SUGARCANE APHID-FORAGE SORGHUM INTERACTIONS AND EFFECTS OF ABIOTIC AND BIOTIC CONDITIONS

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Abstract: The sugarcane aphid (Melanaphis sacchari (Zehnter); (Hemiptera: Aphididae)) is a pest of (Sorghum bicolor (L.) Moench) in the United States. While it was originally found infesting sorghum in Florida in 1977, it was not until 2013 that it started to cause widespread economic loss in sorghum crops. To combat this pest, host plant resistance is being explored. Finding new sterile germplasm is a priority for sorghum breeders to be able to create new resistant sorghum lines twice as fast as using the fertile counterparts. The A3 cytoplasmic male sterile technique was tested and found suitable to improve a known sugarcane aphid resistant sorghum line, TX2783. Often screening trials are conducted in controlled environments using artificial lighting. Light-emitting diodes (LED) are used to reduce cost, space, and time to grow plants. I found that LED lights lowered the photosynthetic rates, altered stomatal conductance, and changed plant characteristics in different cultivars of sorghum. LED lights also altered damage ratings for resistant and susceptible plants when exposed to sugarcane aphids suggesting that these trials should not use LED lighting when searching for plant resistance. Lastly, I compared life history traits of aphids in aggregated groups to solitary aphids. Aphids reproduction was not sig Overall the results of these studies highlight the importance of experimental conditions when evaluating plant resistance traits and document much-needed sugarcane resistant traits in sterile lines of grain sorghum.

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CHAPTER I

Review of the Literature

Sorghum

Sorghum, *Sorghum bicolor* (L.) Moench,), is among the top five cereal crops produced worldwide (Young & Teetes 1997, FAO 1995). It originated in Africa (Undersander, et al 1990) where it was selectively bred for grain sorghum and is predominately consumed as food (Young & Teetes 1997, Anjali et al 2017). Sorghum currently provides food for more than 500 million people around the world (Anjali et al 2017). The crop has 12% protein content (Anjali et al 2017) and is recommended as a gluten-free alternative for celiac patients (Ciacci et al 2007, Kulamarva et al 2009). Other sorghum varieties have been developed, including sweet sorghum are used for syrup production and as a sweetener (Mercer et al 2011). Sweet sorghums can also be used to make alcohol for consumption and for chemical additions to products (Mercer et al 2011, Maw et al 2017). More recently, biomass sorghums have been developed for use in the production of bioenergy to produce fuel (Mercer et al 2011, Maw et al 2017).

Sorghum is considered to be one of the three most-important cereal crops in the United States and is the most-drought resistant cereal grown in the United States (Taylor et al 2006; Davila- Gomez et al 2011). Like maize, sorghum is a C4 plant, but it has a relatively short growing season (3-5 months), is tolerant to temperature fluctuation, needs less water than many crops, and can grow in saline and alkaline conditions (Davila-Gomez et al 2011). It also can more efficiently utilize N, P, K in the soil than many other crops, and thus requires less fertilizer (Yosef et al 2009). Sorghum grows in semi-arid conditions and is typically not irrigated in the United States (Taylor et al 2006). An

additional advantage of sorghum is that it has a more extensive root system than maize, increasing topsoil retention (House 1985).

In the United States, sorghum is primarily grown to produce silage for animals (Young & Teetes 1997; Miron et al 2007, Queiroz et al, 2018) and produces similar amounts of silage per acre as corn (Undersander, et al 1990). Sorghum forage fed to dairy cattle produces similar cattle weight gain and milk production as those fed with maize (Aydin et al 1999, Grant et al 1995, McCuistion et al 2004, Oliver et al 2004, Bean et al 2013). However, sorghum is often less digestible than corn and young sorghum plants contain alkoxides which release prussic acid which is very toxic to livestock (Undersander, et al 1990). Forage sorghum is also fed to poultry, sheep, pigs and other livestock.

Because maize requires more water and fertilizer and is not as hardy as forage sorghum (Howell et al 2008, Marsalis et al 2009, Bean 2013), the amount of planted sorghum is increasing in the United States. In 2018, more than 5.67 million acres of sorghum were planted in the United States (USDA- NASS 2019) and more than 365 million bushels were produced (USDA- NASS 2019) with an average of 72.1 bushels produced per acre (USDA- NASS 2019). Most of the sorghum was produced in the "sorghum belt", from South Dakota to southern Texas. The states with the highest sorghum planted per acre in 2018, were Kansas, Texas and Colorado, respectively (USDA- NASS 2019).

Although sorghum is tolerant to harsher environmental conditions than most other grains, yield is affected by a number of insect pests including caterpillars, grasshoppers, leaf hoppers, midges, and aphids (Okosum et al. 2021). In 2013, the sugarcane aphid,

Melanaphis sacchari, was found damaging sorghum in four states and by 2015, it was reported to be a perennial pest in 17 states.

Aphids

There are approximately 4,000 aphid species. Some aphids specialize on a single plant family and others use a broad range of hosts (Dixon & Kundu 1998). Of the 4,000 species, about 100 species are considered economic pests (Blackman and Eastop 2017). Unfortunately, many of these species are severe pests of the most important crops grown by humans. Aphids feed on plants by using a piercing/sucking mouthpart (Williams & Dixon 2007) that enters the intracellular part of the tissue and ingest phloem nutrients (Dixon & Kundu 1998). This feeding affects the plants' immunity, changes the physiology of the plant, and potentially exposes the plants to viruses and other pathogens (Quisenberry and Ni 2007).

Aphids belong to the hemipteran suborder Sternorrhyncha which have long antennae (Vandermoten et al 2012). Most aphids have the ability to undergo parthenogenetic reproduction when environmental conditions are favorable. Females that reproduce asexually can generate additional sexually mature females in 4 to 12 days (Zapata, et al 2004). Individual females produce between 34 and 96 nymphs (Chang, et al 1982, Singh et al 2004) and live between 10 and 37 days (Chang et al 1982, Singh et al 2004, Zapata et al 2004). Many factors likely influence aphid reproduction and Michaud et al. (2006) suggested that groupings were an important factor. They found that each aphid reproduced more when it was in a group that when it was alone. Aphids have the ability to produce between 10 and 30 generations of clones per year (Dixon and Kundu 1998), allowing them to overwhelm plant defenses and natural enemies. The aphid's unique

parthenogenetic reproduction allows a mix of generations to develop at once (Simon et al 2002, Miura et al 2003) because the parthenogenetic females give birth to pregnant daughters (Simon et al 2002, Miura et al 2003).

If abiotic and biotic stressors increase, the mother aphid will produce offspring that can grow wings (Guo et al 2016). These winged individuals can disperse and continue parthenogenetic reproduction. As conditions deteriorate in the environment, most aphids will switch to sexual reproduction and will form winged individuals that mate and lay eggs that can survive adverse conditions like winter. Winged aphids are possible for an estimated 95% of aphid species and allow dispersal and colonization of new locations (Hardie et al 1996, Guo et al 2016; Braendle et al. 2006). Winged morphs form in response to environment and hormonal signals (Ishikawa et al 2013, Zera 2016)

Sugarcane Aphids

Sugarcane aphids have 14 known plants worldwide on which they can complete development (Singh et al. 2004). These plants include *Cynodon dactylon* (L.), *Miscanthus sinensis* (L.), *Oryza sativa* (L.), *Panicum colonum*, *Panicum maximum*, *Paspalum sanguinale*, *Pennisetum sp.*, *Saccharum officinarum*, *Setaria italic* (L.), *S. bicolor*, *Sorghum halepense* (L.) *S. verticilliflorum* (Steud.), and *Zea mays* (L.) (Singh et al. 2004). In the United States sugarcane aphids have a limited host range to *S. bicolor*, S. *halepense, Saccharum officinarum*, Sudan grass, and Columbus grass (Sorghum × almum) (White et al 2001, Armstrong et al 2015, Medina et al 2016).

In 2013, sugarcane aphids, *Melanaphis sacchari* were reported causing economic damage to sorghum for the first time in the United States (Elliott, et al 2017). Large numbers of aphids on sorghum were reported from sorghum in Liberty County, Texas.

(Bowling, et al 2016) In 1977, *M. sacchari* was reported as an occasional pest on sugarcane, *Saccharum officinarum* (L.) (Mead 1978. White, et al 2001). Previously, it had been reported in sorghum in Florida also in 1977 but was not thought to be an economic pest (Denmark 1988). Sugarcane aphids have also been found on corn and cotton, but do not reproduce on those crops (Bowling, et al 2016).

Sugarcane aphids consume phloem from the plant tissue and produce large amounts of honeydew, which promotes the growth of sooty molds (Singh et al 2004, Bowling et al 2016). The aphids feed primarily on the underside of leaves throughout the development of the sorghum (Brewer, et al 2017). Aphid populations can surpass 10,000 aphids on a sorghum plant with 2,000 aphids on a single leaf (Brewer, et al 2016).

Feeding decreases the leaf chlorophyll content and stunts the height in sorghum (Limajie et al 2018, Backoulou et al 2018). Affected plants produce uneven seed heads and poor grain set of the heads (Rott et al 2008) and the honeydew causes grains to stick together, impeding harvest. A survey that was done in the Rio Grande Valley in Texas, estimated the loss of yield when sugarcane aphids were present in sorghum crops loss was of 64.53%/ac in 2014-2015 (Zapata et al 2018). Most other studies have reported sugarcane aphids feeding on susceptible sorghum can reduce yields from 10% to more than 50% (Bowling, et al 2016).

Sugarcane aphids overwinter in south Texas on remnant sorghum or on Johnson grass (Bowling, et al 2016). Sugarcane aphids need temperatures that are above freezing and need sorghum or Johnson grass to survive the winter (Bowling, et al 2016). In North America, natural enemies of aphids, such as ladybird beetles and lacewings, suppress *M. sacchari* when the aphid population densities are in low to medium ranges (20 to 160

aphids per plant) (Hewlett, Szczepaniec & Eubanks 2019). The value of the impact of natural enemies towards *M. sacchari* population that are below the economic injury level, should be given some consideration.

Integrated Pest Management (IPM) Strategies

Integrated pest management (IPM) is a pest management approach which uses all tools available to keep potential pest populations below the economic threshold of the crops (Ehler 2006) The pest life cycles, development of host preferences and the population dynamics, are considered as are management options. The outbreak of *M. sacchari* on sorghum in 2013 and continued expansion as a pest has spurred research to identify IPM strategies. Chemical management has been researched but it is often not economical (Brewer, et al 2017) because sorghum is a low value crop. Because of its low value, other management strategies including host plant resistance, biological control and cultural methods have been researched. Worldwide, the economic value of cultivars that are aphid resistant is estimated to be more than 400 million (Wiseman & Webster 1999) and resistant cultivars have slowed the spread of aphids and the diseases they can transmit (Kishaba et al 1992, Tanguy & Dedryer 2009).

Currently, host plant resistance is the most-effective and least disruptive IPM technique to combat *M. sacchari.* (Brewer & Elliott 2004). Host plant resistance increases the economic threshold for sorghum, delays the use insecticides, and is combatable with natural enemies (Brewer & Elliott 2004). While sorghum hybrids with genetic resistant to greenbugs have been created (Michels and Burd 2007), the research of resistance to sugarcane aphids is relatively new (Armstrong et al 2015, Mbulwe et al 2015).

Host plant resistance has three categories of plant resistance, antibiosis, Antixenosis, and tolerance (Painter 1951). Antibiosis impacts the biology of the herbivores and lowers the chance of survival of the insect (Painter 1951). Antixenosis lowers the acceptance of the plant and will cause an insect to feed on a different genotype if a choice is available. Tolerance allows a plant to survive and produce similar yield despite the presence of herbivores feeding on it. Pest populations normally are affected by multiple forms and categories of resistance (Hill 2004, van Emden 2017).

Unfortunately, the use of host plant resistance for the control of *M. sacchari* may be challenging because of different aphid genotypes (Nibouche et al. 2018). Multiple multiloces genotypes of sugarcane aphids have been identified worldwide that are designated as MLL-A, MLL-B, MLL-C, MLL-D, MLL-E, and MLL-F (Nibouche et al. 2018). In 2018, it was suggested the lineage of MILL-F is the current pest that is threatening the sorghum industry in the United States (Nibouche et al. 2018). If other genotypes develop, there will be a need to quickly develop resistant plant lines. The mechanism of resistance and resistant sources, especially of sterile lines, is needed.

Objectives:

1) Determine the effects of artificial lights on sorghum physiology and interactions with aphid pests
2) Determine if sterile lines of sorghum exist that show resistance to sugarcane aphid feeding to improve sorghum breeding programs
3) Test aphid life history traits when aphids are raised alone or in groups

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CHAPTER II

Evaluation of A3 cytoplasmic sterile sorghum germplasm for resistance to sugarcane aphid

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the top five cereal crops produced worldwide (Mundia et al. 2019). The uses of sorghum range from feed and forage for livestock as a water-saving alternative to corn maize (Bean et al. 2013), a source for biofuel (Miron et al. 2007), syrup production as an alternative sweetener (Mercer et al 2011), alcohol fermentation (Mercer et al 2011, Maw et al 2017) and grain for human consumption (Anjali et al. 2017, Mundia et al. 2019).

Grain sorghum production in the United States has been impacted by the sugarcane aphid (SCA), *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) across vast acreages beginning in the summer of 2013 (Armstrong et al. 2015, Bowling et al. 2016, Elliott et al. 2017) but was known to exist in Florida by 1977 (Denmark, 1988) and identified on sugarcane in Louisiana in 1999 (White et al. 2001). In 2013, *M. sacchari* infested and reduced yield on sorghum crops in Liberty County South Texas (Bowling, et al 2016). Since the initial reports of damage in sorghum in 2013, the aphid has rapidly expanded its range (Kerns et al. 2015; Bayoumy et al. 2016) and it now colonizes 20 states annually across the sorghum belt.

Sugarcane aphids have been found colonizing and reproducing on Sudan grass, *Sorghum verticilliflorum* (Steud.), Johnsongrass, *Sorghum halepense* (L.), Columbus grass, *Sorghum almum* (Parodi), Sugarcane (*Saccharun officinarum* L.) and Sorghum, *Sorghum bicolor* (L.) (Hall 1987, White et al. 2001, Armstrong et al. 2015, Medina et al. 2016). Sugarcane aphids have also been observed on corn *Zea maize* (L.) and cotton *Gossypium hirsitum* (L.), but no survival and reproduction were observed (Bowling, et al 2016). The aphid overwinters in northern Mexico and south Texas on remnant sorghum

and Johnsongrass (Bowling, et al 2016) with the lower and upper threshold for fecundity estimated to be 9 and 32°C (De Souza et al. 2019).

Sugarcane aphids collected from throughout the U.S. were phenotype and genotyped and determined to be two biotypes: SoSCA, the sorghum preferred sugarcane aphid, and SoSCA, the sugarcane preferred sugarcane aphid (Paudyal et al. 2019). The two biotypes differ in genotype and differ in survival and reproduction when reared on a set of host plant differentials namely resistant and susceptible grain sorghums, Johnsongrass, *Sorghum halepense* (L.), Columbus grass, *Sorghum almum* (Parodi), and sugarcane *Saccharum officinarum* (L.). The two different biotypes (SoSCA, SoSCA) were easily differentiated by genotyping (Paudyal et al. 2019).

Within the U.S. the most damaging sugarcane aphid biotype found on sorghums is SoSCA, however. Since its appearance into the U.S., numerous resistant grain sorghums have been developed that express resistance mechanisms including antibiosis, tolerance, combinations of the two, and Antixenosis (Paudyal et al. 2018, Paudyal et al. 2020). Feeding by *M. sacchari* (SoSCA) to sorghum causes reduced plant height and plant biomass (Limaje et al 2018, Backoulou et al 2018), uneven growth of seed heads that may not produce grain from injury caused during anthesis (Rott et al. 2008), and in some cases, death of the plant (Bowling et al. 2016).

The outbreak of *M. sacchari* in sorghum in 2013 initiated research to develop integrated pest management (IPM) options for the aphid. Host plant resistance to sugarcane aphid in sorghum germplasm has been identified in both commercial and parental breeding lines (Armstrong et al. 2015, Armstrong et al. 2017, Armstrong et al. 2018, Paudyal et al. 2018, Limaje et al. 2018, and Gonzales et al. 2019). Several sources

were first identified from seedling screening in the greenhouse, followed by field evaluations and then breeding efforts for registration and release. In 2016, Tx 3408 and Tx 3409 were registered and released as seed parental lines developed and released by Texas AgriLife Research with sterile counterparts developed using the A1 cytoplasmic male sterility system (A1 CMS) (Mbulwe et al. 2016). In 2018, Peterson (et al. 2018) continued with the release of nineteen lines RTx3410 through RTx3428 male of steriles produced using the A1 CMS system.

Later in that same year, Hayes (et al. 2018) registered and released an additional two lines R.LBk1 and R.LBk2 from the USDA-ARS Breeding program in Lubbock, TX; both R.LBk1 and R.LBk2 were produced using the A1 CMS system. In terms of forage sorghum breeding, industry commonly uses a small set of public seed parents (A/BTx623, A/BTx631, A/BTx378) for the production of forage and Sudan grass sorghum hybrids (Rooney et al. 2011, Armstrong et al. 2017). These females are widely adapted and high yielding but are not resistant to SoSCA.

Therefore, unless the forage pollinator parent is SoSCA resistant, the hybrid generated between the two inbreeds will also be SoSCA susceptible because SoSCA resistance is a dominant genetic trait (Hayes et al., 2018). One of the first resistant sources discovered for sugarcane aphid resistance was TX 2783, initially developed for greenbug C and E resistance (Peterson et al. 1984) with the dominant resistant trait originating from Capbam, a sorghum germplasm introduced from Russia, and SC110-9 a parent of TX 2783 (Peterson et al. 1984).

To broaden the genetic sources of SoSCA resistant sterile sorghum, the USDA sorghum breeding program in Lubbock, TX recently sterilized three pollinator lines (TX

2783, R.LBK1, R.LBK2) in the A3 cytoplasm for the development of SoSCA resistant forage sorghums. Our research evaluated the sterile lines of A3.TX 2783, A3 R. LBk1 and A3 R.LBK2 to determine if the resistant trait to sugarcane aphid was maintained as it is in the fertile counterparts. The purpose of this research was to confirm SoSCA resistance in sterile sorghums developed in a sterilization backcrossing program.

Materials and Methods

Sugarcane aphid resistance and sterile sorghum background In 2017, two USDA sorghum lines R. LBK1 and R. LBK2 were identified as having tolerance and antibiosis to the sugarcane aphid (Limaje et al. 2018) and were registered and released (Hayes et al. 2018). Both R. LBK1 and R. LBK2 were developed using the pedigree method of plant breeding and are confirmed to be restorer lines. R. LBK1 has a pedigree of (SC56-14E/(86EO361/88BE2668)) and was originally tested as R.11259. SC56-14E is a fully converted caudatum landrace derived from IS12556 with good stay-green drought tolerance. 86EO361/88BE2668 is a line developed by and obtained from Texas AgriLife Research. The pedigree of 86EON361 is (R5646/SC326-6) and the pedigree of 88BE2668 is (Tx2783/(SC748/SC630)). R.LBK2 has a pedigree of (Tx2783/PI 567946) and was originally tested as R.11143. TX 2783 was released by Texas A&M AgriLife Research in 1984 (Peterson et al., 1984).

The pedigree of TX 2783 is complex (IS12610C/((((ROKY8/Tx2536)/SC110-9)/SC599)/SC110-14E)) and was originally selected for resistance to biotypes C and E greenbug, *Schizaphis graminum* (Rondani). TX 2783 has also been found to be cross-resistant to the sugarcane aphid (Armstrong et al., 2015, Armstrong et al. 2017, Armstrong et al. 2018). Our objective here was to use the A3 cytoplasmic male sterile

method (Howad et al. 1999) of sterility on R. LBK1, R. LBK 2 and TX 2783 to allow the incorporation of male steriles into forage sorghums.

Aphid culture A known biotype "SoSCA" of sugarcane aphid that were phenotype and genotyped in 2019 (Paudyal et al. 2019) and maintained as parthenogenic female colony was collected from a post harvested grain sorghum field near Bay City, Matagorda County Texas in August of 2013. This colony has been maintained at the USDA-ARS Stillwater, OK Laboratory by rearing them on susceptible TX 7000 sorghum seedlings in pots covered with sleeve cages in the greenhouse at temperatures ranging from 21°C and 28°C. The plants are grown under natural greenhouse light supplemented by two T-8 fluorescent lights. New sugarcane aphid colonies are transferred to new seedling plants every 2 weeks in the greenhouse to maintain viable colonies for experimentation.

Sorghum resistance trials for male sterile counterparts

Nine sorghum entries, including two known SoSCA resistant sorghums TX 2783 and DKS-3707 (Paudyal et al. 2018), and two known susceptibles TX 7000 and KS 585 (Paudyal et al. 2018), were evaluated in a free-choice flat screen trial. Also included were sterile derivatives of TX 2783 labeled A3. TX 2783, R. LBK1 / A3. R. LBK1, R. LBK2 / A3 R.LBK2, and R. LBK2. The sorghum entries were planted in 8 flats (plastic trays 60 cm x 90 cm with 128 individual cells, Growers Supply, Dyersville, IA 52042). Each entry was randomized and replicated 12 times using Research Randomizer (http://www.randomizer.org, 2020).

Four of the eight flats were used for infesting, while a duplicate set of 4 flats were not infested for comparing plant growth characteristics. When the TX 7000 sorghums seedlings used for infesting were in the 4-5 leaf stage (approximately 20 cm in height) they were laid down each row and across each alley of the flats as reported by Starks and Burton (1977). By this procedure, all entries are placed under strong pressure from the infesting aphids so that no ambiguity exists in the evaluation.

The measured variables for infested and non-infested sorghums were plant height (cm), measured at the end of the trial from the surface of the soil, the number of formed leaves on the plant, excluding the lower cotyledon leaf, and difference in plant height between infested and uninfested plants. Difference in plant height is measured by subtracting an infested sorghum versus the same entry which is not infested and is more realistic in determining what the reduction in plant growth may have been due to aphid feeding. Total chlorophyll content (chlorophyll a + b, (Markwell et al. 1995)) measured as µmol m^{-2} was estimated using a SPAD-502 chlorophyll meter (Minolta, Ramsey, NJ 07466). Three Chlorophyll readings were taken from each entry that was infested and subtracted from the non-infested entries so that the percent loss of total chlorophyll was calculated (C-T)/C*100, where C is the SPAD measurement from the non-infested or control, and T is from infested plant. When the known susceptible Tx7000 was 90-100% dead based on the 16 replications of that entry, all plants in each flat were evaluated for damage using a rating of 1-9; where 1 is a completely healthy plant with no chlorotic 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, represents 95-100% chlorotic tissue (Burd et al. 1993).

The variables of damage rating, plant height, difference in plant height, number of true leaves on a sorghum entry, and percent chlorophyll loss were subjected to PROC MIXED model analysis with sorghum entry means compared ($\alpha = 0.05$) using the least-squared means pair-wise comparisons at $P > |t| \leq 0.05$ level (SAS 9.4, SAS Institute, 2016). This experiment was evaluated on December 19, 2020 and repeated on February 24, 2021 to check for consistency in results.

Sugarcane aphid demographics compared for male sterile sorghum counterparts

The reproductive life-table demographics of the SoSCA were compared for the male fertile TX 2783, R. LBK1 and R.LBK2 lines versus the sterile male counterparts A3.Tx2783, A3.R.LBK1, A3.R.LBK2. Also included for comparative purposes were the SoSCA resistant DKS-3707, and the SoSCA known susceptibles TX 7000 and KS 585. A negative effect on the reproductive capacity of an aphid infesting a plant in a no-choice environment determines the expression level of antibiosis (Smith, 2005).

For the evaluation of antibiosis, two seeds of each entry listed above 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. Each cone-tainers™ seeded with an individual entry was considered one of 12 replicates, representing a total of 108 individual containers. Each cone-tainers™ was fitted with an 8 cm diameter Lexan sleeve, 45 cm in height and ventilated with organdy cloth. The cone-tainers™ were placed in a rack to hold them upright in a completely randomized design inside a growth chamber Conviron®, Winnipeg, Canada) set at 21° C and 14:10 L:D photoperiod with lighting provided by seven TS 32W Ecolux® daylight fluorescent lamps (Fairfield, Connecticut, USA) and four 60W incandescent bulbs. This model

of growth chamber is divided in two identical sections. Wherein one section, entries were challenged with SoSCA, while an identical set of entries that were not infested grew in the other section.

When the sorghum entries reached the two-leaf stage or 4-6 cm in height, the most vigorous plant was kept, whereas the other was removed. Remaining seedlings were infested by a single viviparous female which was removed after 24 h. From these nymphs on each entry, a single, 24h old, nymph per seedling was selected to remain on the nine different sorghum entries where the development time to reproductive adult (*d*) and net reproduction *(Md*), female longevity (*L*), and reproductive period (days in reproduction) was recorded. Intrinsic rate of increase (*rm*) was calculated using the formula: r_m=.0738(1*ogeM*$_d$)/*d* (Wyatt and White, 1977). All reproductive life parameters were analyzed using Mixed model analysis (PROC MIXED, SAS Institute 2016) where mean comparisons were made by using the Least Significant Differences Method (LSD) at P > l*t*l ≤ 0.05 level (SAS 9.4, SAS Institute, 2016).

Results & Discussion

Sorghum resistance trials for male sterile counterparts – experiments 1 & 2

There were varying degrees of damage caused by SoSCA feeding on the 9 sorghum entries (Table 1). The two known fertile/susceptible entries KS 585 and TX 7000 were heavily damaged, significantly more than any of the other fertile or sterile counterparts. The fertile LB k1 2019 Hein 8236 and its sterile counterpart A3 LB k1 2019 Hein 8235 were statistically similar and separated from all other entries with damage

scores of 6.8 and 5.5 respectively. The remaining steriles (A3 TX 2783, A3. R. LBK2) and fertile counterparts sustained ratings considered to be resistant and were not significantly different from the fertile TX 2783; however, the DKS-37-07 sustained the lowest damage rating of 2.9 and had the highest level of tolerance in the first evaluation. The susceptible KS 585 sustained 100% loss in chlorophyl followed by 57.5 % loss for the TX 7000 and 50.5 % for the R. LBK. All other entries had < 40% loss in chlorophyll content with A3 R. LBK2 losing 36.3% and DKS 37-07 having 21.2 % loss . Differences in plant height, a variable measured to determine the effects of SoSCA on plant growth within an entry was highest for the fertile/susceptibles KS 585 and TX 7000, with a reduction of 17.4 and 18.3 cm respectively (Table 1).

All other entries were reduced from 12 cm in height for the R. LBK1, to 9.0 cm in height for DKS-37-07. SOSCA feeding also affected the number of true leaves formed over the duration of the trial. The KS 585 and TX 7000 had the fewest true leaves with a mean of 2.1 each (Table 1). In contrast, the R. LBK1, A3 R. LBK1, and A3 TX 2783 had means of at least2.5 and leaves and did not differ when compared to the KS 585 and TX 7000. All remaining sorghum entries A3 R. LBK2, R. Lbk2, TX 2783, and DKS 37-07 had > 3.5 true leaves per plant.

The results of the second trial for damage rating, chlorophyl loss, difference in plant height and number of true leaves were similar to the first trial (Table 2). A notable difference from the first trial was that the A3 LB k1 2019 Hein 8235 had a damage rating of 5.5 as compared to 4.5 in the second trial (Tables 1 & 2).

Demographics of sugarcane aphid on fertile and sterile counterparts

There was a wide numerical difference in the reproductive response for fecundity, nymphs produced /d, and the intrinsic rate of increase when sugarcane aphids fed on fertile susceptible, compared to when they fed on resistant fertile and the sterile counterparts of resistant fertile entries (Table 3). The fertile susceptible KS 585 produced 152 ± 12.2 nymphs, which was significantly greater than TX7000 which produced 131.9 ± 8.5 nymphs. The R. LBK1 produced 60.0 ± 5.1 nymphs, followed by A3 LBK 1 2019 H8235 that produced 40.5 ± 3.6 nymphs. All other entries including the A3 TX 2783, A3. LBk2 2019 H8237, LBk2 2019 Hein 8238, and the fertile TX 2783 resulted in total fecundity (*Md*) of less than 35 nymphs, while DKS 37-07 had the lowest with on average 13 nymphs produced. The expression of antibiosis was also evident in the number of nymphs produced per d, and for the intrinsic rate of increase. The nymphs produced per d were > 5.0 for the two fertile susceptibles KS 585 and TX 7000, and < 3.0 for the remainder of the entries. The intrinsic rate of increase (*rm*) was significantly higher for the KS 585 and TX 2783 followed by decreases starting with the R. LBK1 at 0.30, down to 0.19 for the DKS 37-07 (Table 3).

Antibiosis, a factor in the plant that reduces aphid reproduction and survival, was also indicated as shown by longevity and reproductive period (Table 4). The SoSCA founding female longevity was 28-d for the fertile susceptibles KS 585 and TX 7000, followed by a 6-d decrease in longevity for the R. LBK1, and reduced to 5.5-d for the known resistant DKS 37-07. The reproductive period (*d*) followed the same pattern as

longevity where the fertile susceptibles KS 585 and TX 7000 survived the longest at > 26-d, followed by a decline starting with R. LBK1 at 22-d, down to 5.5-d for DKS 37-07.

These evaluations for SoSCA resistance showed from the free-choice flat screens that tolerance existed in all of the fertile and sterile counterparts when compared to the known fertile/susceptibles KS 585 and TX 7000. The fertile R. LBK1 that was derived from R.11259 (Hayes et al. 2018) was the least tolerant in terms of damage ratings and other plant measurement factors such as chlorophyl loss, difference in plant height, and numbers of true leaves, and was similar to previous results (Limaje et al. 2018). The fertile R. LBK2 was derived from pollinator R. 1443 (Hayes et al. 2018) and was very similar in tolerance to TX 2783 with damage ratings in the 3's on the 9-point rating scale (Limaje et al. 2018). The sterile counterpart of A3 R.LBK2 was just as tolerant as the fertile R. LBK2 and appears suitable for use in development of SoSCA resistant forage sorghums.

Antibiosis was also present and expressed in the fertile and sterile counterparts evaluated, and reduced fecundity by over 2-fold for the R. LBK1 to greater than 3.8-fold for all other entries.

In conclusion, the sterile counterparts developed using the A3 cytoplasmic male sterile system were as tolerant as known resistant varieties and expressed antibiosis that was comparable or better than their fertile counterparts TX 2783, R. LBK1 and R. LBK2.

Tables

Table 1. Mean (± S.E.) sorghum damage ratings, chlorophyll loss, and difference in plant height for sugarcane aphids reared on A3 cytoplasmic sterile lines compared to known fertile susceptible and resistant sorghums.

Germplasm	Sterile/ Fertile	Damage Rating (1-9) Scale[a]	Percent Loss Chlorophyll Content[b]	Reduction in Plant Height (cm)[c]	Number of true leaves [d]
KS 585	fertile	9.0 ± 0.0 a	100.0 ± 0.0 a	17.4 ± 1.3 a	2.1 ± 0.1 b
TX 7000	fertile	8.5 ± 0.2 a	57.5 ± 2.2 a	18.3 ± 1.0 a	2.1 ± 0.3 b
R. LBK1	fertile	6.8 ± 0.5 b	50.5 ± 4.0 c	12.0 ± 1.5 b	2.5 ± 0.2 b
A3 R. LBK1	sterile	5.5 ± 0.8 bc	36.3 ± 5.7 cd	9.4 ± 1.7 de	2.6 ± 0.2 b
A3 TX 2783	sterile	4.3 ± 0.8 c-d	34.0 ± 8.8 d	13.6 ± 2.8 bc	2.5 ± 0.3 b
A3. R. LBK2	sterile	3.9 ± 0.7 d	30.9 ± 2.0 d	14.4 ± 1.4 bc	3.5 ± 0.1 a
R. LBK2	fertile	3.4 ± 0.5 d	31.7 ± 5.3 d	12.8 ± 1.2 d	3.6 ± 0.2 a
TX 2783	fertile	3.3 ± 0.4 d	32.4 ± 2.8 d	10.8 ± 0.9 d	3.5 ± 0.2 a
DKS- 37-07	fertile	2.9 ± 0.2 e	21.2 ± 3.0 d	9.0 ± 1.1 d	3.7 ± 0.1 a

Column means followed by the same lower-case letters are not significantly different $P < 0.05$; LSD.

[a] Damage ratings evaluated on a 1 – 9 scale, df = 8, 103; $F = 23.6$; $P > F = <0.001$.

[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 = 8, 103, $F = 8.1$, $P = <0.001$.

[c] Mean difference in plant height, (controls – infested), df = 8, 103, $F = 4.0$; $P = <0.001$.

[d] Mean number of true leaves per plant, df = 8, 103, $F = 12.9$; $P = <0.001$.

Table 2. Means (± S.E.) sorghum damage ratings, chlorophyll loss, and difference in plant height for sugarcane aphids reared on A3 cytoplasmic sterile lines and compared to fertile known susceptible and resistant sorghums.

Germplasm	Sterile/ Fertile	Damage Rating (1-9) Scale[a]	Percent Loss Chlorophyll Content[b]	Reduction in Plant Height (cm)[c]	Number of true leaves [d]
KS 585	fertile	9.0 ± 0.0 a	100.0 ± 0.0 a	22.2 ± 1.0 a	2.6 ± 0.1 cd
TX 7000	fertile	9.0 ± 0.0 a	59.0 ± 1.6 b	21.2 ± 1.0 a	2.3 ± 0.1 d
R. LBK1	fertile	6.5 ± 0.5 b	52.6 ± 4.0 b	8.7 ± 1.5 cd	2.6 ± 0.2 bc
A3 R. LBK1	sterile	4.5 ± 0.6 bc	40.0 ± 5.7 c	11.2 ± 1.0 bc	2.9 ± 0.1 bc
A3 TX 2783	sterile	3.8 ± 0.5 cd	27.4 ± 6.1 d	12.8 ± 1.5 b	3.3 ± 0.1 ab
A3. R. LBK2	sterile	2.8 ± 0.3 de	24.8 ± 2.3 d	10.0 ± 0.7 bc	3.6 ± 0.1 a
R. LBK2	fertile	3.2 ± 0.4 d	26.0 ± 4.1 d	18.5 ± 0.7 bc	3.6 ± 0.2 a
TX 2783	fertile	3.5 ± 0.6 cd	32.0 ± 3.4 cd	12.5 ± 1.9 b	3.6 ± 0.1 b
DKS- 37-07 (known res.)	fertile	2.8 ± 0.2 e	31.2 ± 2.2 d	5.8 ± 1.0 d	3.2 ± 0.1 a

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, df = 8, 107; $F = 41.9$; $P > F = < 0.001$;

[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 = 8, 99, $F = 42.2$, $P = <0.001$.

[c] Mean difference in plant height, (controls – infested), df = 8, 99, $F = 20.4$; $P = <0.001$.

[d] Mean number of true leaves per plant, df = 8, 107, $F = 11.9$; $P = <0.001$.

Table 3. Demographic statistics for sugarcane aphid reproduction when reared on A3 cytoplasmic sterile lines and compared to fertile known susceptible and resistant sorghums.

Germplasm	Sterile/ Fertile	Fecundity (*Md*)	Nymphs/ ♀/ d	Intrinsic Rate of Increase (*rm*)
KS 585	fertile	152.9 ± 12.2 a	5.1 ± .10 b	0.41 ± 0.01 a
TX 7000	fertile	131.9 ± 8.5 b	5.5 ± .12 a	0.40 ± 0.01 a
R. LBK1	fertile	60.0 ± 5.1c	2.8 ± .07c	0.30 ± 0.01 b
A3 R. LBK1	sterile	40.5 ± 3.6 d	2.3 ± .11 d	0.27 ± 0.01 c
A3 TX 2783	sterile	34.5 ± 1.7 e	2.2 ± 0.78 de	0.26 ± 0.02 d
A3 R. LBK2	sterile	29.5 ± 2.2 ef	2.1 ± .09 de	0.25 ± 0.03 de
R. LBK2	sterile	26.4 ± 2.2 f	2.0 ± .12 e	0.24 ± 0.02 e
TX 2783	fertile	26.4 ± 6.2 f	2.2 ± .08 de	0.24 ± 0.01 e
DKS- 37-07	fertile	13.0 ± 2.2 g	2.3 ± .08 de	0.19 ± 0.02 f

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD.
Fecundity (*Md*) = sugarcane aphids/female, 12 replications, df = 8, 106, F = 569.9; $P > F = < 0.0001$
Nymps/♀/d; = (*Md*/d), df = 8, 106, $F = 197.5$, $P > F = < 0.0001$. *rm* = intrinsic rate of increase, rm = 0.738(ln *Md*/d); df = 8, 106, $F = 197.05$; $P > F = < 0.0001$.

Table 4. Mean longevity and reproduction for sugarcane aphid when reared on A3 cytoplasmic sterile lines compared to fertile known susceptible and resistant sorghums.

Germplasm	Sterile/ Fertile	♀Longevity (d)	Reproductive Period (d)
KS 585	fertile	27.5 ± 0.44 a	26.1 ± 0.50 a
TX 7000	fertile	28.5 ± 0.38 a	26.9 ± 0.41 a
R. LBK1	fertile	22.5 ± 0.34 b	21.8 ± 1.01 b
A3 R. LBK1	sterile	18.4 ± 0.24 d	15.7 ± 0.74 c
A3 TX 2783	sterile	15.9 ± 0.49 e	13.4 ± 0.70 d
A3. R. LBK2	sterile	14.8 ± 0.52 ef	12.5 ± 0.77 d
R. LBK2	fertile	13.8 ± 0.34 fg	11.7 ± 0.51 d
TX 2783	fertile	13.2 ± 0.24 g	11.8 ± 0.52 d
DKS- 37-07	fertile	8.5 ± 0.69 h	5.5 ± 0.53 e

Column means followed by the same lowercase letters are not significantly different, $P > 0.05$; LSD.
Female Longevity (d), df = 9, 110, $F = 186.6$; $P > F = <0.0001$.
Reproductive period (d) df = 8, 106, $F = 122.1$; $P >$ F = < 0.0001.

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CHAPTER III

LED and conventional lighting affect sorghum physiology and sugarcane aphid interactions differently

Introduction

Light is essential for plant growth and development and can be provided to plants by natural sunlight or by other means such as incandescent, fluorescent or light emitting diodes (LEDs). In horticulture, high value plants can be grown under optimal environmental conditions, including altered light spectra and altered light cycles (Mukish et al. 2017). In addition, light sources that conserve energy are longer lasting and can be easily integrated into digital systems that are in high demand such as those found in greenhouses and growth chambers.

The processes for photosynthesis are highly dependent on light characteristics. Wavelengths, light duration, and light intensity all combine to affect plant growth and health. Light can also cause stress on plants. For example, when light intensity is high and plants face other abiotic stressors, the plants can exceed the requirement for metabolic processes in carbon fixing reactions where photosynthesis production decreases plant growth and development is slowed or ceases (Miyake et al. 2009; Gu et al. 2017; Bayat et al. 2018). Previous studies have documented species-specific light stress (Hogewoning et al. 2010; Nanya et al. 2012; Cope & Bugbee 2013), among plants with C3 and C4 photosynthetic pathways.

Light-emitting diode (LED) technology has been at the forefront of horticulture and greenhouse production because of its improved photosynthetic delivery of specific light spectrum and significantly reduced energy cost (Hogewoning et al. 2007; Massa et al. 2008; Trouwborst et al. 2010). Light emitting diodes are the first artificial light source

where the light emitting spectrum is controlled mostly under the blue and red spectra (Morrow 2008) and unlike conventional lights, have low surface operating temperatures.

A growing body of literature has examined the effects of LED lights on various plants. C3 plants have generally been found to perform well under LED lighting. Studies of C3 plants have included tobacco *Nicotiana tabacum* (Solanaceae), spinach *Spinacia oleracea* L. (Amaranthaceae), radish *Raphanus raphanistrum* L. (Brassicaceae), lettuce *Lactuca sativa* L. (Asteraceae) and strawberry *Fragaria* L. (Brown et al 1995; Yorio et al. 2001; Nhut et al. 2003). In addition to successful growth characteristics, lettuce *Lactuca* grown under blue light LEDs had higher antioxidant activity in in addition to enhanced growth of the seedlings (Johkan et al. 2010).

Tobacco is a C3 plant that also has a C4 pathway and has shown positive reactions to LEDs (Jun et al 2014). LED lights were found to promote growth and reduce the membrane lipid peroxidation damage of the plant (Jun et al 2014). Similarly, when wheat, Triticum aestivum L. which is a C4 plant, is grown under LEDs, it produces more tillers, biomass, yield and shows an overall increase in photosynthetic activity (Monostori et al. 2018; Casati et al., Spampinato & Andreo 1997). In contrast, Limaje et al. (2019) found that sorghum had altered plant morphology and reduced biomass when grown under a LED grow panel lights compared to the same cultivars of sorghum, *Sorghum bicolor* (L.) Moench, grown under conventional light sources.

Sorghum is a C4 plant that is grown in semi-arid parts of the world where droughts are common. This plant is grown as food for human consumption, silage for livestock, to make bio-fuel, and as a cover crop (Miron et al 2007, Bean et al. 2013, Pino

and Heinrichs 2017, Anjali et al. 2017). Researchers and breeders often develop S. bicolor (L.) cultivars in greenhouses where agronomic and breeding experiments are conducted under controlled environmental conditions (Armstrong et al. 2015, Paudyal et al. 2019).

Although much research has been conducted to examine plant response to LED lighting, less research has examined plant-insect interactions under LED lights. Rechner et al. (2016) examined cabbage aphids *Brevicoryne brassicas* and green peach aphids *Myzus persicae* (Sulzer) grown on broccoli (*Brassica oleracea* var. italica) under LED lights. The specialist cabbage aphids had decreased performance while the generalist green peach aphid had increased performance (Rechner et al. 2016) under LEDs suggesting that the plant defenses were affected. Limaje et al. (2019) showed that interactions between sugarcane aphid, *Melanaphis sacharri* Zetner, and resistant and susceptible sorghum cultivars differed by lighting conditions with aphids exhibiting altered behaviors under LED lighting. This study examined the effects of standard 9 band LED lights on sorghum morphology and physiology and interactions between known susceptible and resistant cultivars and the sugarcane aphid.

Materials and Methods

APHID CULTURE

Sugarcane aphids were originally collected from Matagorda County, Texas, in 2013 from infested grain sorghum. The colony is maintained as parthenogenic clones on susceptible TX 7000 sorghum seedings at the USDA-ARS Stillwater, OK Laboratory. Susceptible seedlings are used to maintain sugarcane aphids in pots covered with sleeve

cages in the greenhouse where the temperatures ranged between 21° C and 31° C. The clonal sugarcane aphids are transferred to fresh new susceptible seedlings every week in the greenhouse in order to a continual supply of live colonies. The colony plants and aphids are grown under natural greenhouse light that is supplemented with two T-8 fluorescent lights. The supplemented lights are on timers so that the lights come on at 6am CST and turn off at 8pm CST.

SORGHUM ENTRIES AND CULTURE

Four sorghum cultivars were used in this experiment; susceptible cultivated variety KS 585, susceptible parental line variety TX 7000, resistant parental variety TX 2783 and resistant variety DKS 37-07 (Paudyal et al. 2019). All genotypes were planted in Cone-trainers (model SC10; S7S Greenhouse Supply, Tangent, OR). Each Cone-tainer™ was filled with a three layer system of different potting media from the bottom up: 120g potting soil, 60g fitting clay and 30g of sand. Each Cone-tainer™ was housed in an 8-cm diameter Lexan sleeve with a height of 45 cm, which was ventilated with organdy cloth. Both un-infested (Control) and infested plants were planted and sleeved the same way.

Initially, two seeds of each genotype were planted at a depth of 2cm in the Cone-tainer™. The seedlings were grown under 2 T-8 fluorescent lighting (16/8 h L:D) and at 25°C. One week after planting the seedlings were thinned to one seedling per Cone-tainer™. One day after thinning, the plants were transferred from the greenhouse to the growth chambers. All plants where fertilized with Miracle -Gro Garden feeder at the recommend rate of 1 tablespoon per gallon.

Fourteen days after planting, when plants were at the four-leaf stage, the sorghum seedlings where infested with 10 adult sugarcane aphids per plant. All aphids where the same age when put on the plant. To ensure that all aphids where the same age, adult aphids from the main colony were put on extras of the 4 different genotypes and allowed to reproduce for 12 hours. After 12 hours, adult aphids were removed from each plant leaving only the nymphs. After the nymphs reached 7 days of age, they were transferred to the test genotypes in the growth chambers. Thus, all 10 aphids infested per plant were the same age and been reproduced and grown on the sorghum genotype they were to infest.

There were 12 replicates of each genotype for the 4 treatments: LED grow lights with aphids, LED grow lights with no aphids, conventional lights with aphids and conventional lights with no aphids. In total there were 192 sorghum seedlings used in this study. Plant physiological responses were recorded 15 days after infestation of the aphids. All aphids were removed before measurement of the response variables.

GROW CHAMBERS

Four identical growth chambers that provide temperature, light, and humidity control (Percival Scientific, model E30BC8, Perry, IA 50220) were used in this study. Two of the growth chambers were maintained as originally fitted with two Philips (model 7866113, Philips Inc. Guadalajara, Jalisco, Mexico) fluorescent grow lights, and two clear 40-watt appliance lights (Sylvania, Wilmington, Massachusetts, USA). The other two chambers were fitted with 9 band 60w LED grow panels mounted in the top where the conventional lights were originally affixed. The LED lights had input voltage of

85~265V. The power was 600w with a LED Configuration of 288 PCSX3W and 9 bands. The light intensity within the growth chambers lit by conventional lights and the LED panels were measured with a LI-CO light meter (model LI-250, LI-COR, Lincoln, Nebraska, USA). The LED light spectra were measured using a Liconix Model 45PM å

PLANT RESPONSE MEASURES

All plants used in the experiment were examined at 15 days post-infestation. Plant height was measured from the base of the soil line to the longest leaf tip in cm. The number of true leaves was recorded, and the maximum leaf width was measured at the widest point on the widest leaf on the plant.

When the known susceptible hybrids KS 585 and TX 7000 were 90-100% dead, all plants in Cone-tainer were evaluated for damage using a rating of 1-9 (Webster et al. 1990; Burd et al. 2006). In the damage rating scale, 1 is a completely healthy plant with no necrotic tissue; 2 represents 1-5% chlorotic tissue; 3 represents 5-20%; 4 represents 21-35%; 5 represents 36-50%; 6 represents 51-65%; 7 represents 66-80%; 8 represents 81-95%; and 9 represents 95-100% chlorotic tissue or a dead plant.

To quantify chlorosis, a chlorophyll meter (model SPAD-502, Minolta Camera Co., Osaka, Japan) was used to measure chlorophyll content. The chlorophyll meter absorbs light at wavelengths between 430 and 750 nm and provides an estimate of chlorophyll content in the leaf (Wood et al. 1992). Three separate readings from different leaves were taken from each plant. A SPAD chlorophyll index was calculated with the mean SPAD reading for each plant based on the formula: $(C - T)/C$ (Deol et al. 2001)

where C is the SPAD measurement from the control and *T* is the SPAD measurement from infested plants.

GAS-EXCHANGE RESPONSES

A portable photosynthesis system (model LI-6400, LI-COR, Lincoln, NE) was used to measure the photosynthetic responses of all plants in the study. Methods followed closely those of Franzen et al. (2007), Gutsche et al. (2009) and Paudyal et al. (2020). Measures with the Li-COR 6400 where taken outside on a sunny day (day 15) at approximately 25 °C. Readings were taken after the plants were acclimatized for > 1 h.

Measures were taken from a 6-cm^2 area using one or two leaves. When two leaves were used, care was taken to make sure that the two leaves did not overlap. The parameters that were measured where photosynthetic rate (μmol CO 2 m−2 s−1) and stomatal conductance (mol H_20 m^{-2} s^{-1}). The IRGA light intensity was 1200 umol photos m^{-2} s^{-1} and a reference CO_2 concentration of 400 ppm was used. The stomatal conductance was calculated as the rate at which water vapor changed.

DATA ANALYSIS

All analyses were performed using SigmaPlot 11.0. Response to light conditions and to the presence of aphids were examined within cultivar. Significance was judged when $P < 0.05$. Data were first checked for normality and then were compared using ANOVA, followed by a Tukey test when differences were detected. When normality assumptions were not met, a Kruskal Wallis ANOVA was conducted to compare median values followed by a Tukey test when differences were detected. Whether ANOVA or Kruskal-Wallis ANOVA was performed, means $\pm$ 1 S.E. are presented in results.

Results

The LED panel produced two primary emissions that were centered near 450 nm (blue) and 636 nm (red). Both emission peaks had similar widths, with full width at half maximum (FWHM) values of approximately 100 nm and 120 nm for the blue and red emissions, respectively

(Fig. 1). Light intensity measures were 200.9 μmol (15 s avg.) with the quantum sensor and 8,028 lux (15 s avg.) with the photometric sensor for conventional lights and 2111.0 μmol (15 s avg) with the quantum sensor and 48.63 lux (15 s avg.) with the photometric sensor.

Sorghum cultivars grown under conventional light differed morphologically from those grown under LED lights both as controls and when exposed to aphid feeding (Figs. 2 and 3). Plants from all cultivars were tallest when grown under conventional light and were approximately twice as tall as the same plants grown under LED lights (Table 1). When exposed to aphid feeding under conventional lights, plant heights were reduced most for the susceptible cultivars, TX 7000 and KS-585 (30% and 19% respectively) compared to the plant heights of the resistant cultivars, TX 2783 and DKS 37-07 (approximately 7% each).

However, when the plants were grown under LED lights and exposed to aphid feeding, plant heights were significantly increased for both resistant cultivars when exposed to aphid feeding compared to being growing under LED lights alone (Table 1). In contrast, the susceptible variety TX 7000 was shortest overall under LED lights with aphids while DKS 37-07 heights were similar for plants under LED lights alone and when exposed to aphids under LED light.

Populations of the sugarcane aphid differed significantly by light type for three of the four tested cultivars (Fig. 4). Surprisingly, aphid numbers were lower on known susceptible cultivars (TX7000 and KS585) compared to populations on the known resistant cultivars (TX2783 and DKS37-07). Susceptible plant health declined over the 15 day trial and aphid survival diminished. Susceptible plants grown under LED lights had the least aphids because plants could not maintain the aphids. For both resistant cultivars, the numbers of sugarcane aphids increased significantly under LED lights (Fig. 3) reaching more than 300 per seedling for DKS37-07.

All four sorghum entries, both resistant and susceptible to sugarcane aphids produced more true leaves when grown under LED lights by a factor of 2 as for the same entries grown under conventional lights (Table 1). Aphid feeding reduced the number of leaves by an average of 1 to 3 under conventional lights and by 3 to 5 under LED lights; however, the change in leaf number was not significant. Under LED lights, all sorghum cultivars produced wider leaves. Infestation with aphids significantly reduced leaf width under both conventional light and LED light although the leaf width of DKS 37-07, a susceptible cultivar changed least (Table 1).

When sorghum plants were rated for damage, all cultivars were healthy when grown under conventional lights. Aphid feeding significantly increased damage ratings for all cultivars except DKS-37-07, a known resistant variety. Plants grown under LED lights had significantly higher damage ratings and the damage ratings increased significantly when exposed to aphids under LED lights for all cultivars (Table 1).

Damage rating scores for the conventional lighting controls were lower than for the LED control ratings for the susceptible and resistant entries (Table 1), and the same

were true for the infested. Damage ratings were from 1.5 to 4.5 lower for infested conventional lighting as compared to the LED infested damage ratings.

LED lighting reduced the photosynthetic rates of KS-585 (a susceptible variety) and TX-2783 (a resistant variety) but was similar for the other cultivars tested (Fig. 4). As anticipated, infestation with sugarcane aphid significantly reduced photosynthetic rates for the susceptible cultivars but not for the resistant cultivars. For all cultivars tested, including resistant cultivars, infestation with aphids under LED lighting significantly reduced photosynthetic rates (Figure 5).

Similar to observations of photosynthetic response to light type, stomatal conductance was unaffected under LED lights for TX 7000) but was significantly reduced for the other cultivars (Fig. 6). Aphid feeding reduced stomatal conductance for all cultivars except TX 2783 under conventional light.

When the amount of chlorophyll was measured in control plants compared to those exposed to aphid feeding, the resistant cultivars lost about half as much chlorophyll as the susceptible cultivars under conventional lights. All cultivars except KS 585 lost significantly more chlorophyll under LED lighting (Fig. 7). For KS-585, a similar amount of chlorosis was observed for both types of lights while for TX 2783, plants grown under LED light lost 3x as much chlorophyll and nearly as much as the susceptible TX 7000.

Discussion

In this study, four growth chambers were available, and we used two with conventional lights and two with LED lights with one chamber each receiving plants infested with aphids and the other serving as a control, following the methods of Limaje

et al. (2019). This design leads to the possibility of uncontrolled differences among chambers. Ideally, this study would be repeated with replicated chambers to confirm results; however, growth chamber conditions were monitored daily and the chamber receiving each light treatment was randomly assigned. Light measures within chambers were also very similar, supporting the conclusion that our results relate to lighting condition and the presence of aphids.

The LED lighting used in these experiments affected the plant height, number of true leaves, leaf widths and plant physiology (figs. 2 and 3, Table 1) compared to sorghum grown under conventional lighting for both resistant and susceptible cultivars of sorghum. Even though different brands of LED lights were used between the Limaje et al. (2019) study and this one, the light spectra were similar (Fig. 1). Limaje et al. (2019) previously documented unusual sorghum growth and differential response to herbivory by the sugarcane aphid under LED lighting. However, the physiological mechanism for plant differences and determination of consistency of results across cultivars was not elucidated. The light spectra used by Limaje et al. (2019) were different than the one used in the current study, and although results for growth form were similar, sorghum grown under LED lights by Limaje et al. (2019) had unusual colors including purple and pink that were not noted in this study.

In the Limaje et al. (2019) study, similar numbers of sugarcane aphids were observed on sorghum grown under conventional light compared to sorghum grown under LED light. In this study, the light type and sorghum cultivar influenced aphid numbers, reducing aphids on susceptible cultivars under conventional lighting but increasing aphid numbers for resistant varieties under LED lights (Fig. 4). Low aphid numbers on the

susceptible sorghum are likely explained by plant condition. Susceptible varieties had damage ratings of 6 to 8 under conventional and LED lights respectively (Table 1) and likely could not support sugarcane aphid growth and reproduction. In contrast, resistant varieties supported larger numbers of aphids (approximately 100 per plant under conventional lighting). Aphid numbers significantly increased on resistant varieties under LED lights, suggesting that the plant's resistance mechanisms were compromised under the LED lighting conditions (Fig. 4). Despite higher numbers of aphids, the resistant sorghum damage ratings were still lower than those of the susceptible varieties even under LED lights.

Plants grown under LED grew greater numbers of true leaves regardless of being infested or not infested with sugarcane aphid when compared to conventional lights, in some cases 2 to 3 times more true leaves. Damage ratings were increased by 21 and 16 % respectively when the two susceptible sorghums (TX 7000 and KS 585) were grown under LED, infested, and compared to plants grown under conventional lights and infested. Damage ratings for resistant sorghums were 69 and 65 % greater for infested and resistant sorghums TX 2783 and DKS 37-07 respectively. Therefore, both the light source (LED vs conventional) and the known resistant or susceptible sorghum used in the study influenced damage ratings but to a lesser extent for resistant types of sorghum.

When sugarcane aphids were present, both leaf width and number of true leaves were negatively affected as has been observed for many studies where sugarcane aphid damage was assessed in the effort to find host plant resistance (Armstrong et al. 2015, Paudyal et al. 2019, Paudyal et al. 2020) (Table 1). However there exists a confounding factor when aphids are present and when the sorghums are grown under the LED lights,

as both negatively affect leaf width and number of true leaves. This is an important outcome of both Limaje et al. (2019) and the present experiments because plants are often screened for potential resistance in greenhouse or growth chamber studies and depending on light conditions, potentially susceptible or resistant genotypes could be misinterpreted.

The effects of lighting on sorghum physiology were not consistent across cultivars that were either resistant or susceptible. The susceptible KS-585 and the resistant TX 2783 had significantly lowered photosynthetic rates when grown under LED lights without the presence of aphids (Figure 3). Stomatal conductance rates were also differed by cultivar and lighting condition, being highest for DKS37-07 and similar for the other cultivars under conventional light (Figure 4). LED lighting reduced the stomatal conductance rates for all cultivars except TX7000. With infestation of aphids, the resistant cultivars under LED lights had significantly reduced photosynthetic rates, while both resistant cultivars maintained similar rates under conventional lighting (Figure 3). Resistant cultivars also maintained greater stomatal conductance rates under conventional lighting in the presence of aphids. With LED lighting and aphids, all tested cultivars had significantly lower stomatal conductance. The observed differences in photosynthetic rates and stomatal conductance with aphid infestations are not directly explained by loss of chlorophyll from aphid feeding, although chlorophyll losses were higher under LED lights for all cultivars except for the resistant KS 585.

LED lighting has been shown to benefit a number of plant species, including Solanaceae (Brown et al 1995), spinach *Spinacia oleracea* L. (Amaranthaceae), radish *Raphanus raphanistrum* L. (Brassicaceae), lettuce *Lactuca sativa* L. (Asteraceae) (Yorio

et al. 2001), and strawberry *Fragaria* L. (Nhut et al. 2003). In addition to promoting growth and yield, when lettuce seedlings were grown under blue light LEDs, antioxidant activity was promoted which increased the overall growth of the seedlings (Johkan et al. 2010).

Less research has been conducted on plants with C4 photosynthetic pathways grown under LED lighting, although to date, only sorghum has been documented to have negative responses. In C4 photosynthesis there are 3 subtypes of decarboxylation. NADP-ME (NADP- dependent malic enzyme), NAD-ME (NAD- dependent malic enzyme) and PEPCK (phosphoenolpyruvate carboxykinase) as described by Hatch (1987). Wheat, *Triticum aestivum,* which is a C4 plant (Casati et al., Spampinato & Andreo 1997), had increased photosynthetic activity, number of tillers, biomass, and overall yield when grown under LED lighting (Monostori et al. 2018). While sorghum also displayed increased growth in leaf number and leaf width (Table 1) which could be argued to be favorable, decreased photosynthetic rates (Fig. 5) and reduced stomatal conductance (Fig. 6) also occurred when grown under LED even in the absence of aphids. It is important to note that there are different subtypes of the C4 photosynthesis cycle. Sorghum has the NAD-ME C4 pathway (Rao & Dixon 2016), while wheat has the C4 subtype of NADP (Casati et al., Spampinato & Andreo 1997). Thus, the specific differences in decarboxylation may be the key to affecting sorghums growth under LEDs.

Tobacco, *Nicotiana tabacum*, a C3 plant, shows C4 photosynthesis pathways in the vascular bundles of the stem and petioles (Hibberd & Quick 2002). Both sorghum and tobacco use the same NAD-ME C4 pathway (Rao & Dixon 2016). LED lighting promotes growth of tobacco plants and reduces the membrane lipid peroxidation damage

of the plant (Jun et al. 2014). Perhaps unlike sorghum, tobacco experiences positive growth effects from being grown under LEDs because it does not completely rely on the NAD-ME C4 pathway as sorghum does. More work should be done with sorghum and the NAD-ME pathway to determine if the observed negative effects are based on NAD-ME's inability to compensate efficiently when grown under LEDs.

A key outcome of this study is the effects that LED lights had on resistant and susceptible sorghum when aphids were present. Overall, as anticipated when aphids were on the plant the plant displayed reduced measurements across all evaluated characteristics (Table 1). Reasons for this difference, especially in DKS-37-07 are unknown, but the interaction between aphid feeding and plant growth under LEDs should be further investigated especially because these plants had more chlorosis (Figure 5) and lower photosynthetic rates (Figure 3). It is possible that early infestation of aphids promoted plant response leading to taller plants and then damage increased until physiological measures were taken at day 15.

The effects of LED lighting in herbivores also requires more research. Aphids are small and soft-bodied and altered light wavelengths may impact their behavior or physiology. Previously, Limaje et al. (2019) noted differences in behavior of aphids in LED light experiments compared to those in conventional light treatments. The cabbage aphid B.brassicas and green peach aphid M.persicae have also been documented to be affected by LED lighting (Rechner et al. 2016). When grown on broccoli (*Brassica oleracea var. italica*), the cabbage aphid decreased growth and reproduction, while the green peach aphid increased in reproduction and population growth (Rechner et al. 2016).

In conclusion we recommend that studies involving aphid and sorghum interactions be conducted under known types of conventional lighting because aberrant growth occurs under LED lights. At a minimum, in trials aimed at identifying plant resistance to herbivores, the effects of lighting should be considered.

Tables

		Plant Height (cm)			
		TX-7000	KS-585	TX-2783	DKS-37-07
Conventional	Control	57.8 ± 0.64 a	55.8 ± 0.59 a	58.0 ± 1.2 a	56.5 ± 0.79 a
	Infested	40.9 ± 1.85 b	45.5 ± 0.75 b	53.6 ± 1.38 b	52.5 ± 0.74 b
LED	Control	29.5 ± 0.52 c	26.6 ± 0.61 c	23.9 ± 0.71 c	24.9 ± 0.8 c
	Infested	24.7 ± 1.16 d	27.6 ± 0.72 cd	26.3 ± 0.77 cd	33.6 ± 1.18 d
		df = 3, H = 39.046	df = 3, F = 450.19	df = 3, H = 38.844	df = 3, F = 279.76
		P <0.0001	P <0.001	P <0.001	P <0.001
			Leaf Number		
Conventional	Control	8.0 ± 0.0 a	7.0 ± 0.0 a	8.0 ± 0.0 a	7.0 ± 0.0 a
	Infested	5.0 ± 0.0 a	5.0 ± 0.0 a	6.0 ± 0.0 a	6.2 ± 0.11 a
LED	Control	15.3 ± 0.8 b	13.6 ± 0.63 b	15.4 ± 0.50 b	14.0 ± 0.58 b
	Infested	10.8 ± 0.59 b	7.8 ± 0.22 b	12.3 ± 0.46 b	9.6 ± 0.62 b
		df = 3, H = 42.979	df = 3, H = 43.602	df = 3, H = 44.193	df = 3, H = 41.227
		P <0.001	P <0.001	P <0.001	P <0.001
			Leaf width (mm)		
Conventional	Control	2.1 ± 0.06 a	1.6 ± 0.04 a	1.9 ± 0.04 a	1.9 ± 0.04 a
	Infested	1.0 ± 0.03 b	1.3 ± 0.05 b	1.4 ± 0.03 b	1.5 ± 0.04 b
LED	Control	3.0 ± 0.08 c	3.1 ± 0.08 c	2.9 ± 0.03 c	2.9 ± 0.04 c
	Infested	2.3 ± 0.07 d	2.4 ± 0.03 d	2.4 ± 0.06 d	2.7 ± 0.07 d
		df = 3, F = 169.77	df = 3, F - 206.99	df = 3, F = 253.15	df = 3, F =180.905
		P <0.001	P <0.001	P <0.001	P <0.001
			Damage rating		
Conventional	Control	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a
	Infested	6.2 ± 0.29 bc	6.67 ± 0.19 b	2.0 ± 0.0 bc	2.0 ± 0.12 a
LED	Control	3.3 ± 0.13 b	3.8 ± 0.41 a	3.3 ± *0.14 ab*	2.6 ± 0.15 a
	Infested	7.9 ± 0.15 c	7.9 ± 0.15 c	6.5 ± 0.25 abc	5.7 ± 0.39 b
		df = 3, H = 44.17	df =3, H = 42.618	df =3, H = 44.941	df = 3, H = 42.517

Table 1. Response of each cultivar was checked for normality and then compared with either ANOVA or Kruskal Wallis ANOVA followed by a Tukey test when differences were detected. Columns with the same letters are not significantly different (P > 0.05).

Figures

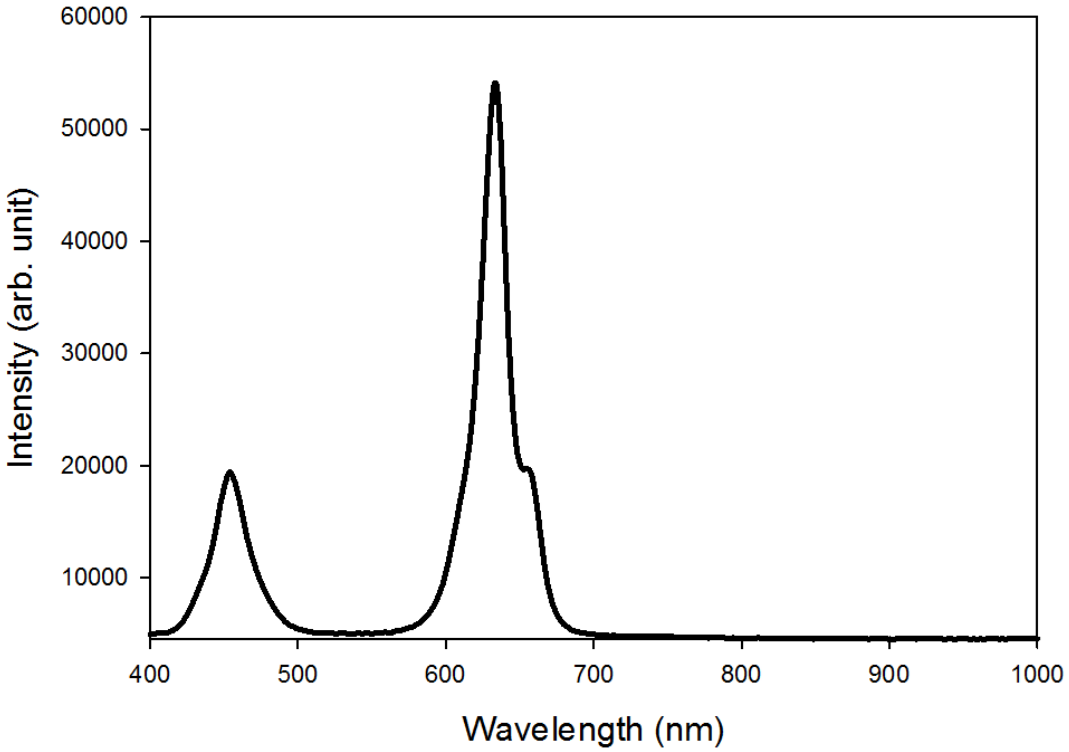


Fig. 1. Light emission spectrum of the 9 band 60w LED grow panels over the visible spectrum and into the near infrared.

Fig. 2. Resistant sorghum variety TX 2783 across four treatments. A. Control under LED lights; B. Infested under LED lights. C. Control under conventional lights; D. Infested under conventional lights. Plants were infested with sugarcane aphids and assessed 15 days post infestation

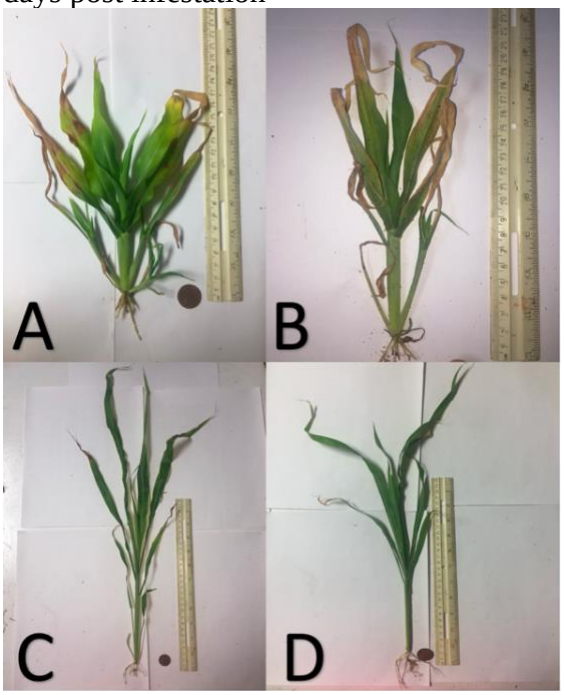
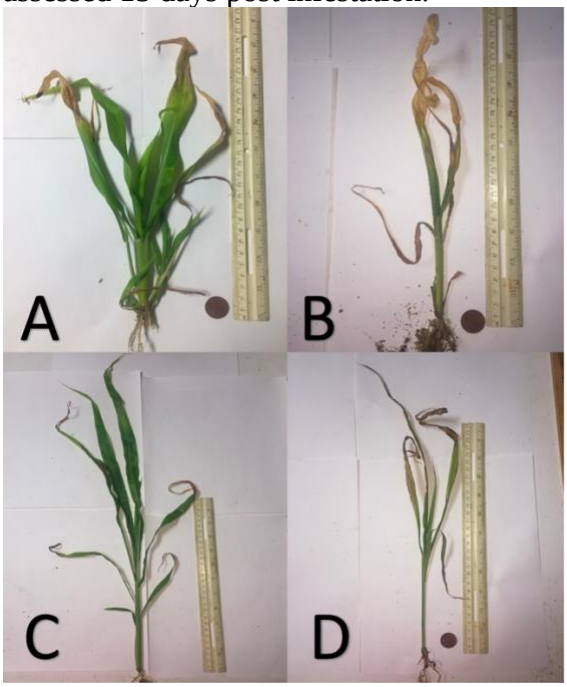


Fig. 3. Susceptible sorghum variety KS 585 across four treatments: A. Control under LED lights; B. Infested under LED lights. C. Control under conventional lights; D. Infested under conventional lights. Plants were infested with sugarcane aphids and assessed 15 days post infestation.



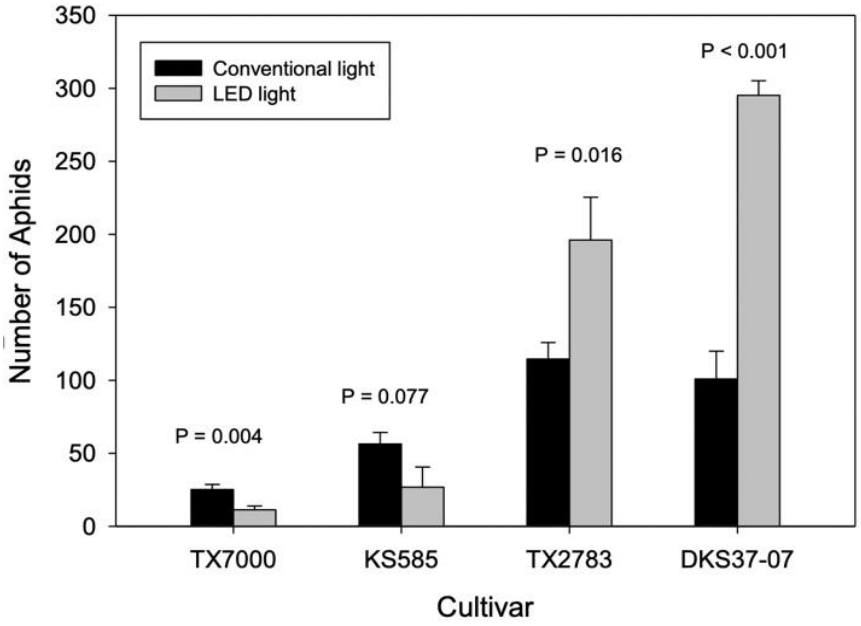


Fig. 4. Mean ± 1 S.E. number of sugarcane aphids per plant 15 days after infestation when grown for resistant (TX 2783 and DKS 37-07) and susceptible (TX7000 and KS585) sorghum cultivars grown under either conventional or LED lighting. P-values represent results of a Student's T-test (df = 22) for each variety.

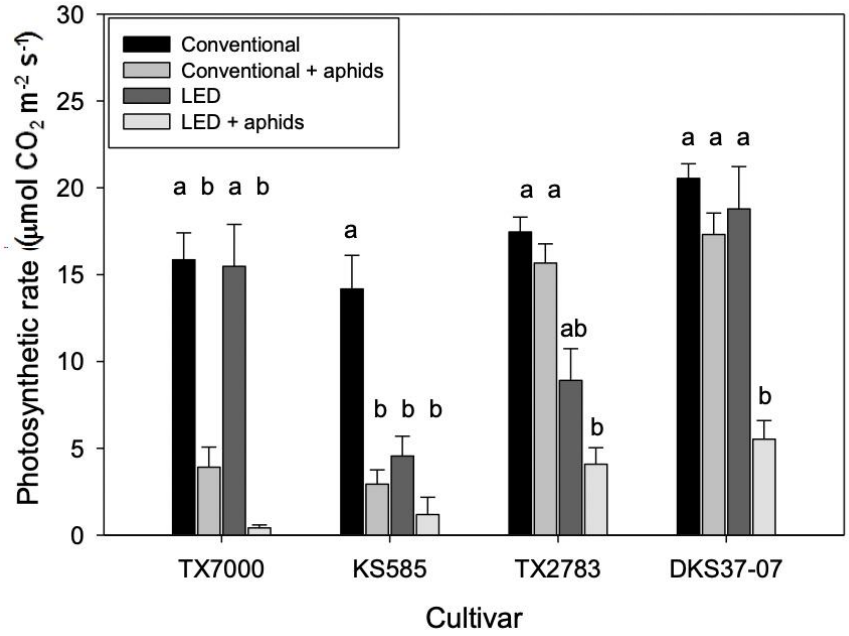


Fig. 5. Mean ± 1 S.E. photosynthetic rates (µmol CO2 m^{-2} s^{-1}) of resistant (TX7000 and KS585) and susceptible (TX 2783 and DKS 37-07) sorghum cultivars grown under either conventional or LED lighting. All plants were measured at 15 days after infestation with sugarcane aphids. Bars with different letters are significantly different (Kruskal-Wallis ANOVA, df = 3, H > 27.14, P < 0.01)

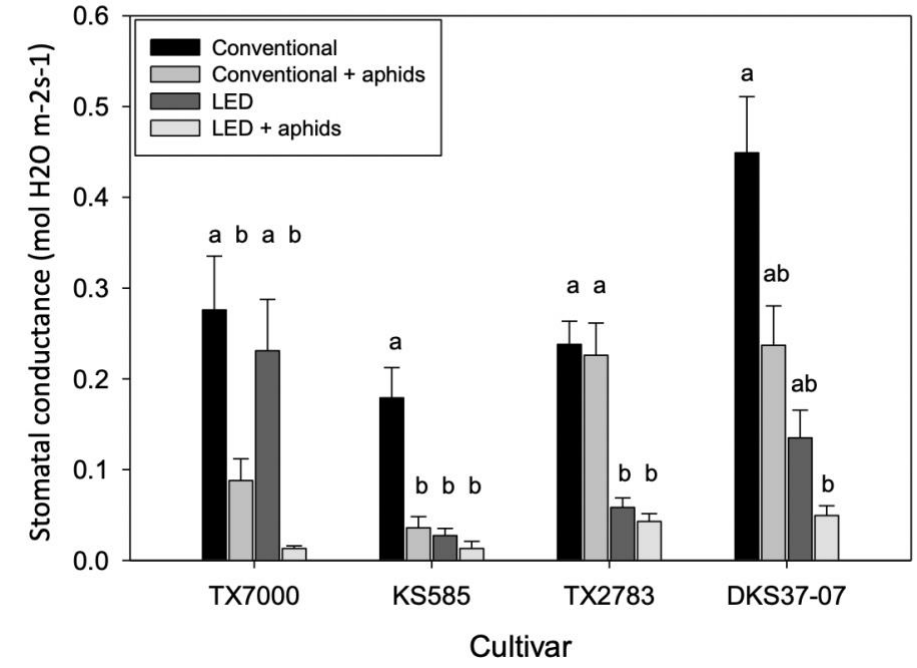


Fig. 6. Mean ± 1 S.E. stomatal conductance (mol H2O m^{-2} s^{-1}) at 15 days after infestation under LED and conventional lights. Bars with different letters are significantly different (Kruskal-Wallis ANOVA, df = 3, H > 24.13, P < 0.01)

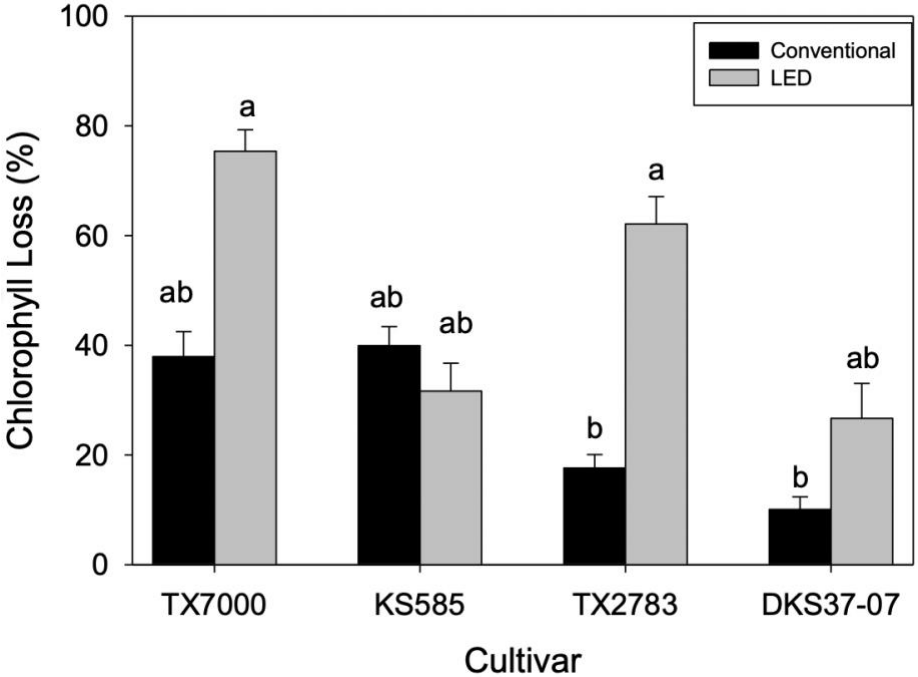


Fig. 7. Mean ± 1 S. E. chlorophyll loss at 15 days after infestation under LED and conventional lights (Control-Infested)/Control. Different letters represent significant differences (P < 0.001) with a Kruskal-Wallis ANOVA followed Dunn's multiple comparison test (H = 62.629 df = 7).

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Appendix

Introduction

Being able to study the movement of animals in a community population is valuable information. In order to have accurate data there needs to be some way to tell individuals apart from the rest of the population. Biologist have been using marking techniques for many years to study birds, mammals and fish. (Hagler & Jackson 2001) In birds, leg bands are often used to identify avian individuals**.** (Griesser et al 2012) In mammals, a wide array of techniques are used from ear tags, freeze brands, hair color dye to high tech ultrasonic detectors and neck collars with video cameras and radio transmitters. (Sikes et al 2011)

In insects it can be difficult to mark them and not injure them due to their small size. A good marking technique should be cost effective, be without basis, weather under environmental conditions and not affect the insect's behavior or physiology. (Southwood 1978, Hagler and Jackson 2001, Lavandero et al 2004)

The ability to mark insects can be highly valuable to a researcher. One example would be Monarch butterflies. Researchers apply a sticker marking technique to the butterflies D cell wing. (Taylor et al 2020) These tags help study the migration success of the butterflies. (Taylor et al 2020) Earlier on it was noted that the weight of the sticker might have been causing issues with the migration success of the butterflies (Taylor et al 2020)

Other marking techniques that have been documented to use for insects are, wire loops placed on a insets body part (Mirenda and Vinson 1979) paint dots (sendova-franks & Franks 1993) Radio tagging for eusocial insects (Summer 2007) and passive pollen uptake by insects (Hagler & Jackson 2001) and burning brands into insects that have a harder exoskeleton, like beetles. (Walker & Wineriter 1981)

Sugarcane aphids *Melanaphis sacchari* (Zehntner) (SOSCA) are soft bodies insects that are only 1 – 10 mm in size with piercing sucking mouthparts. (Dixon & Kundu 1998) The population of aphids is explosive in just a matter of weeks. A single aphid starts reproducing in a matter of 7-9 days with the total life span being anywhere from 20 to 37 days. (Chang et al 1982, Singh et al 2004, Zapata et al 2004). That single aphid can have as many nymphs in her lifetime. (Chang et al 1982, Singh et al 2004). Currently there is no easy and inexpensive way to mark and identify individual aphids in a population. Finding new ways would be highly beneficial and open new research for being able to study population's behavior and individual's interaction in aphid populations.

This study looked at the effects of aphid's reproduction if the individuals were marked with an Ultra Fine point Sharpie®. Another objective of this study looked at if aphid's reproduction is different when aphids are in solitary or in aggregated groups.

Aphids are piercing sucking insects that feed primarily on a plant phloem. The plant sap that is ingested by the aphids has sugars in it that is often excreted by the aphid and left behind on the plant called honeydew. Aphids will also ingest xylem sap to help with the osmotic effects of them feeing off of phloem. (Buchanan et al 2000)

In the aphid's saliva is a feeding mechanism that counteracts the plant's defenses and keeps the plant from closing off the phloem cell the aphid is feeding on. (Will & van Bel 2006) This is called plant conditioning. This feeding can inoculate the plant to many differnt diseases and viruses.

It is thought that during the plant conditioning that the aphids are boosting the plants growth and heath in order to prolong the life of the plant so the aphid can keep living longer. (Michaud, Jyoti & Qureshi 2006) It was observed that *Aphis fabe* Scopoli reproduced at a higher rate when in aggerated groups rather than being in solitary (Way 1967)

Michaud, Jyoti & Qureshi (2006) looked at the reproductive fitness of Russian Wheat Aphids *Diuraphis noxia* (Mordvilko) in colony sizes of one and 10 aphids. Their conclusions were that adults that were reproducing in the colony size of 10 aphids reproduced at a higher rate than aphids reproducing in solitude. Our study of the second objective looks at if Sugarcain aphids reproducing in groups live and reproduce higher than aphids in singles.

Methods and Materials

Aphid Culture All SoSCA from this study are from the USDA-ARS Stillwater, OK Laboratory. The aphids were originally collected from Matagorda County, TX in 2013. The aphids are reared on susceptible TX 7000 sorghum seedlings. The seedlings are in pots that are covered with sleeve cage. All plants and aphids are maintaining in a greenhouse with temperatures that range between 21^{o} C and 31^{o} C. The SoSCA are transferred to new susceptible seedings every week in the greenhouse. The plants are aphids are under both natural light and supplanted light that is from two T-8 fluorescents lights. All supplemented lights are on timers so that the lights come on at 6am CST and turn off at 8pm CST.

Growth Chamber One growth chamber was used for both the aphid reproduction study and the aphid marking study. The chamber provided light, temperature and humidity control (Percival Scientific, model E30BC8, Perry, IA 50220). The growth chamber was fitted with two Philips (model 7866113, Philips Inc. Guadalajara, Jalisco, Mexico) fluorescent grow lights, and two clear 40-watt appliance lights (Sylvania, Wilmington, Massachusetts, USA). The chamber is kept at 27^{o} C and has timers on the lights to come on at 6am CST and turn off at 8pm CST.

Aphid reproduction study There were two different groups of aphids being looked at for this part of the study. Aphid groups were 10 aphids females of the same age per plant versus a single aphid group were there is only one aphid per plant. We had 12 reps of each group in this study (12 reps of 10 aphids per plant and 12 reps of 1 aphid per plant.)

The known susceptible TX 7000 was used to rear the aphids that were used in this experiment . The TX 7000 seedlings were planted in 10.16cm in diameter pots that were filled with a three-layer level of different potting media from the bottom up: potting soil, fritting clay and sand. Each pot was housed in an 8-cm diameter Lexan sleeve with a height of 45 cm, which was ventilated with organdy cloth. Initially, two seeds of each genotype were planted at a depth of 2cm in the Cone-tainer™. The seedlings were grown under 2 T-8 fluorescent lighting (16/8 h L:D) and at 25 °C. One week after planting the seedlings were thinned to one seedling per pot. One day after thinning, the plants were transferred from the greenhouse to the growth chambers. All plants where fertilized with Miracle -Gro® Garden feeder at the recommend rate of 1 tablespoon per gallon.

After 24 hours after transferring the seedlings to the growth chamber they were infested with 15 adult aphids per plant. The adult aphids were given 24 hours to reproduce the first clonal nymphs on the plant before the adult was removed. This was to ensure that all of the baby aphids were the same age. The less than 24 hour nymphs were left on the plant, not to exceed 10 for the multiple treatment group, and a single aphid per plant for the single group. Nymphal aphids were counted daily to ensure the growth progress and make sure all were living. The nymph aphids reach maturity and started reproducing 6 to 7 days after being born, also known as the pre-reproductive period indicated as *d*, (Wyatt and Wight 1977).

After reaching maturity the aphids were evaluated for the number of nymphs on the plant once a day every day until all of the founding female adult aphids were deceased. Nymph aphids were counted and taken off of the plant during the once a day

evaluation. The number of adult aphids were also counted and recorded when they started to die.

Life parameters: like pre-reproductive period (d); is the amount of time it takes the nymphs to reach reproductive maturity. Intrinsic rate of increase (*rm*) was calculated using the formula: r_m=.0738(1*oge*M_d)/*d* (Wyatt and White, 1977) were M_d is the number of nymphs produced for a time that is the same amount of time as d. Longevity and total fecundity were also recorded. All reproductive life parameters were analyzed using Mixed model analysis (PROC MIXED, SAS Institute 2016) where mean comparisons were made by using the Least Significant Differences Method (LSD) at $P > |t| \leq 0.05$ level (SAS 9.4, SAS Institute, 2016).

Aphid marking study There were 4 colors being evaluated in this study, Red, Blue, Green and black. We also had a control with no marking on the aphid. All of the colored aphids were marked with Ultra Fine point Sharpie®. All colored SoSCA were marked in the same place, on the abdomen right above and between the cornicles. There are 6 reps per color treatment plus the 6 reps for the control for a total of 30 plants.

One sorghum cultivar was used in this experiment, susceptible variety TX 7000 was used to rear all aphids in this experiment as previously explained above. All of the seedlings were planted in 10.16cm pots and were filled with a three layer system of different potting media from the bottom up: potting soil, fitting clay and sand. Each pot was housed in an 8-cm diameter Lexan sleeve with a height of 45 cm, which was ventilated with organdy cloth. Initially, two seeds of each genotype were planted at a depth of 5.08cm in the Cone-tainer™. The seedlings were grown under 2 T-8 fluorescent

lighting (16/8 h L:D) and at 25 °C. One week after planting the seedlings were thinned to one seedling per pot. One day after thinning, the plants were transferred from the greenhouse to the growth chambers. All plants where fertilized with Miracle -Gro Garden feeder at the recommend rate of 1 tablespoon per gallon.

After 24 hours after transferring the seedlings to the growth chamber they were infested with 5 adult aphids each. The adult aphids were given 24 hours to reproduce offspring on the plant before the adult was removed. We did this to ensure that all of the baby aphids were the same age. The 24 hour nymphs were left on the plant and culled to 5 nymphs per plant. We did this so that when we marked the aphids later on in the study we had extras. Nymph aphids were counted daily to ensure the growth progress and make sure all were living. The nymph aphids reach maturity and started reproducing 6 to 7 days after being born. We allowed the mature aphids 48 hours of producing nymphs before we marked them. All nymphs produced during the 48 hours were disposed of. When the aphids were marked, only one colored aphid remained per plant. All of the aphids for each treatment group were examined every 24 hours after being marked from the beginning of the experiment. Both color and control aphids were counted every 12 hours and the nymphs were counted and taken off of the plant. The study concluded 2 weeks after the aphids started reproducing.

We evaluated the nymph aphids produced from the colored and control as well. We took 2 nymphs from each plant that was in the 24 hour mark and put them on a single individual plant. When the nymphs started to reproduce, we culled so that only one aphid per plant. We had 6 aphids from each colored aphid (24 aphids) and 6 aphids from the

control (6 aphids). Aphids were evaluated for number of nymphs produced per day. All nymphs were taken off of the plant.

Results and Discussion

Aphid reproduction study

There was not a significant difference between SoSCA that were reared in aggregate as compared to solitary . The intrinsic rate of increase (*rm*) for the groups was 0.410 ± .001 and for the singles 0.409 ± 0.003 (Table 1). Female longevity also had no significant difference. The aphids in groups lived 31.83 ± .46 and aphids in solitary lived 30.33 ±.50 (Table 1). Total Aphid Fecundity had also no significant difference and was very close in numbers with the aphids in groups having 146.0 ± 1.67 and aphids in solitary having 145.08 ± 4.69 (Table 1). Average nymphs produced per female per day had no significant difference with groups 4.24 ± .126 and singles 4.15 ± .125 (Table 1).

Aphid marking study

There was no significant difference between SoSCA that was marked with colored permeant marker and SoSCA that was not marked. It was seen that for total number of nymphs produce by all 6 females in the non-colored control group produced more nymphs than any other group, 414. While green produced 406, black 395, blue 392 and 391 (Table) The mean numbers of nymph that an aphid reproduced every day was also had no significant difference. Control group 5.8, green group 5.6, group 5.5, blue group 5.4 and red group 5.4 (Table 2).

Discussion

Aphid reproduction study

We observed no significant difference in this study with female aphids in groups or in solitary. Our data suggests that the number of nymphs reproduced by a female aphid is not dependent on if she is aggregated with other aphids or not.

Aphid marking study

All of the females across all of the testing colors were very consistent across the 12 days of this study. We saw no significant differnt between the total nymphs reproduced by all 6 females in each group and the average nymphs per day reproduced.

We did see some small differnt in the total nymphs reproduced by all 6 females. The females in the non-color control group did reproduce the highs nymphs in this test, 414. The number is not significant, and since may factors play into aphid's reproduction and they are alive. This will hopefully open up further research in insect's communal behavior and give researchers a chance to study the communal-individual interactions.

Tables

Table 1. Demographic statistics for sugarcane aphid reproduction when in groups and singles

Germplasm	Longevity/(Rpd)	Fecundity (*Md*)	Nymphs/ ♀/ d	Intrinsic Rate of Increase (*rm*)
Single (1 aphid)	30.33 ±.50	145.08 ± 4.69	4.15 ± .125	0.409 ± 0.003
Group (10 aphids)	31.83 ± .46	146.0 ± 1.67	4.24 ± .126	0.410 ± .001

Fecundity (*Md)*=sugarcane aphids/female, 12 replicants, df=1 F= .01 P=.934
Nymphs/♀/d=(*Md*/d)
Longevity/(rpd)= de=1 F=4.92 P=.0371
rm= intrinsic rate of increase, rm= 0.738(In *Md*/d) df=1 F=4.24 P=.0515

Table 2. Demographic statistics for sugarcane aphid marking study when aphids were marked with colored permanent marker

Germplasm	Fecundity (*Md*)	Nymphs/ ♀/ d
Red	391	5.43 ± .101
Green	406	5.63 ± .0913
Blue	392	5.44 ± .0967
Black	395	5.49 ± .0928
Control	414	5.75 ± .104

For color groups, *df* = 4, 359, *F* = 2.05. *P* = 0.087
Fecundity (*Md*) = sugarcane aphids/female, 6 replications
Nymphs/♀/d; = (*Md*/d)
rm = intrinsic rate of increase, rm = 0.738(ln *Md*/d)

Source of Variation	DF	SS	MS	F	P
Between Groups	4	5.572	1.393	2.048	0.087
Residual	355	241.528	0.680		
Total	359	247.100			

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