SCREENING GRAIN SORGHUM FOR PLANT RESISTANCE TO SUGARCANE APHID (MELANAPHIS SACCHARI) AND TESTING THE EFFECTS OF LED GROW LIGHTS

By

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Abstract: Sorghum, *Sorghum bicolor*, is increasing importance as human food, animal forage, and a source for biofuel production. Since 2013, sugarcane aphid, *Melanaphis sacchari* has become a perennial and significant pest in the southern United States. Natural plant resistance to this pest offers the best option for sustainable low-cost management. Screening for resistance of cultivars is usually conducted in greenhouse trials where plants are grown in controlled environments and infested with aphids from a colony of *M. sacchari*. The recent adoption of LED pink growth lights at The United States Department of Agriculture greenhouses in Stillwater OK has reduced energy costs. However, sorghum plants grown under these certain types of lights appeared to be different with more leaves, shorted height, and chlorosis compared to plants grown under normal light. I conducted experiments using known resistant and known susceptible sorghum lines that were grown under normal and LED lights. Plants grown under pink light had more root and aboveground biomass, but were shorter with fewer leaves, and exhibited less chlorophyll than the same plants grown under conventional lights. When infested with aphids, both growing conditions supported similar number of aphids but plants grown under LEDs had higher damage ratings than those under conventional lights. Based on the results of this experiment, greenhouse trials used to detect sorghum resistance should not use pink LED lights. In the second set of experiments, sorghum lines obtained from a Texas breeder were screened for resistance to sugarcane aphid. Replicated trials were conducted with 34 sorghum lines for compared with two known resistants and two known susceptible lines respectively. Based on these trials, two new lines were identified as having resistance. A second set of trials focused on these lines and quantified aphid survival, reproduction and population growth. The two newly identified pollinator lines, R.11143 and R.11259 exhibited substantial antibiosis and are recommended to be used in breeding programs for developing resistant sorghums threatened by the sugarcane aphid.

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CHAPTER I

INTRODUCTION

Sorghum, *Sorghum bicolor* [L.] Moench, is the world's fifth most-important grain in terms of production and acreage. Sorghum grows in hot and dry agro-ecosystems where most other food grains do not survive. These areas are frequently drought-prone and characterized by generally poor soil quality and thus, in the semi-arid tropics, sorghum is a vital source of food for millions of people (House, 1985). It also provides an important source of raw material for many uses including the preparation of beer and other local drinks, sugar, syrup, fodder, fuel, and even fiber for shelters. Traditional foods made from sorghum include unfermented and fermented breads, porridges, couscous and snacks (Anglani, 1998). In marches during festivals of social patterns of some nations where sorghum stalks are carried as decorations. Burundi, an East-African country whose national flag bears a figure of a bundle of sorghum stalks (Wall and Ross, 1970). Sorghum also provides an important feed for animals and livestock in these areas. With the expansion of human populations, the demand for this dependable crop has increased greatly (House, 1985). Because of its importance to as food for the under-nourished people in developing nations and millions of the poorest people living in the semi-arid

tropics of Africa, Asia and Central America, sorghum is one of the five crops that The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has a mandate to improve (House, 1985).

Sorghum is also important in industrialized countries where mechanized production, the use of nitrogen fertilizer and pesticides/ herbicides produce higher yields that are primarily used for animal feed. Ben Franklin in 1757 provides the first record of sorghum being grown in the United States when he wrote about its use in producing brooms. Today, the United States of America leads world production of sorghum followed by Nigeria, India, Mexico and Argentina. Bioethanol produced from sweet sorghum waste is also being researched in many areas (Zegada-Lizarazu and Monti, 2012).

Origin of Sorghum

Although the exact time and place of sorghum domestication is undetermined, the Mande people may have domesticated sorghum around the headwaters of the Niger River between 8,000 and 5,000 years ago. Archaeological evidence suggests that it was introduced from Egypt to Ethiopia about 3000 B.C. (House, 1985). As cultivation spread, local varieties were created in east central Africa, in or near Ethiopia and Sudan. However, similarities in Asia and northern, western, and southern regions of Africa produced fewer cultivars. The general types of sorghum that occur in northeast Africa are also found in southern Asia, and Europe. Other hypothesized origins of Sorghum in India and Australia have not been supported. In Australia there are at least 10 wild grassy types in the genus *Sorghum*, but none of these served as a progenitor of the cultivated sorghum in that country.

Some extinct relatives or one or more types of wild grass of the genus Sorghum, may have been the progenitors of the cultivated crop. Sudangrass (*Sorghum sudanense*) as well as several wild types or species have been found in India or in east-central Africa. These include Tabucki grass (*S. verticilliflorum*), Kamerun grass (*S. effusus*), Tunis grass (*S. virgatum*), and *S. arundinaceum*, *S. plumosum*, and *S. aethiopicum*, which all have 10 haploid chromosomes. Other potential relatives of source species have 5 haploid chromosomes, including *S. versicolor* and *S. purpureo-sericeum*. The species or types that have 10-pair of chromosomes behave as diploids and have been so regarded; however, they may be considered tetraploids if they originated as a result of chromosome doubling of a 5-pair chromosome species or from intercrosses.

Johnsongrass (*Sorghum halepense*), is an allotetraploid, with 20 haploid chromosomes, that possibly originated from a combination of a diploid sorghum and some other species. The diploid grass sorghums prevail in the eastern hemisphere north of the tropical area where sorghum occurs naturally. Some characteristics of cultivated sorghum varieties may be the result of outcrossing between diploid sorghum and Johnsongrass (Wall and Ross, 1970).

Sorghum Classification

The sorghums are members of the Family Poaceae (Chopra, 1982) and subfamily Panicoideae which is characterized by dorsally compressed spikelets. Sorghum is in the Tribe *Paniceae* to which many of the millets including *Panicum miliaceum* (proso millet) and *Pennisetum glaucum* (pearl millet) belong. Sugarcane, *Saccharum officinarum*, is a close relative of sorghum.

In 1753, Linnaeus described three species of cultivated sorghum in his "Species Plantarium" – *Holcus sorghum, H. saccaratus,* and *H. bicolor*. In 1912, sorghums were divided into four groups by Chiovenda under the species name *sorghum*. Sorghums were subsequently divided into the sections *Eu-sorghum* and *para-sorghum* by Snowden. Snowden described 31 cultivated and 17 related wild species; however, these are now considered races of one species. For the classification of *Sorghum bicolor* var. *bicolor*, a simplified classification was developed in 1972 by de Wet and Harlan based on characteristics of the mature spikelets.

This modern revision classifies the variation in *Sorghum bicolor* into the five basic races: 1) bicolor (B), 2) guinea (G), 3) caudatum (C), 4) kafir (K), and 5) durra (D). Hybrids are designated based on the parent cross as follows: 6) guinea-bicolor (GB), 7) caudatum-bicolor (CB), 8) kafir-bicolor (KB), 9) durra-bicolor (DB), 10) guinea-caudatum (GC), 11) guinea-kafir (GK), 12) guinea-durra (GD), 13) kafir-caudatum (KC), 14) durra-caudatum (DC), and 15) kafir-durra (KD). Six spontaneous races of *S. bicolor* spp. *Arundinaceum* are recognized: 1) arundinaceum, 2) aethiopicum, 3) virgatum, 4) verticilliflorum, 5) propinquum, and 6) shattercane (House, 1985). In the past three decades, sorghum has increased in area planted and total yield. In 1986, sorghum was grown on 47 million ha and ranked fifth in importance among the world's cereals with wheat occupying the largest area (229 million), followed by rice (145 million), maize (131 million), and barley (79 million) (Doggett, 1988).

Sorghum in the United States Yield/Production History

In the United States of America, sorghum is traditionally grown on dryland acres throughout the sorghum belt, which runs from South Dakota to South Texas (USCP, 2016). In 2015, the estimated sorghum grain production in the US was at 597 million bushels which was up 38 percent up from the 2014 total. In 2015, sorghum was estimated to cover 8.46 million acres, which was up from the previous year by 19 percent. The average grain yield is approximately 70.0 bushels per acre (USDA, 2016). Of the 21 states where sorghum is grown commercially, the top five in 2015 were: Kansas, Texas, Arkansas, Oklahoma, and Colorado (NSP, 2016) with 3.4 million acres, 2.6 million acres, 450,000 acres, 440,000 acres, and 440,000 acres respectively (WPDN, 2016).

U.S. sorghum production is typically used for the livestock industry where it is used in feeds for poultry, beef, dairy, and swine. After harvest, the stems and foliage are used for hay and silage. New varieties of sorghum are being developed for use in bioethanol production where it offers similar amounts of sugar as corn while requiring a third less water. A growing export market exists where U.S. sorghum has also been shipped to Mexico, China, Japan and many other countries where it is used as animal feed. In addition to traditional animal feeds and silage, sorghum has been increasing in the consumer market as food where it can be substituted for rice.

As the world human population grows, the challenges of providing high quality nutrition increases. Substantial advances have been made by cereal scientists with new varieties and technology to improve crop yields of rice and wheat with most of the progress being made in increasing favorable growing conditions. Because sorghum is grown on poorer

soils with less reliable water, improving the yields of sorghum is more difficult. However, plant breeding has produced cultivars that can tolerate arid or wet climates and many insect pests (Wall and Ross, 1970). However, in 2013, a new sorghum pest in the United States, the sugarcane aphid, *Melanaphis sacchari* caused substantial yield losses and economic damage.

Sugarcane aphid (*Melanaphis sacchari*)

The sugarcane aphid is reported to feed on twenty species of graminaceous plants with its presence recorded in more than thirty countries (Silva, Rocha, and da Silva, 2014). In the United States, the sugarcane aphid *Melanaphis sacchari* (Zehntner) has a history of infesting sugarcane, *Saccharum officinarum* (L.), in Louisiana and Florida, and was first documented on sorghum in 1922 in Florida (Armstrong et al. 2015). Although the sugarcane aphid is considered a pest of sugarcane, it does not usually cause problems on other crops. However, in 2013 near Beaumont, TX this pest species was discovered in high numbers on sorghum and has been a perennial pest of grain and forage sorghum in the United States ever since (Bowling et at. 2016). The source of this sorghum-feeding biotype is unknown and may be a result of genetic change (Knutson et al. 2016).

In 2013, sugarcane aphid was found on sorghum in Louisiana, Mississippi, southern Oklahoma, north Texas, the Rio Grande Valley as well as in the Texas Gulf Coast, this pest species, sugarcane aphid (*Melanaphis sacchari*), was reported to be found (Bowling et al. 2016). By the spring and summer of 2014, it was found throughout much of Texas and 12 other southern states after surviving the 2013 winter in south Texas (Knutson et al. 2016). In 2015, this pest appeared in the major sorghum producing areas of Oklahoma

and Kansas through Texas, and the range included 17 states encompassing 90 percent of sorghum acreage in the United States (Bowling et al. 2016).

Primary feeding occurs by sucking the plant phloem that causes the death of leaf tissue, thus turning the leaves to turn yellow, purple, and then brown (Knutson et al. 2016). When the plant growth progresses towards flowering stages from the flag leaf stage that is when the pest outbreaks usually occur (Van den Berg, Pretorius, and Van Loggerenberg, 2003). Yield reduction is usually a result of infestations during grain development but significant grain loss can also result from infestations on preboot sorghum (Knutson et al. 2016).

Sugarcane aphid feeding causes uneven emergence of the panicle from the boot and heavy infestations can stunt growth of the plants. In forage sorghums, infestations can cause leaf kill, slow growth and reduced forage yields (Bowling et al. 2016). Light absorption of plants infested by sugarcane aphid is often inhibited by the development of black sooty mold reducing the photosynthetic activity (Knutson et al. 2016). The sugarcane aphid can also vector sugarcane yellow leaf virus (ScYLV) (Silva, Rocha, and da Silva, 2014). In sorghum, secondary yield loss results from the accumulation of honeydew that affects the grain separation process. Sticky leaves and stalks can clog combines and may require time to remove the sorghum plants and wash off the honeydew (Knutson et al. 2016). These two forms of damage results in the sugarcane aphid causing yield losses in sorghum between 24 and 73% (Van den Berg, Pretorius, & Van Loggerenberg, 2003).

Management of Sugarcane Aphid in Sorghum

In the United States, there are currently only two pesticides, Sivanto and Transform WG, which are approved to control sugarcane aphids on sorghum. Because sugarcane aphid is a new pest in sorghum, both of these pesticides have not been fully approved and are under 2 ee or section 18 emergency exemption labels. Sivanto 200SL insecticide has a Section 2(ee) label and Transform WG insecticide has a Section 18 Emergency Exemption label for control of sugarcane aphid in grain and forage sorghum. The emergency exemption label for Transform expired April 8, 2017 (Beuzelin and May, 2015). Although more pesticides will become available, the costs of pesticide application coupled with the relatively low value of the sorghum crop limit the usefulness of this approach for management.

An alternative is to find plants that exhibit phenotypic resistance to sugarcane aphid and develop them for commercial use. In the past, plant breeding has been successful in sorghum that has saved millions of dollars to the sorghum industry, especially when used with other management tactics including planting date (Smith, 2005). The first success of sorghum breeders against an aphid pest was for sorghum greenbug, *Schizaphis graminum* (Rondani) (Reese et al. 1994; Kofoid et al. 2012; Michels and Burd, 2007). Plant resistance against sugarcane aphid has also been reported (Teetes et al. 1995; Sharma et al. 2013; Sharma et al. 2014; Armstrong et al. 2015; Armstrong et al. 2016); however, recent work has not fully elucidated the mechanisms or explored hybrids fully.

Studies conducted on the phenotypic expression of plant-insect interactions, especially sugarcane aphids, will better help in characterizing the sources of resistance to aphid (Fartek, et al. 2012). To reduce damage on sorghum grain quality, identifying sorghum

hybrids resistant or tolerant to sugarcane aphid will not only contribute to reducing input costs from pesticide application but also increase yields (Van den Berg, 2002). When the sugarcane aphid settles on a sorghum plant, it must overcome different physical, chemical, and mechanical plant defenses before the phloem is ingested. These characters are under the control of plant genes that contribute to the general expression of resistance and are grouped into three main categories: antibiosis, non-preference or antixenosis, and tolerance (Painter, 1951; Fartek et al. 2012).

It is necessary to determine the possible levels of resistance or tolerance and the yield potential of different sorghum hybrids because integrated pest management of this pest is directly affected by the yield potential in sorghum (Van den Berg, 2002). Selecting the most durable and effective resistance after screening the pools of genetic resources is an important prerequisite in studying plant-aphid interactions (Fartek et al. 2012).

As a starting point, there are commercially available sorghums and parental sorghum germplasm developed to resist greenbug that have cross-resistance to sugarcane aphid (Armstrong et al. 2015; Armstrong et al. 2016). Further testing and characterization of these lines along with additional cultivars bread for different traits will benefit sorghum growers and play a major role in managing this pest species (Knutson et al. 2016).

Plant Resistance Screening Trials

Identification of sorghum resistance to sugarcane aphid begins with replicated studies in a greenhouse where sorghum cultivars are grown under ideal conditions in replicates which are infested with sugarcane aphids from a colony. Positive known resistant cultivars are grown along with susceptible lines and then compared with the potential new strains. After infestation, plants are scored for health, chlorophyll content, and mass. After identifying potentially resistant lines, additional tests are conducted to identify

mechanisms of antixenosis, antibiosis and tolerance by infesting the plants with aphids and characterizing their survival and reproduction (Armstrong et al. 2015).

Greenhouse conditions are likely to influence the results and thus, results from these trials must be confirmed with field trials. Although a number of factors including, constant temperatures, abundant water, and absence of other insect pests under greenhouse conditions may contribute to false conclusions of resistance, light conditions may also play an important role. However, relatively few studies have examined the impact of new light-emitting diodes (LED) on plant characteristics with regard to resistance to aphids. Greenhouses with the USDA have switched to LEDs because of their durability, long lifetime, cool emitting temperature, and the option to select specific wavelengths for a targeted plant response coupled with high energy conversion efficiency (Massa et al. 2008), resulting in lowered energy costs. A number of studies have found these lights to benefit plants, while others have reported changes in plant morphology in response to LED lighting (Folta and Childers, 2008). Sorghum grown under the pink lights at the Stillwater, OK USDA has unusual growth forms (S. Armstrong pers. obs.) and obvious chlorosis. Thus, LED lighting effects may mask or over-express sorghum resistance to sugarcane aphid and should be tested.

Objectives

1) Conduct trials to compare sorghum cultivars grown under LED grow lights and conventional lights and examine the effects of sugarcane aphid feeding on plants in both conditions.

2) Conduct flat screen trials to identify new cultivars of sorghum that exhibit resistance to sugarcane aphid in greenhouse conditions and quantify aphid survival and reproduction on these lines to determine the mechanisms of resistance.

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Chapter II

LED Grow Lights Significantly Alter Sorghum Characteristics and Plant-Aphid Interactions in Greenhouse Trials

Abstract

For normal growth and production of plant tissues, water, carbon dioxide, nutrients and light are required. Light emitting diodes (LED) are increasingly used as a substitute to normal sunlight that support plant growth in greenhouse horticulture because of their small size, durability, wavelength specificity, long operating life, are high efficiency in offering photosynthetically active radiation at lowered energy costs compared to conventional lights. However, studies conducted to test the effects of these lights have revealed that spectral quality of LED's can have dramatic effects on plant morphology as well as nutrient uptake and pathogen development that could seriously limit some crops solely lighted with these narrow band LED's. The suggested use of these LED's at The United States Department of Agriculture greenhouses is to reduce energy costs. Sorghum plants grown under these lights appeared to be different with more leaves unusual coloration and obvious chlorosis compared to plants grown under normal sunlight. The goals of this research were to compare sorghum plants grown under LED light with

traditional plant grow lights and to characterize the effects of the sugarcane aphid, *Melanaphis sacchari* on tolerant and resistant sorghum cultivars under both conditions. Plants grown under LEDs had significantly greater biomass, but significantly reduced height, less leaves, and less chlorophyll. In trials with aphids, both conditions supported similar numbers of aphids but plants grown under LED had higher damage ratings than those under conventional lights. For future trials, sorghum should not be grown using the LED lights when assessing resistance to aphid infestation.

Introduction

It has long been known that plants require water, carbon dioxide, nutrients and light for normal growth and production of gametes. However, recent advances in light-emitting diode (LED) technology has offered improved efficiency in both photosynthetically active radiation and lowered energy costs. LEDs are characterized by relatively narrow-band spectra and have been increasingly used in growth chambers, in greenhouse horticulture, and are being researched for growing plants in space (Hogewoning et al. 2007; Massa et al. 2008; Trouwborst et al. 2010). Their small size, durability, long operating lifetime, wavelength specificity, relatively cool emitting surfaces, and linear photon output with electrical input current make these solid-state light sources ideal for use in plant lighting designs. LEDs have been used to allow year-round production by providing high intensity light during winter months in northern climate greenhouses and can improve plant growth during overcast days in summer (Heuvelink et al. 2006; Moe et al. 2006).

Because the output waveband of LEDs (single color, nonphosphor-coated) is much narrower than that of traditional sources of electric lighting used for plant growth, one challenge in designing an optimum plant lighting system is to determine wavelengths essential for specific crops. Indeed, a number of studies have been conducted in greenhouses and growth chambers that have tested the effects of varied light sources, wavelengths, and plant species. These studies have revealed that like other light sources, spectral quality of LEDs can have dramatic effects on plant anatomy and morphology as well as nutrient uptake and pathogen development (Massa et al. 2008). Foliar intumescence may develop in the absence of ultraviolet light spectra and this and other less understood stimuli could seriously limit some crops lighted solely by narrow-band LEDs. For example, studies on rice have revealed dramatic effects on rice leaves depending on the spectra used (Matsuda et al. 2004).

The quality of light, including proper spectral distribution can affect many characteristics of the plants. For example, blue light spectra are vital for growth and development of higher plants, because photomorphogenesis is triggered by blue photoreceptors (Briggs and Huala, 1999; Christie and Briggs, 2001). A combination of red and blue LEDs promotes production of many vegetable crops, including pepper (Brown et al. 1995), spinach, radish, and lettuce (Yorio et al. 2001). Wheat has been observed to respond with higher photosynthetic rates and greater stomatal conductance leading to increased biomass production (Goins et al. 1997; Vänninen et al. 2010). Light spectra influence a number of plant characteristics including the ratio of chlorophyll *a*/*b* (Senger and Bauer, 1987), along with a number of other physiological systems including chlorophyll *a*/*b*-binding proteins (Leong and Anderson, 1984) and per unit total protein content (Eskins et

al. 1991). Although physiological, morphological, and genetic traits of plants grown under LED lights have been characterized, very few studies have investigated the influence of the LED lights on plant-insect interactions.

Herbivorous arthropods that feed on plants growing under artificial light are often exposed to abnormally long photoperiods and light spectra that are different from sunlight (Vänninen et al. 2010). The plants are often provided high nutrient and optimal water conditions and the environment is maintained at a near constant warm temperature. Because plants produce secondary plant compounds in response to herbivory and because plants under optimal conditions can often tolerate more injury than plants that are stressed, characterizing insect-plant interactions in artificially lighted greenhouse conditions is important. Especially in cases where greenhouse trials are used prior to largescale field implementation. Recently, the United States Department of Agriculture has suggested the used of LED grow panels in an effort to reduce energy costs. Sorghum plants grown under this orange, blue, and red LED appear different with more leaves, and discoloration compared to plants grown under sunlight.

Different lines of sorghum grown in the USDA greenhouses in Stillwater are evaluated for natural resistance to sugarcane aphid, *Melanaphis sacchari* a new aphid pest in the southern United States. The goals of this research were to compare sorghum plants grown under LED light with traditional plant grow lights and to characterize the effects of aphid infestation on tolerant and resistant sorghum cultivars under both conditions.

Materials and Methods

Aphids

Parthenogenic sugarcane aphid adults (*Melanaphis sacchari*) were collected from Matagorda County, TX, in 2015 and maintained on a known susceptible sorghum variety, RTx7000 as a colony (Armstrong et al. 2016). This clonal colony is transferred to new seedling plants every two weeks in the greenhouse. Plants were maintained on greenhouse benches at temperatures between 21°C and 31°C and grown under two T-8 fluorescent lights that provide supplemental light.

Sorghum Entries

To test the effects of light on plants and plant-aphid interactions, two known resistant lines, TX2783 and DKS37-07, and two known susceptible lines, MORHC858 and WSH117 were used. Two seeds of each genotype were planted in cone-tainers™ (model SC10, S7S greenhouse supply, Tangent, Oregon 97389) in a rich three-layer media of potting soil, fritted clay, and sand (from bottom to top respectively). The cones were fitted with a plastic transparent tube (Round Tube–4 feet MW-1 3/8" PETG-Clear-Stock-Polybag) covered on the top with an organdy cloth. The cones were maintained in the growth chambers at constant temperature with a photoperiod of 14:10 (L:D) hours. When the plants reached the three-leaf stage the most vigorous seedling was kept, and the other was removed.

Light Conditions

Experiments were designed to study the effects of LED grow lights (Sunshine Systems, Grow panel 300, Wheeling IL) on sorghum plant growth and interactions with sugarcane aphid infestation. The LED light source was tested against conventional lighting provided within growth chambers (Percival® Model E30B, Perry IA). Controls were lit with two 8 x 17 cm Phillips fluorescent grow lights along with 2 round 40-watt appliance light bulbs. The temperature of the growth chambers was maintained at 25°C $\pm$ (80-84°F) throughout the experiment for a duration of 21 days to test the effects of lighting on plant growth and 20 days to test the effects on aphid reproduction and damage on plants grown under different light conditions. The total distance of lights from the surface of the soil for standard light and LED grow lights was 67 and 46 centimeters respectively.

The light emission from the pink light grow lamps (model number W2238) were analyzed using a 0.3 m spectrometer using a 150 groove/mm diffraction grating and a thermoelectrically cooled CCD camera. Light was coupled into the spectrometer using a 100 μm diameter optical fiber.

Experiments

Four different cultivars of sorghum were used in flat screen trials where 15 replications of each cultivar were grown under LED grow lights and conventional lights. Plant height and the number of leaves were measured every 48 hours. Chlorophyll content was measured using a SPAD device 502 chlorophyll meter (Minolta, Ramsey, NJ 07466) after 7 and 16 days post emergence. Wet and dry weights were taken at the conclusion of the experiment. The upper portion of the plant from the soil line up was clipped and weighed

as were the root system. Then the tissues were labelled and dries in a drying oven at 50°C for 72 hrs.

A second trial was designed to measure sugarcane aphid reproduction and damage under LED grow light and conventional lighting. The same four cultivars of sorghum were used with 11 replications of each cultivar planted in cone-tainers™ (model SC10, S7S greenhouse supply, Tangent, Oregon 97389). Two separate trays were prepared and placed into separate growth chambers, one with the grow lights and the other for conventional lights. Each plant was infested with leaf cuttings that had 5 adult females transferred to each sorghum entry at the three leaf stage, 8 days post emergence. After 2 weeks of infestation, the plants were evaluated for increase in height and number of leaves. Chlorophyll content was measured with the SPAD 502 chlorophyll meter (Minolta, Ramsey, NJ 07466) followed by counting the number of nymphs and winged adults on each plant. Damage ratings were made using Webster's scale of 1-9, where 1 equals no damage and 9 equals a dead plant.

Analysis

The variables of plant height, number of leaves on a sorghum entry, wet and dry mass and chlorophyll loss were subjected to one-way ANOVA, with sorghum entry means compared ($\alpha = 0.05$) using least squared means pair-wise comparisons procedure (SAS 9.3, SAS Institute 2010). For trials with aphids, damage rating scale along with the number of nymphs were analyzed using ANOVA.

Results

As shown in Fig. 1, there were two primary emissions that were centered near 457 nm (blue) and 636 nm (red). Both emission peaks had similar widths, with full width at half maximum (FWHM) values of roughly 24 nm and 20 nm for the blue and red emissions, respectively. There was an additional peak centered at 544 nm, although it was very weak in intensity compared to the red and blue emission.

Plant morphology

The difference in visual appearance of the plants growing under the two different spectra was striking with sorghum grown under LED lights having stunted height, dead leaves, and differences in color. One cultivar, MORHC858, turned completely pink and the second cultivar DKS37-07 showed 70-80% of pink color with leaf chlorosis. While the other two cultivars TX2783 and WSH117 were not as pink but turned light green to yellow or white in some cases. Plant morphology also differed with sorghum grown under LED lights producing more leaves and leaves that were broader as compared to the same cultivars grown under conventional light.

The differences in plant morphology are shown quantitatively in Table 1. The number of leaves was significantly greater for the pink light plants compared with the plants under white lights. Leaf number also contributed to the significant differences between the two lights. The total plant height under LED light was significantly less than the total length of the plants under white lights.

Overall the trends observed for the height and number of leaves of the different plants grown under different spectra also apply for the dry weights (Table 2). The DW

differences between spectral treatments for the plants under pink and white lights are greater in the differences in length and number of leaves and were also thicker and therefore heavier per length unit. This also explains why there are significant differences in DW of pink light grown plants compared to that of plants grown under white lights.

Aphid reproduction

Although not significant, the number of sugarcane aphid nymphs was less on sorghum grown under LEDs lights as compared to conventional lights in all cases (Table 4a). The known susceptible WSH117 produced the most aphids under conventional light and considerably less under the LED. For trials with aphid infestation, I found plants to have the same number of leaves (Table 4b), but significantly reduced height for sorghum grown under LEDs (Table 4c). The chlorophyll content was 2 to 3x less in sorghum under grow lights as compared to conventional lights (Table 4d) which is likely one of the main reasons for the decline in aphid reproduction (Table 4a). The damage rating was higher for all plants grown under LED lights with significant differences observed in one known resistant and one known susceptible line (Table 4e).

Discussion

In greenhouse trials using both known susceptible and known resistant sorghum lines, substantial differences in plant growth forms were observed when sorghum was grown under pink LED lights compared to sorghum grown under conventional lighting. When plants were infested with aphids, those plants grown under LED lights had lowered chlorophyll and less height and two lines, WSH117 and TX2783 had significantly higher damage ratings than the same plants grown under conventional lights. This surprising

result shows that both a resistant and a tolerant cultivar can be scored as having greater loss to sugarcane aphid when grown under LED lights. Because flat screen greenhouse trials are conducted to identify potential resistance prior to field trials (Armstrong et al. 2017), the influence of pink LED lighting on the results is notable. Differences in sorghum growth are attributed to the variation in different light patterns. This study concludes that pink light affects sorghum growth and response to aphids and hence, the grow lights should not be used with sorghum for trials.

The grow lamp had a label that described the color as “Red: Blue: Orange, 7:1:1”. Based on the evidence presented this is incorrect since only two primary emissions were observed (Fig. 1). The intensity ratio of the red emission to that of the blue emission was 6.9, which is close to the value of 7 on the label. The problem is that the quantum efficiency of the camera can vary quite substantially for different wavelengths of light. According to the manufacturer of the camera, the quantum efficiency near the blue emission is likely close to 50% and the quantum efficiency near the red emission is likely closer to 85%. Therefore, the spectrum should be corrected for the response of the specific system (spectrometer and camera) with the use of a known broadband emission before intensity ratios are considered accurate.

The irradiance spectrum to which plants are exposed during growth has effects different aspects of the plant. In plant research and greenhouse horticulture, growth lamps with different spectral outputs have been used for more than a century; however at a high energy cost and with increases in temperature because of inefficiency. More recently LEDs, which are characterized by relatively narrow-band spectra, have become increasingly used in growth chambers, on an experimental basis in greenhouse

horticulture, and in research aimed at growing plants in space (Hogewoning et al. 2007; Massa et al. 2008; Trouwborst et al. 2010). Hogewoning et al. (2007) previously reported lettuce to be physically very different when grown under pink lights compared to white light. Because pink light affected pant physiology of both sorghum and lettuce which are C4 plants, it is possible that the C4 photosynthetic cycle is more affected by the pink LED lights than C3 plants.

Overall the trends observed for the height and number of leaves of the lettuce grown under LEDs were similar to my results for sorghum. In addition, the dry weights for plants grown with LED were greater than the differences in leaf length and number of leaves because leaves grown under LED lights were thicker and therefore heavier per length unit (Hogewoning et al. 2007).

To date only a few studies have examined the effects of LED lights on herbivore or tritrophic interactions (Vanninen et al. 2010) and more research is warranted as LED technology replaces conventional lighting technology. In addition, the results of previous trials examining sorghum resistance to sugarcane aphid where pink LED lights were used should be confirmed.

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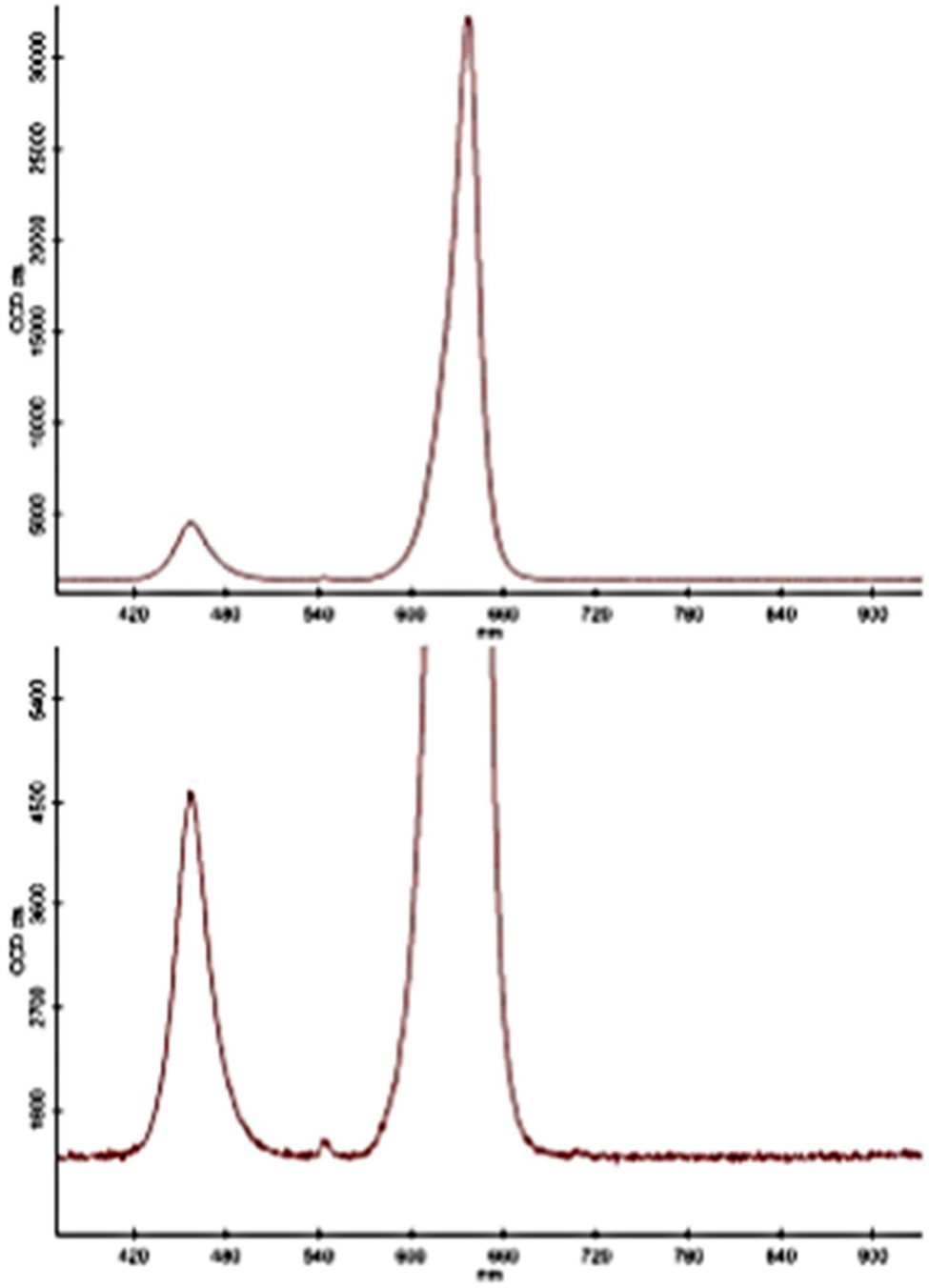

Fig. 1 Emission spectrum of the W2238 grow lamps over the visible spectrum and into the near-IR. The bottom spectrum is zoomed vertically to show details of any weaker emission.

Table 1. Effect of Conventional and LED lights on number of leaves and plant height for four sorghum entries (non-infested)				
Date & Entry	**Leaf Number**		**Plant Height (cm)**	
05/27/2016	Conventional	LED	Conventional	LED
TX2783	1.13 ± 0.19a	0.60 ± 0.13a	1.38 ± 0.33a	0.95 ± 0.22a
DKS3707	1.07 ± 0.18a	0.67 ± 0.13a	1.07 ± 0.18a	0.91 ± 0.18a
MORHC858	1.53 ± 0.22ab	0.93 ± 0.21a	2.87 ± 0.45b	1.41 ± 0.32a
WSH117	1.53 ± 0.17ab	1.13 ± 0.17ab	2.23 ± 0.33ab	1.62 ± 0.23a
05/29/2016				
TX2783	2.27 ± 0.15b	1.27 ± 0.28a	8.23 ± 0.74c	3.47 ± 0.76a
DKS37-07	2.33 ± 0.13b	1.47 ± 0.24a	6.04 ± 0.37ab	3.21 ± 0.53a
MORHC858	2.53 ± 0.24b	1.73 ± 0.30a	10.62 ± 1.20cd	4.68 ± 0.80a
WSH117	2.73 ± 0.21b	2.13 ± 0.26ab	8.98 ± 0.76c	5.03 ± 0.54a
05/31/2016				
TX2783	3.00 ± 0.00b	1.80 ± 0.39a	15.26 ± 0.75c	5.11 ± 1.13a
DKS37-07	3.07 ± 0.07b	2.20 ± 0.35a	12.22 ± 0.60b	4.97 ± 0.81a
MORHC858	3.27 ± 0.15b	2.20 ± 0.35a	18.57 ± 1.44cd	6.36 ± 1.05a
WSH117	3.13 ± 0.13b	2.60 ± 0.27ab	16.56 ± 0.95c	6.67 ± 0.72a
06/02/2016				
TX2783	3.60 ± 0.13b	2.20 ± 0.43a	20.89 ± 1.26b	6.18 ± 1.25a
DKS37-07	3.87 ± 0.09b	2.80 ± 0.46a	17.82 ± 0.75b	6.47 ± 1.12a
MORHC858	3.80 ± 0.11b	2.80 ± 0.46a	23.53 ± 1.30bc	8.09 ± 1.38a
WSH117	3.67 ± 0.13b	3.27 ± 0.32ab	20.63 ± 1.18b	8.65 ± 0.90a
06/04/2016				
TX2783	4.07 ± 0.16ab	3.00 ± 0.49a	25.09 ± 1.38b	7.86 ± 1.33a
DKS37-07	4.33 ± 0.13b	3.33 ± 0.56a	21.90 ± 0.96b	7.51 ± 1.33a
MORHC858	4.47 ± 0.17b	3.33 ± 0.56a	27.73 ± 1.23bc	8.75 ± 1.50a
WSH117	4.13 ± 0.13b	4.27 ± 0.36b	24.45 ± 1.36b	9.93 ± 0.87a
06/06/2016				
TX2783	4.60 ± 0.16ab	3.73 ± 0.52a	27.73 ± 1.57b	9.11 ± 1.30a
DKS37-07	5.00 ± 0.00b	3.93 ± 0.67ab	26.32 ± 1.10b	8.13 ± 1.47a
MORHC858	4.73 ± 0.12ab	4.07 ± 0.65ab	29.75 ± 1.31b	9.54 ± 1.62a
WSH117	4.47 ± 0.22ab	4.60 ± 0.40ab	27.17 ± 1.75b	10.85 ± 0.95a
06/08/2016				
TX2783	4.93 ± 0.21a	5.87 ± 0.91a	30.29 ± 1.87b	10.39 ± 1.38a
DKS37-07	5.53 ± 0.13a	4.67 ± 0.89a	28.36 ± 0.92b	8.25 ± 1.65a
MORHC858	5.00 ± 0.17a	6.07 ± 1.00a	30.77 ± 1.13b	10.47 ± 1.74a
WSH117	4.73 ± 0.27a	6.27 ± 0.65a	28.25 ± 1.82b	11.85 ± 1.05a

Plant Height df = 55, 839, *F*=70.25, P=<.0001; Entry *F*=17.14, P=<.0001; Light Source *F*=1546.53, P=<.0001; Date *F*=291.33, P=<.0001; Entry*Light Source *F*=5.55, P=<.0009; Light Source*Date *F*=81.09, P=<.0001; Entry*Light Source*Date *F*=0.41, P=<0.9993

Leaf Number df = 55, 839, *F*=15.40, P=<.0001; Entry *F*=4.35, P=<.0048; Light Source *F*=31.77, P=<.0001; Date *F*=124.59, P=<.0001; Entry*Light Source *F*=3.65, P=<.0123; Light Source*Date *F*=4.68, P=<.0001; Entry*Light Source*Date *F*=0.44, P=<0.9984

Table 2. Dry matter weights (mg) for two resistant and two non-resistant sorghums grown under conventional and LED Lights (non-infested).			
	Conventional	**LED**	**Conventional vs LED**
Sorghum genotype	Upper Stems and leaves		*P* > *F*
TX2783	140.0 ± 18.8a	246.9 ± 44.3ab	0.0379
DKS37-07	163.3 ± 14.9a	283.6 ± 46.1ab	0.0260
MORHC858	158.0 ± 21.3a	295.0 ± 59.6ab	0.0004
WSH117	113.3 ± 14.1a	240.7 ± 35.9ab	0.0018
All entries	143.7 ± 17.3	266.6 ± 46.5	0.0001
Entry *F*=0.81, *F*=0.494	NLight Source *F*=23.24, *P*>*F*=0.001	Entry by Light Source *F*=0.06, *P*>=0.98	
	Roots		*P* > *F*
TX2783	48.7 ± 7.6a	157.7 ± 33.0b	0.0015
DKS37-07	58.7 ± 5.2a	150.8 ± 28.5b	0.0024
MORHC858	54.0 ± 7.3a	181.4 ± 34.3b	0.0003
WSH117	49.3 ± 7.5a	164.0 ± 32.4b	0.0016
All entries	52.7 ± 6.9	163.5 ± 32.1	0.0001
F=0.21, P>*F*=0.8869	*F*=45.33, *P*<=.0001	*F*=0.19, *P*>F=0.9022	
	Total dry matter		*P* > *F*
TX 2783	188.0 ± 25.8a	404.6 ± 71.8b	0.0099
DKS 37-07	222.0 ± 19.1a	379.2 ± 78.0b	0.0284
MORHC 858	212.0 ± 27.9a	476.6 ± 90.0b	0.0444
WSH 117	162.3 ± 20.8a	388.7 ± 67.8b	0.0014
All entries	196.1 ± 23.4	412.3 ± 76.9	0.0052
F=0.48 P>*F*=0.6983	*F*=28.57P>*F*=0.0001	*F*=0.30P>*F*=0.8283	

Table 3. Difference in Chlorophyll Content (Objective 1 (a) non-infested)			
Entry	LightS	Mean & Std Error	Significance level
TX2783	Conv	25.29 ± 2.28	b
TX2783	Pink	16.91 ± 2.68	a
DKS3707	Conv	28.35 ± 1.4	b
DKS3707	Pink	12.62 ± 2.84	a
MORHC858	Conv	22.41 ± 1.32	ab
MORHC858	Pink	13.00 ± 2.63	a
WSH117	Conv	23.88 ± 1.75	b
WSH117	Pink	14.59 ± 1.87	a

Chlorophyll Content Index df = 7, 119, *F*=7.89, P=<.0001; Entry *F*=0.96, P=<.4157; Light Source *F*=48.73, P=<.0001; Entry*Light Source *F*=1.22, P=<.3059

Table 4 (a): Effects of LED grow light on Number of Nymphs (infested)			
Entry	**Light Source**	**Nymphs**	**P<0.05**
TX2783	Conv	182.0 ± 30.9b	0.394
TX2783	Pink	150.6 ± 18.6b	
DKS37-07	Conv	14.1 ± 2.8a	0.782
DKS37-07	Pink	15.9 ± 5.9a	
MORHC858	Conv	257.0 ± 36.0c	0.774
MORHC858	Pink	244.8 ± 22.7c	
WSH117	Conv	312.0 ± 33.0c	0.079
WSH117	Pink	228.4 ± 31.0bc	

Nymphs df = 7, 86, *F*=19.19, *P*=<.0001; Entry *F*=42.62, *P*=<.0001; Light Source *F*=3.13, *P*=<.0806; Entry*Light Source *F*=1.11, *P*=<.3505

Table 4 (b): Effects of LED grow light on Leaf Number (infested)			
Entry	**Light Source**	**Leaf Number (LeafN)**	**P<0.05**
TX2783	Conv	5.1 ± 0.1b	0.088
TX2783	Pink	4.8 ± 0.1b	
DKS37-07	Conv	4.9 ± 0.1b	1.00
DKS37-07	Pink	4.9 ± 0.1b	
MORHC858	Conv	4.3 ± 0.2a	0.22
MORHC858	Pink	3.9 ± 0.2a	
WSH117	Conv	4.4 ± 0.2a	0.137
WSH117	Pink	3.9 ± 0.2a	

Leaf Number df = 7, 87, *F*=8.50, P=<.0001; Entry *F*= 17.20, *P*=<.0001; Light Source *F*=5.71, *P*=0.0192; Entry*LightS F=0.74, *P*=<0.5309

Table 4 (c): Effects of LED grow light on Plant Height (infested)			
Entry	**Light Source**	**Plant height (Pheight)**	**P<0.05**
TX2783	Conv	43.5 ± 1.6c	0.001
TX2783	Pink	16.0 ± 0.5a	
DKS37-07	Conv	42.5 ± 0.7c	0.001
DKS37-07	Pink	13.8 ± 0.4a	
MORHC858	Conv	31.3 ± 1.3a	0.001
MORHC858	Pink	16.4 ± 0.4a	
WSH117	Conv	36.5 ± 1.6b	0.001
WSH117	Pink	16.5 ± 2.1a	

Plant Height df = 7, 87, *F*=144.33, *P*=<.0001; Entry *F*=11.30, *P*=<.0001; Light Source, *F*=920.77, *P*=<.0001; Entry*Light Source *F*=18.54, *P*=<.0001.

Table 4 (d): Effects of LED grow light on Chlorophyll Content Index (infested)			
Entry	**Light Source**	**Chlorophyll Content Index (CCI)**	**P<0.05**
TX2783	Conv	31.5 ± 0.9b	0.00
TX2783	Pink	14.1 ± 0.9a	
DKS37-07	Conv	29.9 ± 0.7b	0.00
DKS37-07	Pink	13.0 ± 0.5a	
MORHC858	Conv	32.3 ± 0.7b	0.00
MORHC858	Pink	10.9 ± 1.1a	
WSH117	Conv	28.4 ± 1.5b	0.00
WSH117	Pink	11.5 ± 0.6a	

Chlorophyll Content Index df = 7, 86, *F*=115.25, *P*=<.0001; Entry *F*=3.54, *P*=0.0184; Light Source *F*=787.81, *P*=<0.0001; Entry*Light Source *F*=2.77, *P*=<0.0473.

Objective 4 (e): Effects of LED grow light on Damage Rating (infested)			
Entry	**Light Source**	**Damage Rating (DamR)**	**P<0.05**
TX2783	Conv	1.5 ± 0.2a	0.001
TX2783	Pink	2.5 ± 0.2b	
DKS3707	Conv	1.2 ± 0.1a	0.152
DKS3707	Pink	1.0 ± 0.0 a	
MORHC858	Conv	3.5 ± 0.6c	0.107
MORHC858	Pink	4.8 ± 0.5d	
WSH117	Conv	2.5 ± 0.2b	0.00
WSH117	Pink	4.9 ± 0.3d	

Damage Rating df = 7, 87, *F*=24.33, *P*=<.0001; Entry *F*=42.57, *P*=<.0001; Light Source *F*=26.10, *P*=<.0001; Entry*Light Source *F*=5.50, *P*=<.0017.

CHAPTER III

Antibiosis and Tolerance Discovered in USDA-ARS Sorghums Resistant to the Sugarcane Aphid *Melanaphis sacchari* (Hemiptera: Aphididae)

Abstract

The sugarcane aphid, *Melanaphis sacchari*, was discovered infesting grain sorghum near Beaumont, TX close to the Texas and Louisiana state line a few miles inland from the Gulf coast in the late summer of 2013, and has been a perennial pest of sorghum through 2016. Our research was aimed at identifying sorghum germplasm that expresses host-plant resistance to the sugarcane aphid. We originally screened 36 lines including two known resistant sorghums (TX2783 and DKS37-07) and two known susceptible sources (WSH117 and MORHC858). From this screen, two pollinator lines (R.11143 and R.11259) developed by the USDA-ARS in Lubbock, TX showed significant tolerance as indicated by damage ratings and growth characteristics such as plant height, number of leaves and chlorophyll content. The most impressive in the indicator of tolerance was that significantly less chlorophyll loss occurred in R.11143, R.11259, R.TX2783 and DKS37-07, but there was actually a small net gain in chlorophyll content for the R.11143, indicating a highly tolerant sorghum. The same two germplasm (R.11143, R.11259) also expressed significant levels of antibiosis where net reproduction, number of nymphs

produced in a day, intrinsic rate of increase, and the reproductive period in days were all significantly lower than all entries except the resistant controls DKS37-07 and RTx2783. Pollinators R.11143 and R.11259 are recommended to be used in breeding programs for developing resistant sorghums threatened by the sugarcane aphid.

Introduction

The sugarcane aphid *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) was first found infesting sorghum in August of 2013 near Beaumont, TX and within a few days was also discovered in the Rio Grande Valley, Central and Northern Texas, Oklahoma, Louisiana and Mississippi (Villanueva et al. 2014). In 2014, the sugarcane aphid became the most important insect pest of grain and forage sorghums in the United States production regions. Sugarcane aphids exhibit an exponential growth rate of population growth when sorghums and forage sorghums begin the boot stage through grain fill (van Rensburg, 1973). Once the head emerges, large quantities of honeydew is exuded on the leaves causing the secondary effects of sun scorch, sooty mold, and mechanical problems in harvesting due to sticky machinery (Villanueva et al. 2014). Although no specific toxin in the saliva of the sugarcane aphid has been identified, rapid loss of plant assimilate through the phloem sieves causes death to sorghums. If the head is colonized with sugarcane aphids during anthesis, grain fill will be significantly reduced. In terms of host plant resistance, there does exist commercially available sorghum hybrids and parental sorghum germplasm developed for the greenbug plant resistance, but also cross-resistant against the sugarcane aphid (Armstrong et al. 2015, Armstrong et al. 2016). Commercially available hybrids and lines with the cross-resistance have been a major benefit to sorghum production, especially where grown in regions where the maturity of

tolerant hybrids is optimal. A major disadvantage is that Johnsongrass (*Sorghum halepense*) is a significant non-crop host that is universally found throughout North American sorghum production regions. Johnsongrass allows sugarcane aphids to survival before grain sorghum is planted, and in between cropping seasons, dependent upon whether the sugarcane aphid successfully survives the winter. Our specific goal of the following studies was to continue to screen USDA-ARS germplasm from the Plant Stress and Germplasm Research and Development Laboratory Lubbock, TX in the search for host-plant resistance against the sugarcane aphid. Some of these sorghum germplasm in Lubbock are in development for the stay-green characteristic in sorghums that express higher levels of leaf dhurrin, a plant compound that is beneficial to drought tolerance (Hayes et al 2015), while others have parental background from "Capbam" that originated from Russia, and "SC110", a line from South Africa originally used in breeding for head smut, but also found to have resistance against the greenbug. R.Tx2783 was released by Texas A&M Experiment station in Lubbock, TX in 1984 for sorghum breeding programs that were interested in incorporating greenbug biotypes C and E resistance into commercial sorghum hybrids (Peterson et al. 1984). Another know sorghum source that was used as a resistant control in these trials was DKS37-07, also proven to be resistant to greenbug biotypes C, E, with the source of resistance originating from introductions PI 550510 and PI 264453 (Peterson et al. 1996).

Materials and Methods

Aphid Collection and Culture

Sugarcane aphids used in these trials were obtained from a single parthenogenic female collected from infested grain sorghum in Matagorda county Texas in August of 2013.

Sugarcane aphids were cultured on susceptible Tx7000 seedlings in 4.4-L pots fitted with 45-cm-tall x 16 cm diameter cylinders of Lexan™ (SABIC Polymershapes, Tulsa, OK) sleeve cages that were ventilated using organdy cloth covering the top. The sugarcane aphid colonies were transferred to new seedling plants every 2 weeks in the greenhouse. The Tx7000 plants and aphids were maintained on greenhouse benches at temperatures between 26 $\pm$ 5°C under natural greenhouse light supplemented by two T-8 fluorescent lights.

Free-choice flat screen trial – 1

In an effort to evaluate 29 sorghum entries for signs for phenotypic characteristics of host plant resistance, we used a free-choice flat screen where sorghum seedlings were infested with sugarcane aphids and rated for damage, plant height, number of developed leaves and chlorophyll content using the infested compared to the controls (Table 1). Included as known resistant sorghums were TX2783 and DKS37-07. The known susceptible hybrids MORHC858, and WSH117, were compared to the remaining 32 entries obtained from the USDA-ARS Sorghum Breeding Program in Lubbock, Texas. The USDA-ARS sorghum germplasm consisted of mostly elite experimental parent lines used in breeding programs to produce sorghum hybrids for grain and forage. Four flats (plastic trays with 128 individual cells, Growers Supply, Dyersville, IA 52042) were used for infesting, and 4 flats were used for growing non-infested plants for comparison of plant height, number of leaves, and chlorophyll content. Each flat was considered a block, and each sorghum entry was randomized sixteen times using Research Randomizer (http://www.randomizer.org, 2016). The four infested flats were introduced to sugarcane aphids that were reared on the known susceptible Tx7000 by placing the heavily infested

plants down each row, and across each row within the flat (Starks and Burton 1977). When the known susceptible hybrids MORHC858 and WSH117 were 90-100 % dead, all plants in each flat were evaluated for damage using a rating of 1-9; where 1 is a completely healthy plant with no necrotic tissue; 2 represents 1-5% chlorotic tissue; 3, 5-20%; 4, 21-35%; 5, 36-50%; 6, 51-65%; 7, 66-80%; 8, 81-95%; and 9, 95-100% or dead (Webster et al. 1991; Burd et al. 2006). Chlorophyll content was measured using SPAD-502 chlorophyll meter (Minolta, Ramsey, NJ 07466) where chlorophyll type a and b are not detected separately, but summed for total content (Markwell et al. 1995). The top three leaves from each sorghum entry were measured and averaged for one reading that was then compared to an identical non-infested entry for each of the sixteen replications by using the Chlorophyll loss index (C-T)/C*100, where, C is the SPAD measurement from the non-infested or control, and T is from infested plant. Following the chlorophyll content, each plant was cut at the soil surface so plant height could be measured (cm) and the number of leaves could be counted for both infested and non-infested plants. The variables of damage rating, plant height, number of leaves on a sorghum entry, and chlorophyll loss were subjected to one-way ANOVA, with sorghum entry means compared ($\alpha = 0.05$) using least squared means pair-wise comparisons procedure (SAS 9.3, SAS Institute 2010).

Free-choice flat screen trial – 2

To better understand the sugarcane aphid resistance, we included BTx623, BTx399, RTx430, TAM428 in a second trial that also included the resistant check Tx2783 and the known susceptible Tx7000. BTx623 was released by Texas Agricultural Experiment Station in 1977 and is a commonly used grain and forage seed parent. TAM428 is a

Texas A&M parental line found to be resistant to sugarcane aphid in South Africa (Teetes et al. 1995). Tx2783 was released in 1984 because it was resistant to both greenbug biotypes C and E (Peterson, 1984), and also found to be cross resistant to the sugarcane aphid (Armstrong et al. 2015). The parental inbred RTx430 has a history of high yield and for being crossed with brown mid-rib sorghums to produced dual purpose grain and forage sorghum (Yerka et al. 2015).

All plant evaluation variables including damage ratings, difference in plant height, number of leaves, and chlorophyll loss were conducted identical to trial number 1. Additional entries JB18 and JB37 were included in trial 2 because they showed signs of resistance when planted in the field at Lubbock, TX. The primary intent in the development for JB18 and JB37 at the USDA ARS Laboratory in Lubbock is because these sorghums express higher levels of dhurrin, which has shown to be beneficial under abiotic water stressed conditions (Hayes et al. 2015, Emendack et al. 2016).

Antibiosis

From the previous two trials, 8 sorghum entries including BTx623, BTx399, R.11143, R.11259, TX2783, JB18, JB37, and WSH117 were used to determine the presence of antibiosis that results in increased mortality, reduced longevity, and has a negative effect on the reproductive capacity of the insect (Smith et al. 1993). The entry WSH117 was used as the susceptible control, while R.Tx2783 was used as the known resistant for comparative purposes. Two seeds of each entry were planted in cone-tainers™ (model SC10, S7S greenhouse supply, Tangent, Oregon 97389) in a three-layer media of potting soil, fritted clay, and sand (from bottom to top respectively). The cone-tainers™ each

were fitted with an 8 cm diameter Lexan sleeve, 45 cm in height and ventilated with organdy cloth. The containers were maintained in the greenhouse at 23.9°C with a photoperiod of 14 hours of light and 10 hours of dark. When the plants reached the three-leaf stage, or 5-10 cm in height, the most vigorous plant was kept, whereas the other was removed. A single sugarcane aphid adult reared on R.Tx7000 collected from our Matagorda County, TX, collection was transferred to each sorghum entry by using a camel hair brush and allowed to develop on the sorghum until reproduction occurred, followed by removal of all nymphs except the adult. From the time the single nymph turned to adult and started producing offspring, life parameters such as net reproduction (*Ro*), number of nymphs produced per female per day, intrinsic rate of increase (*rm*), female longevity (*L*), and reproductive period (days in reproduction). Intrinsic rate of increase utilizes the [(rm=0.738(logMd/d)] formula where d is the pre-reproductive period of F1 in days, and 0.738 is the slope of Md over "d" days (Wyatt and White, 1977). There were 11 replicates of each sorghum entry and nymphs were counted every 24h until the death of the founding female.

Results

Free-choice flat screen trial – 1

The twenty-nine entries from the free-choice flat screen provided a wide range in phenotypic response to sugarcane aphids in terms of damage rating, differences in plant height, number of leaves, and chlorophyll content for the controls versus the infested plants (Table 2). The three lowest damage ratings were from DKS37-07, R.11143 and TX2783 respectively. It should also be noted that entry R.11259 scored a 5.8 damage

rating which is considered a mid-range of resistance, as opposed to 27 other entries that scored a damage rating 7.0 or higher when challenged with heavy sugarcane aphid pressure. Outside of the damage ratings for DKS37-07, R.11143, TX2783, and R.11259, the remaining sorghum entries appeared to be highly susceptible.

Differences in plant height where the height of the control (non-infested) was subtracted from the infested plants across the sixteen replications indicated that R.11143 has a high degree of tolerance in that there was on average only a 2.6 cm loss in plant height, followed by 9.7 for TX2783, 10.4 for the DKS37-07 and 14.3 cm loss for R.11259. Other notables that resulted in moderate loss were R.13001 and R.13022, however both were greater than 8.0 on the damage rating scale. All other entries ranged from 20 to approximately 30 cm loss in plant height and did not appear to exhibit any degree of tolerance to sugarcane aphid feeding.

Loss of chlorophyll as indicated from the index (C-T) /C*100 was the lowest for the top three entries (R.11143, TX2783, and DKS37-07) that were also lower in damage ratings and difference in plant height (Table 2). Entry R.11143, resulted in a net gain in chlorophyll when compared to the non-infested, and possibly shows a compensatory effect in chlorophyll production when under sugarcane aphid attack. Lower levels of chlorophyll loss, plant height and damage ratings are strong indicators of a high degree of the expression of tolerance. R.13020 and R.11259 lost 24.1 and 25.0 % chlorophyll respectively, however the R.13020 resulted in a damage rating of 7.9, while the R.11259 had a 5.8, indicating that it may express a greater level of tolerance. The remaining entries ranged from 40 to 100% chlorophyll loss.

The number of leaves on the 29 sorghum entries in the first trial were numerically higher for R.11143, followed by DKS37-07, and TX2783, although not statistically higher than a majority of the entries listed in Table 2. DKS37-07 is a medium maturity hybrid, and hybrid vigor combined with sugarcane aphid tolerance might help explain the numerically higher numbers of leaves at the time of the evaluation. Although the overall model for numbers of leaves was significant in Table 2, the limited range of numbers from 2.1 for R.13012 to 5.0 for R.11143 allowed for few statistical separations.

Free-choice flat screen trial – 2

There were three entries in trial 2 that exhibited phenotypic signs of resistance to the sugarcane aphid (Table 3), by scoring less than a 6.0 on the damage rating scale, and those were TX2783 with a rating of 4.6, R.11143 with a rating of 4.8, and R.11259 with a rating of 5.8. However, TAM428 scored a 6.1 and has been reported as resistant to the sugarcane aphid in South Africa, but did not differ from the three resistant sources previously mentioned. The lines JB18 and JB37 that have higher levels of dhurrin do not appear to be resistant to sugarcane aphid, nor do BTx623, BTx399, RTx430, as they were not statistically different than the known susceptible Tx7000. It is possible JB18 and JB37 are moderately tolerant to sugarcane aphid at later maturities, but the JB lines appear to be susceptible in the seedling stage. Most impressive for chlorophyll loss from the ten entries is that R.11143 was only 18.7 %, followed by TX2783 with 35.9 %, and TAM428 with a 49.6 % loss. All other entries were between 60 to 94% loss. In terms of plant height differences, R.11143 showed compensatory growth where the infested plants were on average 0.3 cm taller than the non-infested plants (Table3). R.11143 also had the

highest mean number of leaves on a plant with 3.6, but was not statistically higher than the TX2783, TAM428 or the susceptible check Tx7000.

Antibiosis

The entry WSH117 was included in the antibiosis test because in previous tests it was highly susceptible, and in this evaluation net reproduction was >100 offspring produced in 27 d of reproduction, which was 3.6 nymphs per day, and the highest intrinsic rate of increase of 0.41 (Table 4). A second grouping with lower fecundity was BTx399 and BTx623 with 81.9 and 72.2 nymphs produced respectively, and 2.7 and 2.6 nymphs produced in a day. However, the nymphs/female/day did not differ from WSH117, nor did the intrinsic rate of increase, longevity or reproductive period. The JB lines JB18 and JB37 showed signs of antibiosis in that net reproduction was significantly lower than BTx399 and BTx623 but were also significantly higher than the R.11259, R.11143, and TX2783. The later 3 entries show a significant expression of antibiosis in that they resulted in reductions of 77.7, 86.0, and 94.2 % when compared to the susceptible WSH117. Entry TX2783 was also shown to express both tolerance and antibiosis in sorghum as reported by Armstrong (2016). Most positive from these evaluations is that the R.11143 and R.11259 express both tolerance and antibiosis as a mechanism of resistance to the sugarcane aphid. R.11143 has TX2783 as a parental line on the male side and this evidently carries over in the expression of resistance. However, the pedigree for R.11259 is SC56-14E//86EO361/88BE2668 and the source of tolerance is currently unknown.

Discussion

Plant resistance in sorghums to virulent aphid species has proven to be an economical advent that has saved millions of dollars to the sorghum industry, especially when used with other management tactics such as planting date and judicial use of insecticides (Smith, 2005). This was proven in the long standing battle with the greenbug and the intermittent development of greenbug biotypes in sorghum production over the last 5 decades (Michels and Burd, 2007) and this has especially useful for greenbugs, (Kofoid et al. 2012, Reese et al. 1994) and more recently for sugarcane aphid (Teetes et al. 1995, Sharma et al. 2013, Sharma et al. 2014, Armstrong et al. 2015, Armstrong et al. 2017).

Trial 1 from the free-choice flat screen indicated based on damage ratings that R.11143, R.11259, TX2783, and DKS37-07 exhibited higher levels of tolerance when challenged under heavy sugarcane aphid pressure. Trial 2 confirmed the results from trial 1 where the known resistant TX2783 was relatively equal to R.11143, and both were more resistant than susceptible checks terms of tolerance. BTx623 BTx399 in trial-2 showed no expression of phenotypic resistance as they were no different than the susceptible control Tx7000. The JB lines did not appear to express tolerance to sugarcane aphids based on the results of trial-2.

The reproductive studies confirmed that not only tolerance was expressed in R.11143, R.11259, as was in the TX2783, but antibiosis was clearly a mechanism of resistance as was indicate by significantly less net reproduction, number of nymphs produced per d, the intrinsic rate of increase and the reproductive period in d. R.11143, R.11259. Antibiosis when combined with tolerance is a good combination of resistance mechanism for sorghum

and these two lines should be used in sorghum breeding programs aimed at incorporating plant resistance to the sugarcane aphid.

R.11143 and R.11259 germplasm is maintained at the USDA-ARS Laboratory in Lubbock, TX and can be used in breeding programs that are developing resistant sorghums to sugarcane aphid. TX2783 was released in 1984 for greenbug biotype C and E resistance and is incorporated into several commercially available sorghums that can be purchased. DKS37-07 is currently owned by Monsanto but was developed by DeKalb for greenbug biotype C, E, and I resistance but the gene source for expression is completely different than TX2783. We feel confident that the source of sugarcane aphid resistance in R.11143 comes from TX2783 because of the breeding background. However, the expression of phenotypic resistance in R.11259 must be investigated further based on the SC56-14E//86EO361/BE2668 pedigree. The identification of new sources of tolerance to sugarcane aphid is vitally important to the sorghum seed industry. Identification of both antibiosis and tolerance in new sources, as well as the TX2783 source in different genetic background will continue to be a major goal of sorghum breeding programs.

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Table 1. Background information on sorghum genotypes from the Lubbock, TX USDA-ARS Laboratory evaluated for resistance to sugarcane aphid in 2016-2017.

Designation	Pedigree	Seed	Plant
R. 11012	SC56-14E/86EO361-HF1//02CA5053	White	Tan
R.11018	02CA5053//RTx436/Tx7078/1790E	Red	Tan
R.11079	Sureno/02CA5053	White	Tan
R. 11143	RTx2783/PI567946	Red	Purple
R. 11183	Tx2536/SC1321	Red	Purple
R. 11193	RTx430/SC701	Red	Purple
R. 11259	SC56-14E//86EO361/BE2668	White	Tan
R. 11269	02CA5053//Macia/Dorado	White	Tan
R. 11338	SU1490/IS13076	Red	Purple
R. 12001	RTx2737/RTx436	White	Tan
R. 12004	RTx2737/RTx436	White	Tan
R. 12008	Macia/Dorado/SC414-12E	White	Tan
R. 12010	P40-1/RTx436	White	Purple
R. 12016	SC1154-14E/Koro Kollo	White	Purple
R. 12026	BTx642/Kuyuma	Red	Purple
R. 12028	SC414-12E/Kuyuma	White	Purple
R. 13001	RTx430/1790E	White	Purple
R. 13005	RTx430/1790E	White	Purple
R. 13007	RTx430/Abjabsido	White	Purple
R. 13009	RTx430/R9188	White	Purple
R. 13012	RTx430/SC1014	White	Purple
R. 13015	RTx430/SC1080	White	Purple
R. 13020	RTx430/SC1476	White	Purple
R. 13022	RTx430/SC704	Red	Purple

R.13023	RTx430/SC704	Red	Purple
JB18	RTx436//BTx406/PI568351	Red	Purple
JB37	RTx436//BTx406/PI563145	White	Purple

Table 2. Damage ratings, chlorophyll loss, difference in plant height (cm), and number of leaves for twenty-nine sorghum entries subjected to sugarcane aphid infestations under free-choice flat screen evaluation.

Pedigree	Damage Rating [a]	Chlorophyll Loss [b]	Difference in Plant height [c]	Mean leaves/plant [d]
R.11012	9.0 ± 0.0 a	60.1 ± 4.6 ab	26.0 ± 1.4 o	2.4 ± 0.2 a
R.11018	8.9 ± 0.1 ab	59.4 ± 8.2 bcd	16.5 ± 2.4 d-h	2.8 ± 0.2 a-d
R.11079	8.9 ± 0.1 ab	62.9 ± 8.6 bc	23.7 ± 2.2 mn	2.9 ± 0.2 a-d
R.11143	3.1 ± 0.4 ef	-4.6 ± 4.7 i	2.6 ± 1.3 e-i	5.0 ± 0.2 b-d
R.11183	9.0 ± 0.0 a	65.9 ± 9.8 ab	26.8 ± 2.0 f-k	2.5 ± 0.2 b-d
R.11193	8.9 ± 0.1 ab	57.1 ± 10.5 bcd	21.8 ± 1.9 h-l	2.8 ± 0.2 a-d
R.11259	5.8 ± 0.7 d	25.0 ± 7.2 e-h	14.3 ± 2.2 g-l	3.6 ± 0.2 a-d
R 11269	8.9 ± 0.1 ab	53.7 ± 5.6 bcd	18.4 ± 1.3 i-l	3.0 ± 0.2 a-d
R.11338	8.6 ± 0.3 ab	45.8 ± 6.3 b-f	21.5 ± 1.6 c-f	3.5 ± 0.2 a-d
R.12001	8.9 ± 0.1 ab	58.6 ± 7.5 bcd	20.3 ± 1.2 ab	3.6 ± 0.4 a-d
R.12004	8.1 ± 0.5 abc	53.1 ± 10.1 bcd	24.8 ± 2.3 a-d	2.7 ± 0.1 a-c
R.12008	9.0 ± 0.0 a	89.8 ± 4.4 a	28.9 ± 2.3 f-k	2.3 ± 0.2 b-d
R.12010	7.1 ± 0.6 a	36.7 ± 5.2 d-g	18.5 ± 2.3 e-j	3.1 ± 0.1 a-d
R.12016	7.8 ± 0.7 bc	49.7 ± 10.8 bcd	17.3 ± 3.3 d-h	3.3 ± 0.2 c-d

R.12026	7.8 ± 0.4 bc	39.6 ± 7.6 c-g	18.1 ± 2.3 b-e	2.8 ± 0.1 a-d
R.12028	8.9 ± 0.1 ab	59.4 ± 10.0 bcd	23.0 ± 1.5 h-l	2.8 ± 0.1 a-d
R.13005	8.1 ± 0.7 abc	38.8 ± 17.7 c-g	27.0 ± 4.799 a	2.6 ± 0.4 a-d
R.13001	8.5 ± 0.4 ab	58.4 ± 10.2 bcd	16.4 ± 2.4 j-l	3.4 ± 0.3 a-d
R.13007	8.7 ± 0.3 ab	51.7 ± 5.6 bcd	22.2 ± 1.4 lm	3.3 ± 0.3 a-d
R.13009	8.9 ± 0.1 ab	48.8 ± 4.7 b-e	22.9 ± 1.8 no	2.9 ± 0.2 a
R.13012	8.4 ± 0.6 ab	57.1 ± 39.2 bcd	18.6 ± 3.1 d-i	2.1 ± 0.284 a-d
R.13015	9.0 ± 0.0 a	100.0 ± 0.0 bcd	22.7 ± 1.9 k-m	2.6 ± 0.202 a-d
R.13020	7.9 ± 0.7 abc	24.1 ± 8.4 f-h	18.7 ± 2.6 mn	3.1 ± 0.205 c-d
R.13022	8.7 ± 0.3 ab	53.2 ± 6.8 bcd	14.56 ± 0.9 k-m	2.8 ± 0.1447a-d
R.13023	9.0 ± 0.0 a	68.6 ± 0.0 ab	23.0 ± 2.1 mn	2.6 ± 0.157 a-d
TX2783	4.3 ± 0.8 e	11.6 ± 6.5 hi	9.7 ± 2.6 mn	4.2 ± 0.2 a-d
MORHC858	8.1 ± 0.5 abc	45.8 ± 5.5 b-f	27.3 ± 3.1 mn	3.7 ± 0.119 a
WSH117	8.6 ± 0.4 ab	52.4 ± 5.4 bcd	24.3 ± 2.1 f-k	3.1 ± 0.154 d
DKS37-07	2.5 ± 0.4 f	15.6 ± 4.4 ghi	10.4 ± 2.2 a-c	4.8 ± 0.100 a-d

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD

[a] Damage ratings evaluated on a 1 – 9 scale (Webster 1992), df = 28, 390; $F = 19.11$; $P >$F= <0.0001;

[b] Chlorophyll loss index (C-T) /C*100, where, C is the SPAD reading from the non-infested control, and T is from infested plant, df =28, 316, $F = 6.1$, $P = <0.0001$.

[c] Mean difference in plant height, (controls – infested), df = 28, 390, $F = 7.11$; $P = <0.0001$.

[d] Mean number of leaves per plant, df = 28, 390, $F = 11.77$; $P = <0.0001$.

Table 3. Damage ratings, chlorophyll loss, difference in plant height (cm), and number of leaves for ten sorghum entries subjected to sugarcane aphid infestations under free-choice flat screen evaluation, 2017.

Germplasm	Damage Rating	Chlorophyll Loss	Difference in Plant Height	Leaf Number
BTx623	7.2 ±0.7 ab	80.9 ± 6.3 ab	14.3 ± 2.0 bc	2.7 ± 0.2 bc
BTx399	8.2 ± 0.4 a	89.9 ± 4.6 ab	19.4 ± 1.3 ab	2.9 ± 0.2 bc
TX7000	8.7 ± 0.3 a	93.5 ± 3.7 a	20.6 ± 1.1 ab	3.0 ± 0.1 abc
R.Tx430	8.4 ± 0.4a	82.6 ± 7.3 ab	10.1 ± 1.4 cd	2.7 ± 0.1 bc
TAM428	6.1 ± 0.8 bc	49.6 ± 13.6 cd	3.0 ± 2.8 ef	3.3 ± 0.3 ab
R.11143	4.8 ± 0.9 c	18.7 ± 17.5 e	-0.3 ± 3.3 f	3.6 ± 0.4 a
R.11259	5.8 ± 0.9 bc	64.8 ± 14.0 bc	11.57 ± 2.6 cd	2.8 ± 0.3 bc
TX2783	4.6 ± 0.7 c	35.9 ± 8.5 de	6.3 ± 2.4 de	3.3 ± 0.3 ab
JB18	7.3 ± 0.7 ab	72.7± 9.3 abc	10.3 ± 1.9 cd	2.8 ± 0.2 bc
JB37	8.3 ± 0.3 a	86.6 ± 5.5 ab	19.1 ± 1.1 ab	2.5 ± 0.1 c

Column means followed by the same lowercase letters are not significantly different, $P >$ 0.05; LSD.

Mean Damage Rating, 20 replications, df =9, 165, F = 6.8; $P > F = < 0.0001$.

Difference in plant height, df = 9, 165, $F = 12.7$, $P > F = < 0.0001$.

Chlorophyll loss index (C-T) /C*100, where, C is the SPAD reading from the non-infested control, and T is from infested plant, df = 9, 165, $F = 7.8$; $P > F = < 0.0001$.

Leaf number, df = 9, 165, $F = 2.2$; $P >$ F = < 0.025.

Table 4. Demographic statistics for sugarcane aphid reproduction on eight sorghum entries

Germplasm	*Net Reproduction* (*Ro*)	Nymphs/ ♀/ d	Intrinsic Rate of Increase (*rm*)	♀ Longevity (d)	Reproductive Period (d)
WSH117	101.0 ±12.2 a	3.6 ± 0.22 a	0.41 ± 0.01 a	30.3 ± 1.8 abc	27.2 ± 2.5 abcd
BTx399	81.9 ± 8.5 b	2.7 ± 0.27 b	0.39 ± 0.01 a	32.5 ± 1.9 a	30.4 ± 2.2 a
BTx623	72.2 ± 6.2 b	2.6 ± 0.20 b	0.38 ± 0.01 a	30.8 ± 0.9 ab	27.8 ± 1.7 abc
JB18	43.1 ± 5.1c	1.8 ± 0.13 c	0.33 ± 0.01 b	27.0 ± 1.3 bc	23.8 ± 1.5 bcd
JB37	35.6 ± 4.0 c	1.3 ± 0.12 cde	0.32 ± 0.01 b	33.5 ± 0.9 a	28.9 ± 2.0 ab
R.11259	22.5 ± 3.6 de	1.1 ± 0.10 cd	0.26 ± 0.02 c	26.1 ± 3.0 bc	22.4 ± 3.5 cd
R.11143	14.2 ± 2.2 e	0.7 ± 0.10 de	0.22 ± 0.03 cd	25.3 ± 2.2 c	20.8 ± 2.4 d
TX2783	5.8 ± 1.2 e	1.3 ± 0.34 e	0.19 ± 0.02 d	19.6 ± 1.8 d	13.6 ± 2.1 e

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD.

Net Reproduction (*Ro*) = mean sugarcane aphids per female, 11 replications, df = 7, 83, F = 28.1; $P > F = < 0.0001$.

Nymphs/♀/d; = (*Md*/d), df = 7, 83, $F = 24.1$, $P > F = < 0.0001$.

rm = intrinsic rate of increase, rm = 0.738(log *Md*/d); df = 7, 83, $F = 23.6$; $P > F = < 0.0001$.

Pre-reproductive period (d) df = 7, 83, $F = 28.1$; $P >$ F = < 0.0001.

Female Longevity (d), df = 7, 83, $F = 5.9$; $P > F = <0.0001$.

VITA

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Master of Science

Thesis: SCREENING GRAIN SORGHUM FOR PLANT RESISTANCE TO SUGARCANE APHID (MELANAPHIS SACCHARI) AND TESTING THE EFFECTS OF LED GROW LIGHTS

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