SCAG and SCAF	21 Aug to 10 Aug.	Aphid colonies
Method III	SCAG and SCAF	28 Aug to 6 Sep.	Nymphs
Method IV	3 Lines	20 Aug.	Migrating alates
Method V	SCAG and SCAF	21 Aug. to 21 Nov.	Plant Phenotype

Method I trial planted on 7th April and Method II-V trials planted on 26th July. Method I = Average weight per aphid and number of aphids per leaf (antibiosis). Method II = Average number of aphids per leaf and field rate of increase (reproductive capacity) (antibiosis). Method III = Nymphoal mortality rate (antibiosis). Method IV = Average number of alates per leaf (antixenosis). Method V = Correlation and principal component analysis (aphid damage vs phenotype). SCAG = Sugarcane Aphid Grain Hybrid Trial. SCAF = Sugarcane Aphid Forage Hybrid Trial.

Antibiosis (method I) average weight per aphid and number of aphids per leaf

Eight sorghum germplasm were used to assess average weight per aphid and number of aphids per leaf. Four resistant lines (B.Tx3408, B.Tx3409, R.TAM428 and R.Tx2783), three susceptible lines (B.Tx631, R.Tx436, R.Tx7000) and one susceptible hybrid (Pioneer 84P80) were used. A/B.Tx3408 and A/B.Tx3409 were released as resistant lines to SCA (Mbulwe et al., 2016), R.Tx2783 was released as a resistant line to greenbug (Peterson et al., 1984) and R.TAM428 was reported as having resistance to SCA in Africa and India (Manthe, 1992; Sharma et al., 2014). R.Tx7000, R.Tx436 and Pioneer 84P80 are susceptible to SCA (Armstrong et al., 2016; Mbulwe et al., 2016; Pekarcik, 2016). B.Tx631 is resistant to greenbug biotype E (Miller, 1986) but not necessarily resistant to SCA.

The eight sorghum germplasm were replicated twice in a randomized complete block design. The germplasm were planted late at the Texas A&M AgriLife Research Farm on 7th April 2016. The late planting coordinated reproductive growth stages (stage 6-9) with peak aphid infestations and enabled effective evaluation of the reproductive capacity of aphids on susceptible and resistant germplasm.

In this study aphids were first detected on 23rd June 2016 and after the aphid population was heavy (> 1000 SCA leaf^{-1} plant^{-1}) on 8th July, SCA weights were taken. A heavy population of aphids was necessary to measure aphid weights more accurately. Heavy SCA pressure was also essential to effectively characterization resistance. When aphid pressure is heavy and no alternate host-plant is available this is considered no-choice feeding. The reasoning being that when aphids have no alternate host they are

forced to survive on the existing host-plant. At sampling time, ten plants were selected at random in a plot and five fully expanded leaves with a heavy infestation of aphids (> 1000 SCA $leaf^{-1}$ $plant^{-1}$) were removed for analysis per plant giving a total of 100 samples per genotype or 50 samples per replication.

To count the total number of aphids per leaf, a high quality picture of each leaf was taken immediately after sampling using a digital camera (Panasonic Lumix G DMC-GF2K) in macro mode. Aphids were counted from the captured images using a desktop computer (Asus model X555LA). The windows image viewer program was used with the help of a digital tally hand counter (Control company counter, model # 3129). Alternatively, the total number of aphids was also estimated using the total area covered by aphids divided by the average area covered by one aphid using Microsoft visual basic 2010™ software (Emesu and Chenamani, 2013).

The two methods of counting aphids were compared using Spearman's correlation. Counting aphids using Microsoft visual basic was correlated to hand counts by $\rho = 0.90$ (Spearman's). But since this was only a preliminary attempt to use software to count aphids, only hand counts of aphids were used in the analysis of aphid weights. Counting aphids using Microsoft visual basic was an initial attempt to automate counting hoping that it will make counting more efficient and less strenuous in future studies.

The total weight of the leaf with aphids was measured using a portable electronic balance (MS-600 Digital Pocket Scale, 600 x 0.1G) that was sensitive enough to measure a minimum weight of 500 aphids. After initial weight, the aphids were immediately swept off the leaf using a soft horsehair hand brush. The cleaned leaf was

weighed again immediately. The difference between weight of leaf with aphids and weight of leaf without aphids was the total weight of aphids on that leaf. Average weight of aphids per leaf was calculated as the total weight of aphids divided by the total number of aphids on the leaf.

The null hypothesis (**H_0**) was that sorghum lines with active defense against SCA would have lower aphid weights compared to plants without. Since aphid colonies consist of varying stages of aphid development (all morphs) whose weight distributions are not known, the univariate normal (Gaussian) distribution curve in probability theory (Durrett, 2010 and Klenke, 2013) was used to determine the variances around the mean aphid weights. This is possible because of the central limit theory, which states that averages of random variables independently drawn from independent distributions have a normal distribution given by the probability density function as: $f(x|\mu,\sigma^2) = (1/\sqrt{2\sigma^2\pi})((e^{-(\bar{x}-\pi)(\bar{x}-\pi)})/2\sigma^2))$. If active defense exists, a difference or shift in the weight distribution of aphids between the resistant and susceptible lines was expected. To test this difference, a paired unequal variance Student's test statistic (Student's *t*-test) was used (Yuen, 1974; Cressie and Whitford, 1986; Ruxton, 2006).

Antibiosis (method II) average number of aphids per leaf and field rate of increase (reproductive capacity)

The reproductive capacity of SCA was determined on resistant and susceptible grain hybrids (SCAG) and forage hybrids (SCAF). Both SCAG and SCAF consisted of 12 entries (Table 21) replicated eight times. The two trials were planted side by side, each in a randomized complete block design. In the SCAG trial, eight resistant and four

susceptible hybrids were used. In the SCAF trial five resistant and seven susceptible hybrids were used (Table 21). The trial was planted on 26th July 2016, next to (10 meters apart) a forage hybrid crop (SCA refuge crop, 250 by 50 meters), that was used as a source of SCA to inundate the SCAG and SCAF trials with aphids naturally.

Table 21 List of grain sorghum hybrids (SCAG) and forage sorghum hybrids (SCAF) used to evaluate antixenosis and antibiosis using method II and III in fall, College Station, Texas, 2016.

No.	SCAG (Pedigree)	Genotype	SCAF (Pedigree)	Genotype
1.	A.Tx3408/R.Tx2783	Resistant	A.Tx3408/R.Tx2785	Resistant
2.	A.Tx3408/R.Tx436	Resistant	A.Tx3408/R.Tx2785	Resistant
3.	A.Tx3408/R.Tx437	Resistant	A.Tx3408/R.Tx2909	Resistant
4.	A.Tx3409/R.Tx2783	Resistant	A.Tx3408/R.Tx2909	Resistant
5.	A.Tx3409/R.Tx436	Resistant	A.Tx3408/R.Tx2910	Resistant
6.	A.Tx3409/R.Tx437	Resistant	A.Tx631/R.Tx2785	Susceptible
7.	A.Tx631/R.Tx2783	Resistant	A.Tx631/R.Tx2909	Susceptible
8.	A.Tx645/R.Tx2783	Resistant	A.Tx631/R.Tx2910	Susceptible
9.	A.Tx631/R.Tx436	Susceptible	A.Tx645/R.10781	Susceptible
10.	A.Tx631/R.Tx437	Susceptible	A.Tx645/R.Tx2785	Susceptible
11.	A.Tx645/R.Tx436	Susceptible	A.Tx645/R.Tx2909	Susceptible
12.	A.Tx645/R.Tx437	Susceptible	A.Tx645/R.Tx2910	Susceptible

Antibiosis method II (average number of aphids per leaf and field rate of increase (reproductive capacity). Antibiosis method III (nymphoal mortality rate).

In both the SCAG and SCAF, six plants were randomly selected from every plot, and the topmost leaf of each plant was used to count aphids. The initial number of aphids per leaf was counted in the field by hand on 1st September 2016, and the total number of aphids after ten days was recorded on 10th September 2016. On the 10th day the topmost leaf was photographed and aphids counted using the same method as described in

Method I using Microsoft Windows Photo program. The reproductive capacity (fecundity) of aphids was reported as total number of aphids per leaf and field rate of increase (fm) after 10 days.

The field rate of crease for r-strategists or r-selected species (Dixon, 1998) was calculated using the formula**: (dN/dt)*(1/N) = fm**, where **d** = delta or change, **N** = population size and **fm** = field rate of increase. The difference between the initial aphid population (N_1) and population after ten days (N_2) is **dN**. **N** is the population size of aphid colonies on the leaf after 10 days. The difference in time between the first and the second aphid count is **dt**. This formula is essentially the same as the one used to calculate intrinsic rate of increase under controlled conditions (Lewontin, 1965) but because hypothetical rates of aphid increase under controlled conditions are usually different from field growth rates (Ragsdale et al., 2007), the term field rate of increase was used.

The null hypothesis $\mathbf{H_0}$ was that there should be no differences in the reproductive capacity of SCA on resistance and susceptible sorghum germplasm if antibiosis did not exist. Consequently, sorghums plants expressing antibiosis would have a lower number of aphids than plants without antibiosis. Analysis of variance was done using PROC GLM in SAS v 9.3 (SAS Institute, 2011), and means were compared with test trial LSD values ($P < 0.05$).

Antibiosis (method III) nymphal mortality rate

To determine the mortality rate of newly born nymphs, which were approximately one-tenth (0.1) of the average size of adults, three hybrids were used

(A.Tx3408/R.Tx2783, A.Tx3409/R.Tx2783 and A.Tx631/R.Tx436). A.Tx631/R.Tx436 was described as a susceptible hybrid and the other two as resistant. The three were planted in a randomized complete block design on 26th July 2016. Aphid infestation were first observed on this experiment at the vegetative stage of plant development (stage 2) on 17th August 2016. Mass migrations of alates were observed from the refuge forage crop onto these hybrids on 19th August 2016. After migrations had ceased from the refuge crop to these lines, evaluations commenced on 20th August 2016.

One newly alighted alate (settling and beginning to reproduce) was trapped to a leaf using a clip cage (BioQuip clip cage # 1458). The clip cages were white, circular, diameter 3.7cm and thickness of 1.0 cm. Two experiments were set, in the first experiment the alate and offspring were caged for five days. In the second experiment, the alate and offspring were caged for 10 days. Once the caged alate had given birth to nymphs, which were about one-tenth (0.1) the average size of adults, the total number of births (live aphids) and the total number of deaths (dead aphids) was calculated in each cage. The nymphal mortality rate of newly born SCA was recorded from 21st to 25th August 2016. The other recording was from 28th August to 6th September 2016.

The advantages of using only one winged aphid per cage were: (i) they were abundant in fall, (ii) all nymphs in each clip cage arose from one alate, (iii) it was easy to keep track of the nymphs and determine their age, (iv) cages kept out natural enemies to ensure that mortality was due to host-plant resistance and (v) they helped to shelter aphids from weather elements.

Because clip cages could only accommodate a finite population, rather than use intrinsic rate of increase (rm) to assess the reproductive capacity (fecundity) of SCA the mortality rate was used instead. The null hypothesis $\mathbf{H_0}$ was that hybrids with active resistance would cause a higher mortality on newly born nymphs than hybrids without resistance. The mortality rate was calculated by the formula: $\mathbf{M_r = D/N}$, where $\mathbf{M_r}$ = mortality rate, **D** = deaths and **N** = population size. The means were analyzed for variance using PROC GLM in SAS v 9.3 (SAS Institute, 2011), and means were compared with test trial LSD values ($P < 0.05$).

Antixenosis (method IV) average number of alates per leaf

In this experiment winged aphids had a natural choice to colonize their preferred host-plant. Their mobility allowed them to choose which plants were more suitable based on their perception of cues from the host-plants. After alate migration from the refuge crop had stopped, six plants were randomly selected and alates were counted on the top, fully expanded leaf of each plant. The top leaf was used because the colonization of the host-plant by the alates was from top to bottom, and only the top three leaves were colonized to a great extent throughout the field. Rather than physically count aphids individually in the field a high resolution picture of the leaf with alates was taken using a digital camera (Panasonic Lumix G DMC-GF2K) in macro mode. Alates were counted using Microsoft Windows Photo program on a desktop computer (ASUS) with the help of a hand tally counter. The total plant count per plot, multiplied by three leaves, multiplied by the average number of alates per leaf was the estimate of the aphid population per plot (cumulative total per plot).

The hypothesis stated that if morphology or chemical cues of germplasm altered the behavior of winged aphids, the alate population density would be lower on resistant plants than susceptible plants. A lower alate population would reflect the non-preference for that genotype by SCA. Analysis of variance was used to calculate differences using the statistical software PROC GLM in SAS v 9.3 (SAS Institute, 2011), and means were compared with test trial LSD values ($P < 0.05$).

Phenotype (method V) correlation and principal component analysis

Phenotypic traits (days to 50% anthesis, biomass yield ha^{-1}, dry matter yield ha^{-1}, plant height and number of leaves per plant) were collected on forage sorghum hybrid trials in Lubbock and College Station in summer and Weslaco in fall. Plant composition components (present lignin, protein, cellulose and sucrose) were estimated using Near-Infrared Spectroscopy. Using Spearman's correlation analysis and Principal Component Analysis (PCA), the relationship between these traits and aphid damage was established.

Statistical analysis (correlation)

Correlation analysis is a statistical measure of a linear relationship between two variables. Given by the formula $\boldsymbol{\rho = 1 - (6\ \Sigma d^2_i)/n(n^2-1)}$, where **n** is the number of variables and $\mathbf{d^2_i}$ is the squared difference of the ranked differences. Correlation can be either positive or negative. Spearman's correlation, **ρ**, was used to measure correlation and is equivalent to Pearson correlation of ranked values of variables. Correlations between different measured traits within each test were completed using PROC GLM correlation in SAS version 9.2 (SAS Institute, 2011).

Statistical analysis (PCA)

PCA was used to cluster variables (lignin, protein, cellulose, days to 50% anthesis, sucrose, biomass yield ha^{-1}, dry matter yield ha^{-1}, height, No. Leaves) according to environment, genotype and aphid damage. In large data sets PCA made interpretation of results easier because data was reduced to a smaller number of variables that account for most of the variation. The formula for PCA using covariance method is given by the general formula $\boldsymbol{cov(X,Y) = \Sigma^{n}_{i=1} (X_i - \bar{X})(Y_i - \bar{y})/(n-1)}$ (Smith, 2002) and the generalized linear model for covariate analysis is given by: $\boldsymbol{Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_t X_t + e}$. The null hypothesis is given by $\mathbf{H_0: \beta_2 = \beta_3 = \ldots = \beta_t = 0}$ (Ott and Longnecker, 2015). The analysis was implemented using the statistical software PROC GLM in SAS v 9.3 (SAS Institute, 2011).

Results

Antibiosis (method I) average weight per aphid and number of aphids per leaf

In this study, the aphid pressure was heavy (> 1000 SCA $leaf^{-1}$ $plant^{-1}$) and distinct variation for aphid weight in milligrams (mg) was detectable (Figure 9). The lines designated as resistant, colored dark gray, (B.Tx3408, B.Tx3409, R.Tx2783 and R.TAM428), had significantly lower aphid weights 0.060mg, 0.090mg, 0.080 and 0.048mg respectively. Aphid weights on susceptible germplasm colored light gray and red (R.Tx436, R.Tx7000, Pioneer 84P80 and B.Tx631) were significantly higher 0.252mg, 0.129mg, 0.181mg and 0.488mg respectively. Susceptible genotypes consisted of three lines and one hybrid. Two susceptible lines (R.Tx436 and R.Tx7000) and one hybrid Pioneer 84P80 are jointly colored light gray and B.Tx631 is colored red.

The Gaussian distribution revealed a shift in the distribution of mean weights (0.388mg on susceptible and 0.070mg on resistant germplasm). Mean weight of SCA on resistant lines centered at 0.070mg whereas the mean of susceptible germplasm centered at 0.338 milligrams (Figure 9). This shift in weight distribution was significant using the students-*t* test (*t*-test). The test statistic was -213.03, $p > |t| < 0.0001$, $p < t < 0.0001$, degrees of freedom = 399, Standard deviation 0.000022 with an actual estimate of the mean on resistant hybrids of 0.00007mg.

The bimodal distribution in the susceptible germplasm may have been influenced by differences in the germplasm or may have been influenced by the hybrid and could be broken down into two means: (i) 0.188 milligrams, for the combined means of R.Tx436, R.Tx7000 and Pioneer 84P80 and (ii) 0.488 milligrams for B.Tx631. Why there was such a significant difference between B.Tx631 and the other three susceptible germplasm is not definitively known. The means of individual resistant genotypes did not differ. These shifts in the mean weight distribution of SCA on resistant lines suggests an antibiosis effect which was impending the growth of the insect.

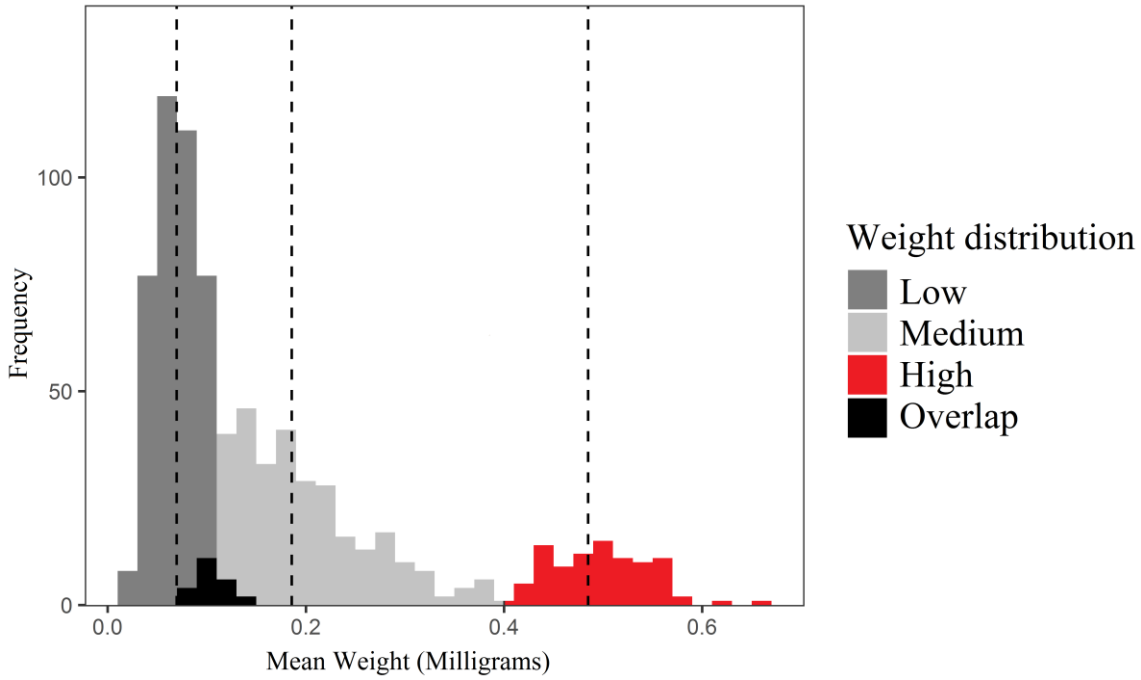

Figure 9 Gaussian distribution of aphid weight in milligrams (antibiosis (method I)) on four resistant and four susceptible sorghum germplasm in summer, College Station, Texas, 2016. Weight distribution; Low (B.Tx3408, B.Tx3409, R.Tx2783, R.TAM428), Medium (Pioneer84P80 (hybrid), R.Tx7000, R.Tx436), and High (B.Tx631). Means weights for high, medium and low were 0.488mg, 0.188mg and 0.07mg respectively. *t-test*; p > |t| < 0.0001, p < t < 0.0001, DF = 399, Std. Dev. 0.000022.

Analysis of variance was performed on the eight sorghum germplasm consisting of four resistant lines, three susceptible lines and one susceptible hybrid (Table 22). Differences in weight distribution was significant $p < 0.0001$* and R.Square of 0.95. The lowest mean aphid weight was on the resistant lines and highest mean aphid weight was on the susceptible lines (Table 22).

Table 22 Analysis of variance for antibiosis (method I) using average weight per aphid on resistant and susceptible germplasm. Mean separation of aphid weights on eight sorghum germplasm evaluated in summer, College Station, Texas, 2016.

Pedigree	Genotype	Mean Separation	Standard Error
R.TAM428	Resistant	0.048f	0.00000309
B.Tx3408	Resistant	0.060f	0.00000309
R.Tx2783	Resistant	0.080e	0.00000309
B.Tx3409	Resistant	0.090e	0.00000309
R.Tx7000	Susceptible	0.129d	0.00000309
Pioneer 84P80	Susceptible	0.181c	0.00000309
R.Tx436	Susceptible	0.252b	0.00000309
B.Tx631	Susceptible	0.488a	0.00000309

Randomized completely block design. Eight entries by two replications. Column means followed by the same lowercase letters are not significantly different. DF = 7, R.Square = 0.95, Observations = 800, F. Ratio = 2263.4, P. > F = < 0.0001*.

Antibiosis (method II) average number of aphids per leaf and field rate of increase (reproductive capacity)

Among the hybrids evaluated for the number of aphids and field rate of increase, resistant hybrids accumulated fewer aphids (160-215) per leaf than susceptible hybrids. Susceptible hybrids had more aphids (351-427) per leaf (Table 23). Given the reduced number, it can be inferred that either reproduction rates are reduced or survivability of the SCA is more limited on the resistant genotypes as estimated by number of aphids per leaf and field rate of increase (fm) (Table 23).

Table 23 Mean separation for antibiosis (method II), using average number of aphids per leaf and field rate of increase. Grain sorghum hybrids in fall, College Station, Texas, 2016. Average no. of aphids per leaf, field rate of increase (fm), no. of aphids per plant, plant count and cumulative no. of aphids per plot on resistant (R) and susceptible (S) hybrids.

Genotype	Aphids Leaf^{-1}	fm Leaf^{-1}	Aphids Plant^{-1}	Plant Count	Aphids Plot^{-1}
A.Tx631/R.Tx2783 (R)	160d	0.097d	480d	15a	9240d
A.Tx3408/R.Tx437 (R)	168d	0.097d	504d	20a	11602d
A.Tx3409/R.Tx437 (R)	195d	0.097d	585d	27a	16929d
A.Tx3409/R.Tx2783 (R)	196c-d	0.097c-d	588c-d	25a	14700c-d
A.Tx3408/R.Tx2783 (R)	200c-d	0.098c-d	600c-d	27a	12891c-d
A.Tx3408/R.Tx436 (R)	209c-d	0.098c-d	627c-d	21a	10668c-d
A.Tx3409/R.Tx436 (R)	210b-d	0.098b-d	630b-d	25a	14800b-d
A.Tx645/R.Tx2783 (R)	215c-d	0.098c-d	645c-d	24a	26772c-d
Mean (R)	**194**	**0.098**	**582**	**23**	**14700**
A.Tx631/R.Tx437 (S)	351a-c	0.099a-c	1053a-c	19a	19656a-c
A.Tx645/R.Tx436 (S)	368a-b	0.099a-b	1104a-b	22a	25146a-b
A.Tx645/R.Tx437 (S)	381a	0.099a	1143a	25a	32025a
A.Tx631/R.Tx436 (S)	427a	0.099a	1281a	29a	18383a
Mean (S)	**382**	**0.099**	**1145**	**24**	**23803**

Randomized complete block design. Twelve entries by eight replications. Column means followed by the same lowercase letters are not significantly different fm = field rate of increase. P > 0.0001*; DF = 11, F.Ratio, LSD, Mean response. The asterisk (*) indicates significance.

In the forage hybrid study, resistant hybrids accumulated 119-134 aphids per leaf while the susceptible genotypes ranged from 309-356 (Table 24). Interestingly, A.Tx631/R.Tx2785 and A.Tx645/R.Tx2785 although classified as susceptible, had lower numbers of aphids per leaf, implying that they have some form of resistance to SCA. Further studies are needed to confirm this observation. The reproductive capacity or field rate of increase (fm) on resistant forage hybrids was also lower than on susceptible hybrids (Table 24).

Table 24 Mean separation for antibiosis (method II), using average number of aphids per leaf and field rate of increase. Forage sorghum hybrids in fall, College Station, Texas, 2016. Average no. of aphids per leaf, field rate of increase (fm), no. of aphids per plant, plant count and cumulative no. of aphids per plot on resistant (R) and susceptible (S) hybrids.

Genotype	Aphids Leaf^{-1}	fm Leaf^{-1}	Aphids Plant^{-1}	Plant Count	Aphids Plot^{-1}
A.Tx3408/R.Tx2785 (R)	119c	0.096c	357c	32a-b	11424c
A.Tx3408/R.Tx2910 (R)	116b-c	0.096b-c	348b-c	27a-b	9396b-c
A.Tx3408/R.Tx2909 (R)	116c	0.096c	348c	35a	12180c
A.Tx3409/R.Tx2910 (R)	128c	0.096c	384c	30a-b	11520c
A.Tx3409/F10762-3dw (R)	133c	0.096c	399c	23b	9177c
A.Tx3409/R.Tx2785 (R)	134c	0.096c	402c	25a-b	10050c
Mean (R)	**124**	**0.096**	**373**	**29**	**10625**
A.Tx631/R.Tx2785 (S)	148c	0.097c	444c	33a	14652a
A.Tx645/R.Tx2785 (S)	158c	0.097c	474c	35a	16590a
ES5200 (S)	309a-b	0.098a-b	927a-b	33a	30591a-b
A.Tx645/R.Tx2910 (S)	321a	0.098a	963a	35a	33705a
A.Tx631/R.Tx2910 (S)	334a	0.099a	1002a	35a	35070a
A.Tx645/R.Tx2909 (S)	356a	0.099a	1068a	34a	36312a
Mean (S)	**271**	**0.098**	**813**	**34**	**27820**

Randomized complete block design. Twelve entries by eight replications. Column means followed by the same lowercase letters are not significantly different fm = field rate of increase. P > 0.0001*; DF = 11, F.Ratio, LSD, Mean response. The asterisk (*) indicates significance.

Antibiosis (method III) nymphal mortality rate

Analysis of antibiosis by nymphal mortality rate revealed significant differences among genotypes, and least squares means detected differences between resistant and susceptible germplasm for mortality rate of nymphs. Replication effects could not be detected by the model as well as duration and genotype by duration interactions (Table 25).

Table 25 Analysis of variance for antibiosis (method III), using mortality rate of newly born nymphs the size of one-tenth the average size of an adult, in clip cages in a five and ten day duration in two resistant (R) and one susceptible (S) grain sorghum hybrid (SCAG trial) in fall, College Station, Texas, 2016.

Sources of Variance	Combined Duration (5 and 10 days)	5 day Duration	10 day Duration
Genotype	0.0003*	0.0269*	0.0347*
Replication	0.3128	0.2709	0.3647
Duration (5 and 10 days)	0.1143		
Genotype x Duration	0.5047		
LS Mean Contrast: (R) vs (S)	0.0004*	0.0138*	0.0366*

Randomized complete block design. Three entries by four replications. R = resistant hybrids, S = susceptible hybrids. The asterisk (*) indicates significant differences.

In a 24 hour period, an alate produced on average five nymphs. In a five day period, mortality was not observed on susceptible germplasm (Table 26). In fact, trends in the five and ten day test were essentially identical. In the resistant backgrounds, the mortality of nymphs averaged 34% (0.34). In addition, differences among genotypes in each classification were observed as well (Table 26).

Higher mortality rates reduced the exponential growth rates of SCA on resistant hybrids and largely explained differences in aphid numbers and possibly reduced aphid weight reported earlier. SCA host-plant interaction resulted in adverse effects on the SCA newly born nymphs, which were about one-tenth (0.1) the size of an average adult. It appears that the antibiosis effect was direct but it is unknown if this adverse effect is constitutive (inherent) or induced (triggered by SCA herbivory activity).

Table 26 Mean separation for antibiosis (method III), using mortality rate, for newly born nymphs (one-tenth the average size of an adult), in clip cages, in two resistant (R) and one susceptible (S) grain sorghum hybrids (SCAG trial) in fall, College Station, Texas, 2016.

Pedigree	Genotype	Mortality Rate Mean Separation
A.Tx3408/R.Tx2783	Resistant	0.24 a
A.Tx3409/R.Tx2783	Resistant	0.44 a
Mean	**Resistant**	**0.34**
A.Tx631/R.Tx436	Susceptible	0.00 b
Mean	**Susceptible**	**0.00**

Randomized complete block design. Three entries by four replications. Column means followed by the same lowercase letters are not significantly different. P > 0.0003*; DF = 2, LSD, F.Ratio, Mean response. The asterisk (*) indicates significance.

Antixenosis (method IV) number of alates per leaf

In College Station fall, in the grain hybrid (SCAG) trial and forage hybrid (SCAF) trial, resistant grain hybrids had fewer alates (34-64) than susceptible hybrids (82-86) (Table 27). On average susceptible hybrids had nearly twice as many winged aphids as resistant hybrids per leaf. The same was true for the estimated number of winged aphids per plant and also the total accumulated estimate of winged aphids per plot.

Table 27 Mean separation for antixenosis (method IV), using number of alates per leaf on grain sorghum hybrids in fall, College Station, Texas, 2016. Average no. of alates per leaf, no. of alates per plant, plant count and no. of alates per plot on resistant (R) and susceptible (S) hybrids.

Genotype	Alates Leaf^{-1}	Alates Plant^{-1}	Plant Count	Alates Plot^{-1}
A.Tx3408/R.Tx436 (R)	34c	102c	22a-b	2244c
A.Tx3408/R.Tx437 (R)	35c	105c	31a	3255b-c
A.Tx631/R.Tx2783 (R)	35c	105c	23a-b	2415c
A.Tx3408/R.Tx2783 (R)	41c	123c	25a	3075c
A.Tx645/R.Tx2783 (R)	44c	132c	23a-b	3036c
A.Tx3409/R.Tx2783 (R)	48b-c	144b-c	23a-b	3312c
A.Tx3409/R.Tx437 (R)	50b-c	150b-c	26a	3900b-c
A.Tx3409/R.Tx436 (R)	64a-c	192a-c	13b	2496c
Mean (R)	**44**	**132**	**23**	**2967**
A.Tx645/R.Tx437 (S)	82a-b	246a-b	26a	6396a-b
A.Tx631/R.Tx436 (S)	83a	249a	18a-b	4482a-c
A.Tx631/R.Tx437 (S)	85a	255a	19a-b	4845a-c
A.Tx645/R.Tx436 (S)	86a	258a	27a	6966a
Mean (S)	**84**	**252**	**23**	**5672**

Randomized complete block design. Twelve entries by eight replications. Column means followed by the same lowercase letters are not significantly different. P > 0.0001*; DF = 11, LSD, F.Ratio, Mean response. The asterisk (*) indicates significance.

Similar to grain hybrids, forage sorghum resistant hybrids had fewer numbers of alates per leaf (24-49) while the numbers were higher on susceptible hybrids (60-89) (Table 28). Almost twice as many alates were present on susceptible hybrids per leaf than on resistant hybrids per leaf. The estimated number of alates per plant and also the total accumulated estimate of alates per plot were fewer on resistant forage sorghums than of susceptible forage sorghums.

Table 28 Mean separation for antixenosis (method IV), using number of alates per leaf. Forage sorghum hybrids in fall, College Station, Texas, 2016. Average no. of alates per leaf, no. of alates per plant, plant count and no. of alates per plot on resistant (R) and susceptible (S).

Genotype	Alates Leaf^{-1}	Alates Plant^{-1}	Plant Count	Alates Plot^{-1}
A.Tx3408/R.Tx2909 (R)	24e	72e	35a	2520e
A.Tx3408/R.Tx2785 (R)	26e	78e	32a	2496e
A.Tx3409/R.Tx2785 (R)	48a-e	144a-e	23a	3312a-e
A.Tx3409/R.Tx2910 (R)	49b-e	147b-e	32a	4704b-e
Mean (R)	**37**	**110**	**31**	**3258**
A.Tx631/R.Tx2785 (S)	33d-e	99d-e	35a	3465d-e
A.Tx645/R.Tx2785 (S)	38c-e	114c-e	35a	3990c-e
A.Tx645/F10762-3dw (S)	60a-e	180a-e	35a	6300a-e
A.Tx645/R.Tx2910 (S)	64a-d	192a-d	35a	6720a-d
A.Tx631/R.Tx2910 (S)	66a-d	198a-d	35a	6930a-d
A.Tx645/R.Tx2909 (S)	69a-c	207a-c	33a	6831a-c
A.Tx631/R.Tx2909 (S)	86a-b	258a-b	35a	9030a-b
ES5200 (S)	89a	267a	32a	8544a
Mean (S)	**63**	**189**	**34**	**6476**

Randomized complete block design. Twelve entries by eight replications. Column means followed by the same lowercase letters are not significantly different. P > 0.0001*; DF = 11, LSD, Mean response. The asterisk (*) indicates significance.

Phenotype (method V) correlation and principal component analysis

Correlation analysis

In forage sorghum hybrids yield components were positively correlated with aphid damage. SCA damage was measured using Sharma's chlorosis scale of 1-9 (Sharma et al., 2014). Plant height and average number of leaves per plant were negatively correlated to aphid damage (chlorosis) at -0.8 while yield per hectare and dry matter yield per hectare were moderately negatively correlated to aphid damage at -0.6 and -0.5 respectively. Days to 50% anthesis was not correlated to aphid damage. Protein

and lignin were highly positively correlated to aphid damage at 0.8 and 0.7 respectively while cellulose was fairly positively correlated to aphid damage at 0.3 (Table 29).

Table 29 Forage sorghum correlation analysis of nine phenotypic traits (by-variable) with aphid damage (variable) in Lubbock and College Station (summer) and Weslaco (fall) in Texas, 2016 using Spearman's (ρ) and Pearson's (r) correlations and the associated probability for (ρ). Aphid damage was measured using Sharma's chlorosis scale of 1-9.

Variable	By Variable	Spearman (ρ)	Prob. > |ρ|	Pearson (r)
Aphid damage	Lignin	0.6842	< 0.0001*	0.7
Aphid damage	Protein	0.7421	< 0.0001*	0.8
Aphid damage	Cellulose	0.3752	< 0.0001*	0.3
Aphid damage	Days to 50% anthesis	-0.1823	0.318	-0.2
Aphid damage	Sucrose	-0.6600	< 0.0001*	-0.7
Aphid damage	Biomass yield ha^{-1}	-0.6227	< 0.0001*	-0.6
Aphid damage	Dry matter yield ha^{-1}	-0.6834	< 0.0001*	-0.6
Aphid damage	Height	-0.7394	< 0.0001*	-0.8
Aphid damage	Number of Leaves	-0.8234	< 0.0001*	-0.8

Asterisk (*) indicates significance for Spearman correlation test.

Principal component analysis

Principal component analysis resulted in seven PCA variance components which accounted for most of the variation. The first two components accounting for 81.3% of the total variation (Figure 10). The PCA components beyond PCA3 were of minimal value and not shown in the figure. In the PCA analysis, the individual traits aligned similarly to the correlations between the individual traits (Table 29). Height, number of leaves and yield (biomass and dry matter ha^{-1}) were tightly associated and had opposite reactions compared to lignin, sucrose and aphid damage (Figure 10).

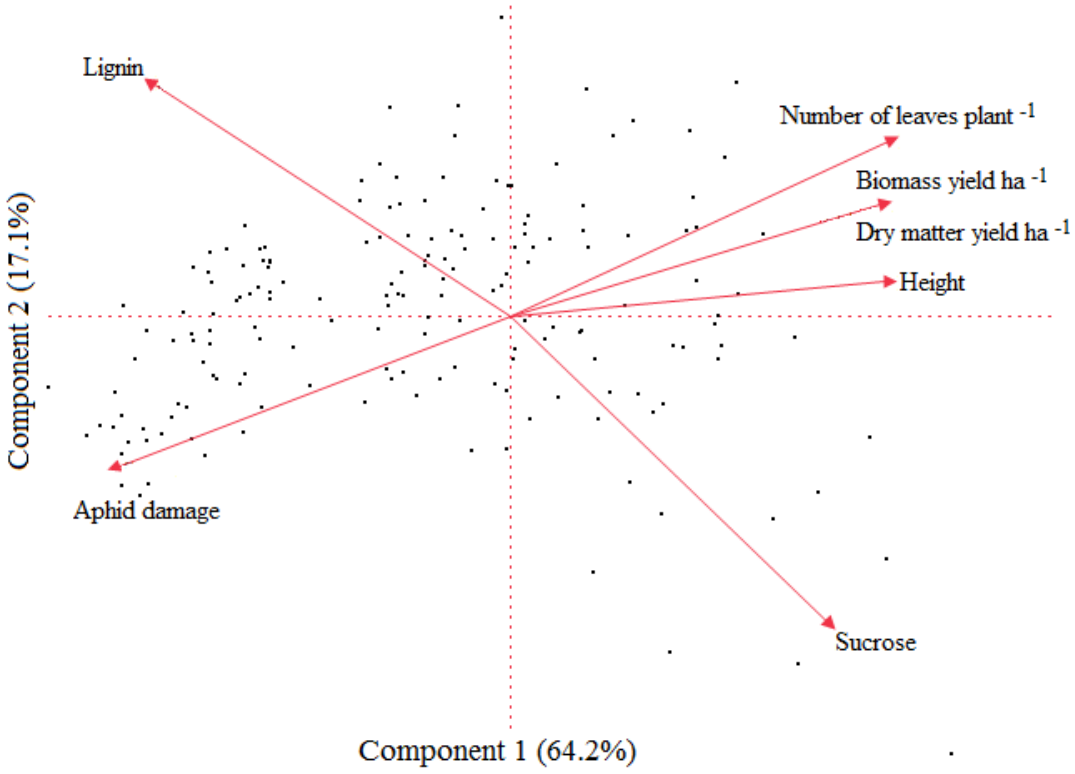

Figure 10 Principal component analysis (PCA) of lignin, sucrose, number of leaves plant^{-1}, biomass yield ha^{-1}, dry matter yield ha^{-1} and height in forage sorghums in relation to aphid damage. Lubbock and College Station (summer) and Weslaco (fall) in Texas, 2016. Aphid damage measured using Sharma's chlorosis scale of 1-9.

Discussion

Antibiosis

Sorghum lines and hybrids manifested antibiosis in the form of reduced aphid weights and number of sugarcane aphid on resistant genotypes compared to susceptible genotypes (Method I and II antibiosis evaluation). In addition, the mortality rate of SCA (Method III antibiosis evaluation) on resistant germplasm was greater than on the susceptible genotypes. The reproductive capacity (fecundity) of the SCA was lower on

resistant germplasm. These results support earlier studies of antibiosis against SCA on sorghum (Manthe, 1992; Armstrong et al., 2016). Additional studies on aphid weight gain done in the current work further support that resistant germplasm is expressing antibiosis for SCA actively.

There appears to be sufficient antibiosis defense in the resistant lines to reduce the fecundity of SCA, this allows resistant plants to continue growing under SCA pressure while susceptible plants succumb to aphid pressure. The study suggest that resistant germplasm have some advantage over susceptible germplasm under SCA infestations. Hybrids made by crossing resistant lines (B.Tx3408, B.Tx3409 and R.Tx2783) with susceptible lines also expressed antibiosis in both grain and forage sorghums. This mode of resistance appears to be repeatable, transferable and dominant.

Studies on greenbug resistance on sorghum germplasm R.Tx2783 also concluded that antibiosis was one of the defense mechanisms towards biotype E and H (Gorena, 2004). Molecular studies of defense against insect herbivores have implicated cyanogenic glucosides in antibiosis reaction (Darbani et al., 2016). Studies on greenbug resistance suggest that salicylic acid and jasmonic acid are involved in defense pathways including dhurrin, a cyanogenic glucoside which is essentially converted into hydrogen cyanide at the wounding site (Zhu-Salzman et al., 2004). Several other genes have been implicated in conditioning resistance to greenbug and are believed to be mostly additive in nature (Katsar, 2002).

The role of anthocyanins (3-deoxyanthocyanidins) in the fight against fungal and bacterial infections is well documented of which the major candidate gene is

Sb06g029550 (Poloni and Schirawski, 2014). Yellow sugarcane aphid (*Sipha flava* (Forbes)) infestations have been associated with an increase in the production of red and purple pigmentation usually associated with anthocyanins (Gonzáles et al., 2002; Poloni and Schirawski, 2014). Since *Sipha flava* transmits pathogens it is not surprising that 3-deoxyanthocyanidins are associated with Yellow Sugarcane Aphid infestation.

However, an increase in red or purple pigmentation (anthocyanin production) does not necessarily mean resistance to SCA. It may be a secondary effect due to opportunistic infections as a result of plant stress. Increase in pigmentation was observed on R.Tx2783 as plants began natural senescence (programed cell death) at the same time as R.Tx7000 succumbed to heavy aphid pressure. Further B.Tx3408, which has high levels of resistance to the SCA, is a tan plant and does not produce any significant quantity of anthocyanins. Plants may mistake aphid activity for pathogen infection (Jaouannet et al., 2014) and this may explain the anthocyanin production.

Different aphid species and biotypes trigger different defense pathways because of their variable herbivory activity (Jaouannet et al., 2014). That could partially explain why a resistant host-plant to one aphid biotype may be susceptible to another. That could also explain why a host-plant may be susceptible to one aphid species and resistant to another, as was the case with R.TAM428 resistant to SCA (Manthe, 1992, Sharma et al., 2014) but susceptible to greenbug. Conversely, R.Tx2783 resistant to greenbug also expressed resistance to SCA. In this situation, this may imply that genes conditioning greenbug and SCA resistance in B.Tx3408, B.Tx3409 and R.Tx2783 are related. However, even when genes are related defense pathways may be different. Aphids are

capable of evading even very effective host-plant defense pathways (Zhu-Salzman et al., 2004). Elucidating the molecular mechanisms of antibiosis resistance will require further molecular analysis.

Antixenosis

Antixenosis was evident due to differences in the population densities of alates on leaves for resistant and susceptible genotypes. Since aphids are able to pick up cues on the suitability of a host-plant (Dixon, 1998), the lower population densities of alate SCA at the onset of colonization on resistant plants implies antixenosis or non-preference. This documented behavior (Blum 1968; Heftmann, 1975; Seigler and Price, 1976; Kogan and Ortman, 1978; Atkin and Hamilton; 1982; Maiti and Gibson, 1983; Campbell and Dreyer, 1985; Smith, 2005), may be due to plant volatiles or plant morphology that alert SCA of the unsuitable nature of the resistant host-plant for their survival.

Many other traits that may be involved in antixenosis resistance (Sharma et al., 2014) could not be measured such as leaf thickness, size and wax. Plant phytochemicals could not be measured either. This was because some equipment were not suitable for field measurements and not all traits could be measured accurately under field conditions that are useful for a plant breeding program. Future studies should include additional tools to measure antixenosis under field conditions on sorghum. It would be worthwhile to also include studies to measure plant phytochemicals.

Phenotype

Positive correlation between SCA damage and any trait implies that these traits are not helpful to improve resistance. Conversely, traits negatively correlated with SCA help the plant to cope with SCA infestations. Therefore, forage sorghums with higher amounts of sucrose, yield per hectare, fresh biomass, taller plants and plants with more leaves were among traits that helped resistant plants cope with SCA better in a production environment. PCA analysis indicated that taller plants, plants with more leaves or plants with a higher yield contributed to resistance plants being less damaged under heavy aphid pressure. This is because height, number of leaves, and yield were highly negatively correlated with aphid damage (chlorosis).

CHAPTER VII

CONCLUSIONS

Variation for SCA resistance was found in breeding lines and led to the release of A/B.Tx3408 and A/B.Tx3409 as sources of resistance to SCA. Additional sources of resistance are being evaluated. R.Tx2783, a resistant check, was originally developed for greenbug resistance (Peterson et al., 1984) and was also resistant to the sugarcane aphid. Greenbug resistance does not necessarily mean SCA resistance since other sources of greenbug resistance were susceptible to SCA (Armstrong et al., 2015). Ultimately, resistance is valuable in mitigating the effect of SCA on sorghum productivity in both grain and forage systems. To quantify this conclusion more research is needed.

B.Tx3408 and B.Tx3409 were derived from a common parent 08PR047. This line was originally selected for greenbug biotype C resistance and E. Additional studies are required to know whether resistance to SCA and greenbug comes from a common source. Some R.Tx2783 derived lines are both resistant to SCA and greenbug biotypes C and E. R.Tx2783 has a complex pedigree, with a significant proportion of its parentage from SC110-9. While R.Tx2783 demonstrates resistance to both pests, SC110-9 (a parent in R.Tx2783) is resistant to SCA but susceptible to all greenbug biotypes. What causes this difference is intriguing and further work will be indispensable.

Resistant germplasm exhibited all three mechanisms of resistance described by Painter (Painter, 1951). Evidence of antibiosis included reduced aphid weight, aphid numbers and aphid fecundity. Antibiosis effect was direct as observed by death of newly

born nymphs. Antixenosis was demonstrated by reduced alate frequency on resistant genotypes compared to susceptible genotypes. Finally tolerance was manifested by resistant lines and hybrids that maintained some level of growth and productivity (Smith, 2005) despite the presence of SCA. It is noteworthy that resistance does not imply immunity; none of these resistance sources are completely immune.

Resistance was consistently expressed as a dominant trait. Hybrids of resistant and susceptible lines were always resistant regardless of which parent was male or female. This supports studies in Southern Africa on resistance to SCA which was inherited as a dominant trait. Segregation rations of an F_2 population, from a cross between a resistant line R.TAM428 and a susceptible line A.Tx3048, showed a Mendelian inheritance pattern. Segregation rations of resistant versus susceptible was 3:1 (Manthe, 1992). R.Tx2783 was among the lines identified as a resistant source to SCA.

The inherent genetic qualities of resistant hybrids enabled the plants to outgrow SCA infestations. Genes involved in the production of anti-nutritive proteins associated with resistance to aphid attacks have been identified in sorghum and are believed to be highly conserved (Wang et al., 2013). Perhaps the high level of conservation might also mean that this resistance is narrow (vertical resistance/qualitative) and hence the dominant effect.

Taller sorghum genotypes suffered relatively less aphid damage. So were plants with higher yields and number of leaves. Thus, plant phenotypic traits also contributed to resistance to a great extent. In conclusion, for practical application of this information

the ultimate goal in plant breeding is to reduce plant damage and increase yield and quality of the crops. In the end, measurements of insect damage to plants are more useful than measurements of insect growth or population development on plants. Often, measurements of yield reduction manifest direct insect feeding injury in plants.

IPM strategies are important to reduce the likelihood of SCA developing resistance. Tritrophic interactions between the resistant host-plant, the insect herbivore and the natural enemies also contribute to host-plant resistance under field conditions (Brewer and Elliott, 2004). Preserving natural habitats for the natural enemies is just as important. Weather has a major influence on SCA prevalence and expression of resistance. Hot-dry weather favored SCA rather than rainy weather. But in the end host-plant resistance should be less dependent on external factors but on the genetics of the plant.

Detailed studies in heritability and QTL mapping are under way. This will help to determine specific genes and pathways conditioning resistance in B.Tx3408, B.Tx3409 and R.Tx2783 or if these genes are similar to other already identified genes. Populations of Recombinant Inbreed Lines (RILs) have already been developed. The next step is to extract deoxyribonucleic acid (DNA) from RILs and collect additional data (genotypic and phenotypic) accurately. QTL mapping combined with bioinformatics will help to establish the role of specific genes and phytochemicals in SCA resistance.

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APPENDIX I

LIST OF 500 LINES SCREENED FOR SUGARCANE APHID

No.	Pedigree	No.	Pedigree
1	((B1*B9501)-V60*B024)	38	(B.05134/B.05165)-CSF1
2	(03BRON171*R.TAM428)	39	(B05134/B05165)-CSF1
3	(03BRON171*R.TAM428)	40	(B.05136/B.Tx2752)-CSF1
4	(08BRON295	41	(B.05167/B.Tx2752)-CSF1
5	(88B943*91BE7414)	42	(B.05173/B.Tx2752)-CSF1
6	(88V1080/	43	(B.05193/B.Tx645)-CSF1
7	(90EON362-4/B.05330)	44	(B.05221/B.Tx623)-CSF1
8	(91BE7414*R.Tx2917)	45	(B.05221/B.Tx623)-CSF1
9	(96CD677*87EO109)	46	(B.1*B.9501)
10	(B.03093-CS4-WF2/B.Tx378)	47	(B.807*(KS22*P9516))-F1
11	(B.03093-CS4-WF2/B.Tx378)	48	(B.807*(KS24*BON34))-F1
12	(B.05001/B.Tx645)	49	(B.807*(KS24*BON34))-F1
13	(B.05001/B.Tx645)-CSF1	50	(B.807*B35)-F1-WFF2
14	(B.05001/B.Tx645)-CSF1	51	(B.9817*B402)-F1
15	(B.05057/B.01021)-CSF1	52	(B.9817*B9108)-F1
16	(B.05058/B.01021)-CSF1	53	(B.9817*B9108)-F1
17	(B.05067/B.01021)-CSF1	54	(B.DLT125*B402)-F1
18	(B.05067/B.01021)-CSF1	55	(B.Tx3042*(BTx625*B35))
19	(B.05070/B.01074)-CSF1	56	(B.TX399*98CA4779)-F1
20	(B.05070/B.01074)-CSF1	57	(B.Tx631/(GB102A*Tx631))
21	(B.05070/B.01074)-CSF1	58	(B.Tx643*B.Tx635)-HF8
22	(B.05070/B.01074)-CSF1	59	(B.Tx643*B.Tx635)-V6
23	(B.05070/B.01074)-CSF1	60	(DL0N357/(GB102A*R.Tx631))
24	(B.05070/B.01074)-CSF1	61	(DL0N357/(GB102A*R.Tx631))
25	(B.05070/B.Tx642)-CSF1	62	(Macia*TAM428)-LL9
26	(B.05070/B.Tx642)-CSF1	63	(R.01171*R.01180)-F1
27	(B.05070/B.Tx642)-CSF1	64	(R.01171*R.01180)-F1
28	(B.05075/B.01074)-CSF1	65	(R.02107/R.Tx436)-CSF1
29	(B.05075/B.01074)-CSF1	66	(R.02107/R.Tx436)-CSF1
30	(B.05075/B.01074)-CSF1	67	(R.02107/Tx436)-CSF1
31	(B.05075/B.01074)-CSF1	68	(R.04164/R.Tx436)-CSF1
32	(B.05077/B.01074)-CSF1	69	(R.04164/R.Tx436)-CSF1
33	(B.05098/B.05071)-CSF1	70	(R.9603*SC1251)-F1
34	(B.05098/B.05071)-CSF1	71	(R.9645*97BRON304)-F1
35	(B.05117/B.05165)-CSF1	72	(R.9645*97BRON304)-F1
36	(B.05117/B.05165)-CSF1	73	(R.9645*97BRON304)-F1
37	(B.05134/B.05165)-CSF1	74	(R.9733*97BRON304)-F1

No.	Pedigree	No.	Pedigree
75	(R.TX436*(87EO366*R.TAM428)	112	07BRON269/
76	(R.Tx436/ICSV745)	113	07BRON273-R
77	(R.Tx437/(96GCPOB124*P851171)	114	07BRON273/
78	(SC35-14E/R04104)	115	07BRON274-R
79	(SV1*Sima	116	07BRON274/
80	(SV1*Sima/IS23250)-LG15	117	07BRON280-R
81	(A.Tx2536/B.05005)	118	07BRON280/
82	(R.Tx2783*SC414-12E)	119	07BRON283/
83	(R.Tx2963	120	07BRON296/
84	01BRON184	121	07BRON298/
85	01BRON186	122	07BRON300/
86	01BRON186-R	123	08BRON277-R
87	02BRON166	124	08BRON277/
88	03BRON172	125	08BRON290/
89	04BRON254	126	10BRON276/
90	04BRON257	127	11BRON237-R
91	04BRON257-R	128	11BRON251-R
92	04BRON262	129	11BRON251/
93	04BRON262-R	130	11BRON255-R
94	04BRON267	131	11BRON287-B
95	04BRON267-R	132	12BRON276/
96	04BRON271	133	12BRON289-R
97	04BRON271-R	134	13BRON268-R
98	04BRON273	135	13BRON272/
99	04BRON273-R	136	13BRON273-R
100	04BRON291/	137	13BRON273/
101	05BRON279/	138	13BRON278-R
102	05BRON287-R	139	13BRON278/
103	05BRON287/	140	13BRON279
104	05BRON289-R	141	13BRON279-R
105	05BRON289/	142	13BRON280
106	06BRON274/	143	13BRON280-R
107	06BRON277-R	144	13BRON281
108	06BRON277/	145	13BRON282
109	06BRON287-R	146	13BRON285-R
110	06BRON287/	147	13BRON287-R
111	06BRON289/	148	13BRON291

No.	Pedigree	No.	Pedigree
149	13BRON291-R	186	A.Tx631/R.Tx2910
150	14BRON270-R	187	A.Tx631/R.Tx436
151	14BRON287-B	188	A.Tx631/R.Tx437
152	14BRON288-B	189	A.Tx642/R.Tx2783
153	14BRON290-B	190	A.Tx642/R.Tx436
154	14BRON291-B	191	A.Tx645/R.10781
155	14BRON292-B	192	A.Tx645/R.12169
156	14BRON293-B	193	A.Tx645/R.Tx2783
157	14BRON294-B	194	A.Tx645/R.Tx2785
158	14BRON295-B	195	A.Tx645/R.Tx2909
159	1790E	196	A.Tx645/R.Tx2910
160	1BRON195/(91BE146*Tx2864)	197	A.Tx645/R.Tx436
161	A3.Tx436/R.Tx437//B.11055	198	A.Tx645/R.Tx436
162	A3.Tx436/R.Tx437//B.11070	199	A.Tx645/R.Tx437
163	A3.Tx436/R.Tx437//B.11071	200	B.DLO357
164	A3.Tx436/R.Tx437//B.11072	201	B.DLO357
165	A/B.11055-WF1-CS1/R.Tx436	202	B.OK11
166	A/B.11055-WF1-CS1/R.Tx437	203	B.Tx2921
167	A.F7301	204	B.Tx2923
168	A.Tx2752/R.Tx2783	205	B.TX3197
169	A.Tx2752/R.Tx436	206	B.Tx3408
170	A.Tx2752/R.Tx437	207	B.Tx3409
171	A.Tx2928/R.Tx2783	208	B.TX378
172	A.Tx2928/R.Tx436	209	B.Tx399
173	A.Tx2928/R.Tx437	210	B.Tx623
174	A.Tx3408/R.Tx2783	211	B.Tx631
175	ATx3408/RTx2909	212	B.Tx635
176	A.Tx3408/R.Tx2910	213	B.TxARG-1
177	A.Tx3408/R.Tx436	214	B.11055
178	A.Tx3408/R.Tx437	215	B.11055-WF1-CS1-WF3
179	A.Tx3409/R.12169	216	B.11055-WF1-CS1-WF3-CS2
180	A.Tx3409/R.Tx436	217	B.11070
181	A.Tx3409/R.Tx437	218	B.11070-CS2-CS1-WF3
182	A.Tx631/R.Tx2783	219	B.11071
183	A.Tx631/R.Tx2785	220	B.11072
184	A.Tx631/R.Tx2909	221	B.11094
185	A/B.11055-WF1-CS1/R.Tx436	222	B.13001

No.	Pedigree	No.	Pedigree
223	B.13002	260	B.13043
224	B.13003	261	B.13044
225	B.13005	262	B.13045
226	B.13005	263	B.13046
227	B.13006	264	B.13047
228	B.13007	265	B.13048
229	B.13008	266	B.13049
230	B.13009	267	B.13050
231	B.13010	268	B.13051
232	B.13011	269	B.13052
233	B.13012	270	B.13053
234	B.13013	271	B.13054
235	B.13014	272	B.13055
236	B.13018	273	B.13055
237	B.13019	274	B.13056
238	B.13020	275	B.13057
239	B.13021	276	B.13058
240	B.13022	277	B.13059
241	B.13023	278	B.13060
242	B.13025	279	B.13061
243	B.13026	280	B.13063
244	B.13027	281	B.13064
245	B.13028	282	B.13065
246	B.13029	283	B.13066
247	B.13030	284	B.13067
248	B.13031	285	B.13068
249	B.13032	286	B.13069
250	B.13033	287	B.13070
251	B.13034	288	B.13071
252	B.13035	289	B.13076
253	B.13036	290	B.13077
254	B.13037	291	B.13078
255	B.13038	292	B.13079
256	B.13039	293	B.13080
257	B.13040	294	B.13081
258	B.13041	295	B.13082
259	B.13042	296	B.13083

No.	Pedigree	No.	Pedigree
297	B.13085	334	B.13137
298	B.13086	335	B.13138
299	B.13087	336	B.13139
300	B.13090	337	B.13140
301	B.13091	338	B.13141
302	B.13092	339	B.13142
303	B.13093	340	B.13146
304	B.13094	341	B.13147
305	B.13095	342	B.13148
306	B.13096	343	B.13149
307	B.13097	344	B.1778
308	B.13098	345	B.4R
309	B.13099	346	B.Tx2752
310	B.13100	347	B.Tx3042
311	B.13101	348	B.Tx378
312	B.13102	349	B.Tx623
313	B.13103	350	B.Tx631
314	B.13104	351	B.Tx642
315	B.13105	352	B.Tx643 (B1)
316	B.13106	353	B.Tx645 (B807)
317	B.13108	354	CE151
318	B.13109	355	DK37-07
319	B.13110	356	Ent62/SADC
320	B.13111	357	EPSON 2-40/E#15/SADC
321	B.13121	358	FGYQ336
322	B.13122	359	ICSV745
323	B.13123	360	ICSV745
324	B.13124	361	ICSV745
325	B.13125	362	IS1144C/SC451/Dur
326	B.13126	363	IS1212158C/SC984/ZZ
327	B.13127	364	IS12609C/SC109/ZZ
328	B.13131	365	IS12610C/SC110/ZZ
329	B.13132	366	IS12612C/SC112/ZZ
330	B.13133	367	IS12661C/SC170/ZZ
331	B.13134	368	IS12664C/SC173/ZZ
332	B.13135	369	IS1266C/SC210/Dur
333	B.13136	370	IS5887C/SC248/Rox

No.	Pedigree	No.	Pedigree
371	JS222	408	R.13247
372	M 627	409	R.13248
373	PGRC/E#222878	410	R.13249
374	PGRC/E#222879	411	R.13250
375	PGRC/E#69414	412	R.13251
376	Pioneer 84P80	413	R.13252
377	R.08270	414	R.13253
378	R.11131	415	R.13261
379	R.11159	416	R.13262
380	R.11239	417	R.13263
381	R.13208	418	R.13264
382	R.13209	419	R.13265
383	R.13210	420	R.13266
384	R.13211	421	R.13267
385	R.13212	422	R.TAM2566
386	R.13213	423	R.TAM428
387	R.13214	424	R.TX2737
388	R.13215	425	R.Tx2783
389	R.13219	426	R.Tx2908
390	R.13220	427	R.Tx2913
391	R.13221	428	R.Tx2935
392	R.13222	429	R.Tx434
393	R.13223	430	R.Tx435
394	R.13224	431	R.Tx436
395	R.13226	432	R.Tx437
396	R.13227	433	R.Tx7000
397	R.13228	434	R.9188
398	R.13229	435	R.Tx430
399	R.13230	436	R.Tx437
400	R.13231	437	Sarvasi (s)
401	R.13240	438	Sarvasi (t)
402	R.13241	439	SC1047-9
403	R.13242	440	SC1080/IS9370C/Caf
404	R.13243	441	SC110
405	R.13244	442	SC110/IS12610C/ZZ
406	R.13245	443	SC113/IS2655C/CauNig
407	R.13246	444	SC1211C/CauKaf/Cacho de

No.	Pedigree	No.	Pedigree
445	SC124/IS12615C/DurDoc	482	R.Tx2954
446	SC1429	483	R.Tx2956
447	SC170	484	R.Tx2973
448	SC170/IS12661C/ZZ	485	R.Tx2974
449	SC170-6-17	486	R.Tx430
450	SC20/IS12540C/DocAmb	487	R.Tx436
451	SC222/IS1098C/Dur	488	R.Tx7000
452	SC243/IS3956C/Rox	489	WM#177
453	SC259/IS2510C/Con	490	WM#322
454	SC266-6	491	WSV187
455	SC28/IS12548C/Dur	492	R.SC 2-251
456	SC301/IS3817C	493	R.SC 2-252
457	SC373/IS7461C	494	R.SC 2-253
458	SC455/IS5479C/Dur	495	R.SC 2-254
459	SC517-9	496	R.SC 2-255
460	SC54/IS2535C/Cau	497	R.SC 7-406
461	SC56/IS12568C/CauNig	498	R.SC 7-407
462	SC582	499	R.SC 7-408
463	SC599/IS17459/CauKaf	500	R.SC 7-409
464	SC610/IS1220C		
465	SC626/IS8004C/Caf		
466	SC659/IS2225C/CafRox		
467	SC702		
468	SC756/IS6920C/CauKaf		
469	SC782/IS6057C/CauNig		
470	SC847/IS1108C		
471	SC963/IS2864C/Cau		
472	SCS2690-2		
473	R.TAM428		
474	TanGbRW		
475	R.Tx2737		
476	R.Tx2783		
477	R.Tx2794		
478	R.Tx2859		
479	R.Tx2860		
480	R.Tx2868		
481	R.Tx2952		

APPENDIX II

SPLIT PLOT DESIGN OF TWELVE SORGHUM HYBRIDS (FOUR RESISTANT AND SIX SUSCEPTIBLE) WITH FOUR REPLICATIONS USED TO EVALUATE THE EFFECT OF SUGARCANE APHID ON THE YIELD AND QUALITY OF FORAGE USING INSECTICIDE AND NO INSECTICIDE TREATMENTS

ATx631/RTx2785	ATx645/RTx2910	ATx3408/RTx2785	ATx645/BTx623/R10781
ATx645/RTx2910	ATx3408/RTx2909	ATx631/RTx2909	ATx3408/RTx2785
ATx645/RTx2909	ATx3408/RTx2785	ATx3408/RTx2909	ATx631/RTx2785
ATx3408/RTx2785	ATx645/RTx2909	ATx3408/RTx2910	ATx645/RTx2785
ATx631/RTx2910	ATx631/RTx2785	ATx645/BTx623/R10781	ATx631/RTx2909
ATx645/BTx623/R10781	ATx631/RTx2909	ATx631/RTx2910	ATx3408/RTx2910
ATx3408/RTx2910	ATx631/RTx2910	ATx645/RTx2909	ATx3408/RTx2909
ATx645/RTx2785	ATx3408/RTx2910	ATx645/RTx2910	ATx645/RTx2910
ATx3408/RTx2909	ATx645/BTx623/R10781	ATx645/RTx2785	ATx631/RTx2910
ATx631/RTx2909	ATx645/RTx2785	ATx631/RTx2785	ATx645/RTx2909

BLOCK I

Insecticide (spray)

ATx3408/RTx2785	ATx645/RTx2910	ATx645/RTx2910	ATx3408/RTx2785
ATx3408/RTx2909	ATx3408/RTx2910	ATx631/RTx2785	ATx631/RTx2909
ATx3408/RTx2910	ATx645/RTx2785	ATx631/RTx2910	ATx3408/RTx2909
ATx631/RTx2785	ATx631/RTx2909	ATx3408/RTx2910	ATx645/RTx2909
ATx631/RTx2909	ATx631/RTx2785	ATx645/RTx2909	ATx645/RTx2910
ATx631/RTx2910	ATx645/RTx2909	ATx3408/RTx2785	ATx631/RTx2910
ATx645/RTx2785	ATx645/BTx623/R10781	ATx631/RTx2909	ATx645/BTx623/R10781
ATx645/RTx2909	ATx3408/RTx2909	ATx645/BTx623/R10781	ATx631/RTx2785
ATx645/RTx2910	ATx631/RTx2910	ATx645/RTx2785	ATx3408/RTx2910
ATx645/BTx623/R10781	ATx3408/RTx2785	ATx3408/RTx2909	ATx645/RTx2785

BLOCK II

No-insecticide (NO-spray)

APPENDIX III

AGRONOMIC TRAITS MEASURED IN COLLEGE STATION 1 COLLEGE STATION 2 LUBBOCK AND WESLACO IN 2016 ON RESISTANT AND SUSCEPTIBLE HYBRIDS ON FORAGE SORGHUM HYBRID TRIALS FOR SUGARCANE APHID RESISTANCE PLANT HEIGHT DAY TO 50% ANTHESIS SEED COLOR PLANT COLOR AND DESIRABILITY

Genotype	Plant Height	Days to 50% Anthesis	Desirability	Seed Color	Plant Color
College Station-1					
A.Tx3409/R.Tx2785 (R)	2.8ab	180	1	P	P
A.Tx3408/R.Tx2785 (R)	2.8a	179	1	W	T
A.Tx3408/R.Tx2909 (R)	2.5bc	>180	1	W	T
A.Tx3408/R.Tx2910 (R)	2.6a-c	>180	1	T	T
A.Tx645/R.Tx2785 (S)	2.7a-b	177	1	P	P
A.Tx631/R.Tx2785 (S)	2.8a	177	1	T	T
A.Tx645/R.Tx2909 (S)	2.7a-b	>180	1	P	P
A.Tx631/R.Tx2909 (S)	2.6a-c	>180	1	T	T
A.Tx631/R.Tx2910 (S)	2.6a-c	>180	1	T	T
A.Tx645/R.Tx2910 (S)	2.6a-c	>180	1	P	P
College Station-2					
A.Tx3409/R.Tx2785 (R)	1.7b	180	1	P	P
A.Tx3408/R.Tx2785 (R)	2.0a	178	1	W	T
A.Tx3408/R.Tx2909 (R)	1.2c	>180	1	W	T
A.Tx3408/R.Tx2910 (R)	1.1c-d	>180	1	T	T
A.Tx645/R.Tx2785 (S)	1.9a-b	177	1	P	P
A.Tx631/R.Tx2785 (S)	1.7b	177	1	T	T
A.Tx645/R.Tx2909 (S)	1.0c-e	>180	1	P	P
A.Tx631/R.Tx2909 (S)	0.9d-e	>180	1	T	T
A.Tx631/R.Tx2910 (S)	0.8e	>180	1	T	T
A.Tx645/R.Tx2910 (S)	0.9e	>180	1	P	P
Lubbock					
A.Tx3409/R.Tx2785 (R)	N/A	N/A	1	P	P
A.Tx3408/R.Tx2785 (R)	1.9a	N/A	1	W	T
A.Tx3408/R.Tx2909 (R)	1.9a	N/A	1	W	T
A.Tx3408/R.Tx2910 (R)	N/A	N/A	1	T	T
A.Tx645/R.Tx2785 (S)	1.9a	N/A	1	P	P

Genotype	Plant Height	Days to 50% Anthesis	Desirability	Seed Color	Plant Color
A.Tx631/R.Tx2785 (S)	2.1a	N/A	1	T	T
A.Tx645/R.Tx2909 (S)	2.0a	N/A	1	P	P
A.Tx631/R.Tx2909 (S)	1.9a	N/A	1	T	T
A.Tx631/R.Tx2910 (S)	2.0a	N/A	1	T	T
A.Tx645/R.Tx2910 (S)	1.9a	N/A	1	P	P
Weslaco					
A.Tx3409/R.Tx2785 (R)	N/A	N/A	1	P	P
A.Tx3408/R.Tx2785 (R)	2.8a-b	N/A	1	W	T
A.Tx3408/R.Tx2909 (R)	3.0a-b	N/A	1	W	T
A.Tx3408/R.Tx2910 (R)	N/A	N/A	1	T	T
A.Tx645/R.Tx2785 (S)	2.9a-b	N/A	1	P	P
A.Tx631/R.Tx2785 (S)	2.8a-b	N/A	1	T	T
A.Tx645/R.Tx2909 (S)	3.1a	N/A	1	P	P
A.Tx631/R.Tx2909 (S)	3.0a-b	N/A	1	T	T
A.Tx631/R.Tx2910 (S)	3.0a-b	N/A	1	T	T
A.Tx645/R.Tx2910 (S)	3.0a-b	N/A	1	P	P

Randomized complete block design. Twelve entries by four replications by two whole plots by three environments. Column means followed by the same lowercase letters are not significantly different. P > 0.0001*, Location P > 0.0001*, Treatment P 0.0707, LSD. There was not enough seed to plant A.Tx3408/R.Tx2910. Days to 50% anthesis not collected in Lubbock and Weslaco. In Weslaco, data collected in fall.

APPENDIX IV

MEAN SEPARATIONS OF EFFECT OF SUGARCANE APHID (SCA) PRESSURE ON YIELD HA^{-1} (TONS HA^{-1}) OF FORAGE SORGHUM HYBRIDS BETWEEN INSECTICIDE AND NO INSECTICIDE TREATMENTS IN FOUR ENVIRONMENTS IN TEXAS 2016

College Station-1 Summer				
Genotype	**Insecticide**	**No-insecticide**	**Difference**	**% Difference**
A.Tx3408/R.Tx2910 (R)	132.4a	133.1a	0.7a	0.5a
A.Tx3408/R.Tx2909 (R)	128.0a	135.6a	7.6a	5.9a
A.Tx3409/R.Tx2785 (R)	62.1b	56.1b	-6.0a	-9.7a
A.Tx3408/R.Tx2785 (R)	54.5b	52.9b	-1.6a	-2.9a
Mean (R)	**94.3**	**94.4**	**0.1**	**0.1**
A.Tx645/R.Tx2909 (S)	134.8a	127.8a	-7.1a	-5.3a
A.Tx631/R.Tx2910 (S)	127.8a	121.2a	-6.5a	-5.1a
A.Tx645/R.Tx2910 (S)	126.4a	128.6a	2.2a	1.7a
A.Tx631/R.Tx2909 (S)	125.6a	128.3a	2.7a	2.1a
A.Tx631/R.Tx2785 (S)	57.8b	63.7b	6.0a	10.4a
A.Tx645/R.Tx2785 (S)	54.2b	55.1b	0.8a	1.5a
Mean (S)	**104.4**	**104.1**	**-0.3**	**-0.3**
College Station-2 Summer				
Genotype	**Insecticide**	**No-insecticide**	**Difference**	**% Difference**
A.Tx3409/R.Tx2910 (R)	20.8a-e	17.9b-e	-2.9b	-13.9b
A.Tx3408/R.Tx2910 (R)	20.8a-e	20.1b-d	-0.7c	-3.4c
A.Tx3408/R.Tx2909 (R)	26.9a-d	21.5b-d	-5.4a	-20.1a
A.Tx3409/R.Tx2785 (R)	28.0a-b	24.9a-b	-3.1	-11.1b
Mean (R)	**24.1**	**22.8**	**-1.3**	**-5.4**
A.Tx645/R.Tx2785 (S)	23.1a-e	21.0b-d	-2.0b	-8.7b
A.Tx631/R.Tx2785 (S)	18.1c-e	14.5b-e	-3.6a	-19.9a
A.Tx645/R.Tx2909 (S)	15.9d-e	10.4d-e	-5.5a	-34.6a
A.Tx645/R.Tx2910 (S)	13.1d-e	7.2e	-5.9a	-45.0a
A.Tx631/R.Tx2909 (S)	12.2d-e	7.5e	-4.7a	-38.5a
A.Tx631/R.Tx2910 (S)	8.6e	5.2e	-3.3a	-38.4a
Mean (S)	**15.1**	**10.6**	**-4.5**	**-29.8**

Split-plot-design. Twelve entries by four replications by two whole plots. R = resistant hybrid, S = susceptible hybrid. Column means followed by the same lowercase letters are not significantly different. SCA pressure in College Station-1 summer was low (< 350 SCA leaf^{-1} plant^{-1}). SCA pressure in College Station-2 summer was high (500-1000 SCA leaf^{-1} plant^{-1}).

Weslaco Fall				
Genotype	**Insecticide**	**No-insecticide**	**Difference**	**% Difference**
A.Tx3408/R.Tx2909 (R)	59.7a	57.4a	-9.5b	-15.9b
A.Tx3408/R.Tx2785 (R)	54.8a	45.3a-b	-2.3c	-4.2c
A.Tx3409/R.Tx2910 (R)				
A.Tx3408/R.Tx2910 (R)				
Mean (R)	**57.3**	**51.3**	**-6.0**	**-10.5**
A.Tx631/R.Tx2910 (S)	65.9a	44.0a-b	-22.0a	-33.4a
A.Tx645/R.Tx2910 (S)	65.3a	50.2a-b	-15.1a-b	-23.1a-b
A.Tx645/R.Tx2785 (S)	62.3a	54.5a-b	-7.9b	-12.7b
A.Tx631/R.Tx2785 (S)	51.5a	40.7a-b	-10.8b	-21.0b
A.Tx631/R.Tx2909 (S)				
A.Tx645/R.Tx2909 (S)				
Mean (S)	**61.3**	**47.3**	**-14.0**	**-22.8**
Lubbock Summer				
Genotype	**Insecticide**	**No-insecticide**	**Difference**	**% Difference**
A.Tx3409/R.Tx2785 (R)				
A.Tx3408/R.Tx2785 (R)				
A.Tx3408/R.Tx2909 (R)	72.8a	42.3a	-30.5a	-41.9a
A.Tx3408/R.Tx2910 (R)	69.6a	49.2a	-20.3a	-29.2a
Mean (R)	**71.2**	**45.8**	**25.4**	**-35.7**
A.Tx645/R.Tx2785 (S)	72.2a	42.7a	-29.5a	
A.Tx631/R.Tx2785 (S)				
A.Tx645/R.Tx2909 (S)	73.8a	49.2a	-24.6a	-33.3a
A.Tx631/R.Tx2909 (S)	85.3a	53.1a	-32.2a	-37.7a
A.Tx631/R.Tx2910 (S)	78.4a	52.8a	-25.6a	-32.7a
A.Tx645/R.Tx2910 (S)	73.5a	61.7a	-11.8a	-16.1a
Mean (S)	**76.6**	**51.9**	**-24.7**	**-32.2**

Split-plot-design. Twelve entries by four replications by two whole plots. R = resistant hybrid, S = susceptible hybrid. Column means followed by the same lowercase letters are not significantly different There was not enough seed to plant A.Tx3409/R.Tx2910, A.Tx3408/R.Tx2910, A.Tx631/R.Tx2909 and A.Tx645/R.Tx2909 in Weslaco. SCA pressure in Weslaco-fall was moderate (350-500 SCA leaf^{-1} plant^{-1}). There was not enough seed to plant A.Tx3409/R.Tx2785, A.Tx3408/R.Tx2785, and A.Tx631/R.Tx2785 in Lubbock. SCA pressure in Lubbock was high (500-1000 SCA leaf^{-1} plant^{-1}).

APPENDIX V

MEAN SEPARATION OF EFFECT OF SUGARCANE APHID (SCA) ON PERCENT PROTEIN QUALITY OF FORAGE SORGHUM HYBRIDS BETWEEN INSECTICIDE AND NO INSECTICIDE TREATMENTS IN FOUR ENVIRONMENTS IN TEXAS SUMMER 2016

College Station-1 Summer				
Genotype	**Insecticide**	**No-insecticide**	**Difference**	**% Difference**
A.Tx3409/R.Tx2785 (R)	4.1a	4.0a	0.0a	0.0a
A.Tx3408/R.Tx2785 (R)	4.0a	3.9a	-0.1a	-2.5a
A.Tx3408/R.Tx2909 (R)	3.6a	3.6a	0.0a	0.0a
A.Tx3408/R.Tx2910 (R)	3.5a	3.4a	-0.1a	-2.9a
Mean (R)	**3.8**	**3.7**	**-0.1**	**-2.6**
A.Tx645/R.Tx2785 (S)	4.4a	4.3a	-0.1a	-2.3a
A.Tx631/R.Tx2785 (S)	4.3a	4.2a	-0.1a	-2.3a
A.Tx645/R.Tx2909 (S)	3.5a	3.5a	0.0a	0.0a
A.Tx631/R.Tx2909 (S)	3.3a	3.3a	0.0a	0.0a
A.Tx631/R.Tx2910 (S)	3.1a	3.0a	-0.1a	-3.2a
A.Tx645/R.Tx2910 (S)	3.0a	3.0a	0.0a	0.0a
Mean (S)	**3.5**	**3.4**	**-0.1**	**-2.9**
College Station-2 Summer				
Genotype	**Insecticide**	**No-insecticide**	**Difference**	**% Difference**
A.Tx3408/R.Tx2910 (R)	6.8a	6.4a-c	-0.4a-b	-5.9a-b
A.Tx3408/R.Tx2909 (R)	6.7a	6.4a-c	-0.3a-b	-4.5a-b
A.Tx3409/R.Tx2785 (R)	6.1a	5.3c	-0.8a	-13.1a
A.Tx3408/R.Tx2785 (R)	5.9a	5.6b-c	-0.3a-b	-5.1a-b
Mean (R)	**6.4**	**5.9**	**-0.5**	**-7.8**
A.Tx631/R.Tx2909 (S)	8.1a	7.8a	-0.3a-b	-3.7a-b
A.Tx645/R.Tx2909 (S)	8.0a	7.3a	-0.7a	-8.8a
A.Tx631/R.Tx2910 (S)	8.0a	6.8a-b	-1.2b	-15.0b
A.Tx645/R.Tx2910 (S)	8.0a	7.7a	-0.3a-b	-3.8a-b
A.Tx645/R.Tx2785 (S)	7.3a	7.0a-b	-0.3a-b	-4.1a-b
A.Tx631/R.Tx2785 (S)	6.7a	6.6a-c	-0.1a-b	-1.5a-b
Mean (S)	**7.7**	**7.2**	**-0.5**	**-6.5**

Split-plot-design. Twelve entries by four replications by two whole plots. R = resistant hybrid, S = susceptible hybrid. Column means followed by the same lowercase letters are not significantly different. SCA pressure was low in College Station-1 summer (< 350 SCA $Leaf^{-1}$ $Plant^{-1}$). SCA pressure in College Station-2 summer (500-1000 SCA $leaf^{-1}$ $plant^{-1}$).

Weslaco Fall				
Genotype	**Insecticide**	**No-insecticide**	**Difference**	**% Difference**
A.Tx3408/R.Tx2785 (R)	3.6b	3.1b	-0.5a	-13.9a
A.Tx3408/R.Tx2909 (R)	3.4b	3.3b	-0.1a	-2.9a
A.Tx3408/R.Tx2910 (R)				
A.Tx3409/R.Tx2785 (R)				
Mean (R)	**3.5**	**3.2**	**-0.3**	**-8.6**
A.Tx645/R.Tx2910 (S)	4.2a-b	3.5a-b	-0.7a	-16.7a
A.Tx631/R.Tx2910 (S)	3.9b	3.2b	-0.7a	-17.9a
A.Tx645/R.Tx2785 (S)	3.6a-b	3.8a-b	0.2a	5.6a
A.Tx631/R.Tx2785 (S)	3.5b	3.3b	-0.2a	-5.7a
A.Tx631/R.Tx2909 (S)				
A.Tx645/R.Tx2909 (S)				
Mean (S)	**3.8**	**3.5**	**-0.3**	**-7.9**
Lubbock Summer				
Genotype	**Insecticide**	**No-insecticide**	**Difference**	**% Difference**
A.Tx3408/R.Tx2909 (R)	4.9a-b	4.6d	-0.3a-c	-6.1a-c
A.Tx3408/R.Tx2785 (R)	4.4b	5.3b-d	1.0c	22.7c
A.Tx3408/R.Tx2785 (R)				
A.Tx3408/R.Tx2910 (R)				
Mean (R)	**4.6**	**5.0**	**0.3**	**6.5**
A.Tx645/R.Tx2909 (S)	6.1a	5.5a-d	-0.6a-b	-9.8a-b
A.Tx631/R.Tx2909 (S)	6.0a	6.6a	0.6a-b	10.0a-b
A.Tx631/R.Tx2910 (S)	5.9a	4.8c-d	-1.1a	-18.6a
A.Tx631/R.Tx2785 (S)	5.3a-b	5.8a-c	0.5a-c	9.4a-c
A.Tx645/R.Tx2785 (S)				
A.Tx645/R.Tx2910 (S)				
Mean (S)	**5.8**	**5.7**	**-0.1**	**-1.7**

Split-plot-design. Twelve entries by four replications by two whole plots. R = resistant hybrid, S = susceptible hybrid. Column means followed by the same lowercase letters are not significantly different. There was not enough seed to plant A.Tx3408/R.Tx2910, A.Tx3409/R.Tx2785, A.Tx631/R.Tx2909 and A.Tx645/R.Tx2909 in Weslaco fall. SCA pressure was moderate in Weslaco fall (350-500 SCA leaf^{-1} plant^{-1}). There was not enough seed to plant A.Tx3408/R.Tx2785, A.Tx3408/R.Tx2910, A.Tx645/R.Tx2785 and A.Tx645/R.Tx2910 in Lubbock summer. SCA pressure was high in Lubbock (500-1000 SCA leaf^{-1} plant^{-1}).

APPENDIX VI

WORK PLAN (I) EVALUATION OF SORGHUM GERMPLASM FOR RESISTANCE TO SUGARCANE APHID AND LIST OF TRAITS MEASURED IN FOUR ENVIRONMENTS WESLACO (SUMMER AND FALL) IN CORPUS CHRISTI AND COLLEGE STATION

Objective: I				**To Evaluate Sorghum Germplasm for Resistance to Sugarcane Aphid.**
Experimental details:				**(i) Planting Date:** Weslaco - 20th February 2015. Corpus Christi - 10th March 2015. College Station - 6th April 2015. Weslaco Fall - 15th August 2015. **(ii) Treatment:** Natural infestation with sugarcane aphid. **(iii) Design:** Randomized complete block. **(iv) No. Entries:** 500. **(v) Plots:** 1 row. **(vi) Replications:** **(vii) Plot length:** 22ft, 20ft, and 20ft (College Station, Corpus Christi and Weslaco respectively).
Measurements:				The primary measurement in screening/evaluating for sugarcane aphid resistance will be the chlorosis rating on a scale of 1 - 9.
Traits	**Weslaco**	**Corpus Christi**	**College Station**	**Description**
DY	**x**	**x**	**x**	**D**a**ys** to 50% anthesis (Julian days using Julian calendar).
HT	**x**	**x**	**x**	Plant **h**eigh**t** (inches using height stick).
EX	**x**	**x**	**x**	Panicle **ex**ertion (inches ruler).
PL	**x**	**x**	**x**	**Pl**ant color (purple = P, red = R and tan = T).
SD	**x**	**x**	**x**	Grain **c**olor (red = R, white = W, yellow = Y and brown = B).
DS	**x**	**x**	**x**	**D**esirability **r**ating (1-9 scale).
LG	**x**	**x**	**x**	**L**od**g**ing rating (fallen and un-harvestable grain/plant) (1 - 9).
SCAC	**x**	**x**	**x**	**S**ugar**c**ane **a**phid **c**hlorosis-Aphid damage approximately 2 weeks pre-flowering (based on 1 - 9 scale).

Objective: II				**To determining Effect of Sugarcane Aphid on Yield and Quality of Forage Hybrids**
Experimental details:				**(i) Planting Date:** Weslaco - 17th February 2016. Corpus Christi - 29th March 2016. College Station; Field 213 - 23rd March 2016. Field 213 - 25th April 2016. **Second planting (fall):** College Station; Field 405 - 30 June 2016 **(ii) Treatment:** Two blocks. One block sprayed (insecticide) for aphids (control) and the other will not in order to determine the effects of aphids on **grain yield and quality**. Each block has 12 entries x 1 rows x 4 replications. **(iii) Design:** Split-plot with two factors (insecticide). **(iv) No. Entries:** SCAF = 12. **(v) Plots:** SCAF = 1 row. **(vi) Replications:** SCAF = 4 reps. **(vii) Plot length:** 22ft, 20ft, and 20ft (College Station, Corpus Christi and Weslaco respectively).
Measurements:				The primary measurement in SCAF are yield components (grain yield). The secondary measurement of importance is quality components of grain and the tertiary measurements will be the standard phenotypic measurements and if opportunity arises additional measurements may be taken.
Traits	**WE**	**CC**	**CS**	**Description**
DY	**x**	**x**	**x**	**D**a**y** to 50% anthesis (Julian days using Julian calendar).
HT	**x**	**x**	**x**	Plant **h**eigh**t** (inches using height stick).
EX	**x**	**x**	**x**	Panicle **ex**ertion (inches ruler).
PL	**x**	**x**	**x**	**Pl**ant color (pigmented = P, red=R and tan=T).
SD	**x**	**x**	**x**	Grain **c**olor (red= R, white = W, yellow = Y and brown = B).
DS	**x**	**x**	**x**	**D**esirability **r**ating (1 - 9 scale).
LG	**x**	**x**	**x**	**L**od**g**ing rating (fallen and un-harvestable grain/plant) (1 - 9).
YD	**x**	**x**	**x**	**Y**iel**d** (grams/plant or grams/plot).
DI	**x**	**x**	**x**	**D**ate of aphid **i**nfestation.

Objective: II				**To Determining Effect of Sugarcane Aphid on Yield and Quality of Forage Hybrids**
Measurements:				The primary measurement in SCAF are yield components (grain yield). The secondary measurement of importance is quality components of grain and the tertiary measurements will be the standard phenotypic measurements and if opportunity arises additional measurements may be taken.
Traits	**WE**	**CC**	**CS**	**Description**
AR			**x**	**A**phid **r**eproduction (count the number of aphids in the clip cage everyday on mature plants at growth stage 2-5 stage just before flowering (number of aphids per clip cage per day) **(SCAF).**
SCAC	**x**	**x**	**x**	**S**ugar**c**ane **a**phid **c**hlorosis-Aphid damage approximately 2 weeks pre-flowering (based on 1 - 9 scale).
AP				Estimate **a**phid **p**opulations (density) weekly on all germplasm.
TR				Presence of **tr**ichomes (Yes = 1, no = 0).

Objective: III	**To Determine the Performance of Grain Sorghum Lines and Hybrids Under Sugarcane Aphid Pressure.**
Experimental details:	**(i) Planting Date:** Weslaco - 17th February 2016. Corpus Christi - 29th March 2016. College Station; Field 213 - 23rd March 2016. Field 213 - 25th April 2016 **(ii) Treatment:** Two blocks. One block will be sprayed (insecticide) for aphids (control) and the other will not in order to determine the effects of aphids on **forage yield and quality**. Each block has 12 entries x 1 row x 4 replications. **(iii) Design:** Split-plot with two factors (insecticide). **(iv) No. Entries:** SCAG (12), SCAP (20), SCAH (15) **(v) Plots:** 1 row. **(vi) Replications:** SCAG (8), SCAP (9), SCAH (3). **(vii) Plot length:** 22ft, 20ft, and 20ft (College Station, Corpus Christi and Weslaco respectively).

Objective: III				**To Determine the Performance of Grain Sorghum Lines and Hybrids Under Sugarcane Aphid Pressure.**
Measurements:				The primary measurement on SCAF are yield components (forage-biomass yield). The secondary measurement of importance is quality components by looking at the nutrient composition. The tertiary measurements will be the standard phenotypic measurements and if opportunity arises additional measurements may be taken.
Traits	**WE**	**CC**	**CS**	**Description**
DY	**x**	**x**	**x**	**D**a**y** to 50% anthesis (Julian days using Julian calendar).
HT	**x**	**x**	**x**	Plant **h**eigh**t** (inches using height stick).
EX	**x**	**x**	**x**	Panicle **ex**ertion (inches ruler).
PL	**x**	**x**	**x**	**Pl**ant color (pigmented = P, red = R and tan = T).
SD	**x**	**x**	**x**	Grain **c**olor (red = R, white = W, yellow = Y and brown = B).
DS	**x**	**x**	**x**	**D**esirability **r**ating (1 - 9 scale).
LG	**x**	**x**	**x**	**L**od**g**ing rating (fallen and un-harvestable grain/plant) (1-9).
YD	**x**	**x**	**x**	**Y**iel**d** (grams/plant or grams/plot).
DI	**x**	**x**	**x**	**D**ate of aphid **i**nfestation.
SCAC	**x**	**x**	**x**	**S**ugar**c**ane **a**phid **c**hlorosis-Aphid damage approximately 2 weeks pre-flowering (based on 1 - 9 scale).
AP				Estimate **a**phid **p**opulations (density) weekly on all germplasm.
TR	**x**	**x**	**x**	Presence of **tr**ichomes (Yes = 1, no = 0).

Objectives: IV				**To Determine Categories of Resistance and Correlation Between Phenotype and Resistance.**
Experimental details:				**(i) Planting Date:** College Station 7th April 2016 and 26th July 2016. **(ii) Treatment:** N/A **(iii) Design:** Randomized complete block. **(iv) No. Entries:** SCAG (12), SCAF (12), Breeding lines (8), SCAG-Subset (3). **(v) Plots:** 1 row plots. **(vi) Replications:** SCAG (8), SCAF (8), Breeding lines (2), SCAG-Subset (4). **(vii) Plot length:** 22ft (College Station).
Measurements:				The primary measurement is the standard phenotypic measurements and if opportunity arises additional measurements may be taken on SCAG, SCAF and on 8 breeding lines of interest.
Traits	**WE**	**CC**	**CS**	**Description**
DY		**x**	**x**	**D**a**y** to 50% anthesis (Julian days using Julian calendar).
HT		**x**	**x**	Plant **h**eigh**t** (inches using height stick).

Measurements:				The primary measurement is the standard phenotypic measurements and if opportunity arises additional measurements may be taken on SCAG, SCAF and on 8 breeding lines of interest.
Traits	**WE**	**CC**	**CS**	**Description**
PL		**x**	**x**	**Pl**ant color (pigmented = P, red = R and tan = T).
SD		**x**	**x**	Grain **c**olor (red = R, white = W, yellow = Y and brown = B).
DS		**x**	**x**	**D**esirability **r**ating (1 - 9 scale).
LG		**x**	**x**	**L**od**g**ing rating (fallen and un-harvestable grain/plant) (1-9).
DI		**x**	**x**	**D**ate of aphid **i**nfestation.
AP		**x**	**x**	Estimate **a**phid **p**opulations (density) weekly on all germplasm.
SCAC		**x**	**x**	**S**ugar**c**ane **a**phid **c**hlorosis-Aphid damage approximately 2 weeks pre-flowering (based on 1 - 9 scale).