

**SIGNIFICANCE OF PHOTOPERIOD ON THE FITNESS OF THE  
SUGARCANE APHID AND APPLICABILITY OF BRIX REFRACTOMETRY  
AS A POTENTIAL METHOD TO PREDICT AND DETECT  
RESISTANCE OF SORGHUM**

**by**

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## ABSTRACT

Little is known of the biology of the sugarcane aphid, *Melanaphis sacchari* (Zehntner), that became a persistent insect pest of sorghum, *Sorghum bicolor* (L.) Moench, in North America in 2013. Understanding the fitness of sugarcane aphid in relation to environmental conditions and developing a method to predict resistance would aid in understanding how sugarcane aphids develop and evaluating sorghum for resistance to this major insect pest. Effects of three photoperiods of 14:10, 13:11, and 12:12 light:dark hours were evaluated on sugarcane aphids on susceptible 'ATx399 x RTx430' sorghum at daily temperatures of 30 (light or day) and 20°C (dark or night), respectively, in an incubator. A sugarcane aphid from a pure colony was placed individually into a clip cage, with two clip cages per each of four plants in six pots. When each aphid produced a nymph, the mother aphid was discarded and the nymph was retained and allowed to mature and produce offspring until it died in the clip cage. The nymphs produced were counted and removed each day.

The average pre-reproductive period for the sugarcane aphid was approximately 53% (6.6 days) longer at a photoperiod of 14:10 light:dark hours than at the two other photoperiods. The reproductive period of each aphid at the 14:10 photoperiod was 13.9 days, 2 days longer than the 11.8 days at a photoperiod of 13:11 light:dark hours. The post-reproductive period for each sugarcane aphid averaged 2.4 days at a photoperiod of 12:12, 4.7 days shorter than the 7.1 days at a photoperiod of 13:11 light:dark hours.

Total fecundity (87.7 aphids) at the 13:11 photoperiod was 24 aphids more than the 63 aphids at 12:12 and 31 aphids more than the 56.3 aphids at 14:10 light:dark hours. Developmental times and fitness of sugarcane aphids differed significantly at the different photoperiods.

Efficacy of Brix refractometry as a potential method for determining the resistance of sorghum against sugarcane aphids was evaluated in a greenhouse and field. Resistant sorghum line 'RTx2783' was compared to susceptible sorghum line 'RTx430' at the boot stage. The °Brix values were collected by crushing a mature sorghum leaf and subsequently pipetting the liquid into a Brix reader. The average °Brix of the resistant sorghum did not differ from the susceptible sorghum line in the greenhouse ( $F_{1,38} = 0.0016$ ;  $P = 0.8985$ ) nor in the field ( $F_{1,18} = 0.3424$ ;  $P = 0.5656$ ). The difference between the °Brix of the two sorghum lines in the greenhouse was only 0.01% and merely 0.03% in the field. Brix refractometry was not an applicable method to use for determining resistant sorghums to sugarcane aphids.

A Brix refractometer, although a common tool in plant breeding, was incapable of detecting resistance and susceptibility in two common sorghum lines. The °Brix values were too similar; therefore, a plant breeder should not use °Brix to define resistance or susceptibility of the two sorghum lines. The mechanism of resistance in 'RTx2783' was not related to the total sugar content and was undetectable by Brix refractometry. Consequently, the mechanism of resistance must be researched further.

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# CHAPTER I

## INTRODUCTION

### Background to the Study

Sorghum, *Sorghum bicolor* (L.) Moench, is a grass crop cultivated primarily for grain, silage, and ethanol (Smith 2001). Approximately 120 species of insect pests in 29 families infest sorghum worldwide (Young and Teetes 1977). The sugarcane aphid, *Melanaphis sacchari* (Zehntner), is an insect pest of sorghum in many areas of the world. The sugarcane aphid has been a threat to sorghum, as well as sugarcane, *Saccharum officinarum* (L.), for decades (van Rensburg 1973). However, the sugarcane aphid only recently became a major pest of sorghum in North America.

The sugarcane aphid was first reported on sorghum in the United States, and categorized as an occasional pest, in Louisiana in 1999 (White et al. 2001). Dr. MO Way discovered sugarcane aphids damaging sorghum at Beaumont, TX, during the growing season of 2013. The sugarcane aphid rapidly increased in abundance and spread to infest thousands of acres of sorghum throughout the Great Plains. By 2015, sugarcane aphids were in 17 states and threatened more than 90% of the sorghum grown in North America (Bowling et al. 2016).

Sorghum is damaged by the feeding by sugarcane aphids. The aphid feeds by intracellularly inserting its mouthparts into vascular tissues of the plant (Minks et al. 1989). Feeding can cause severe necrosis, stunting, and death of plants. Sugarcane

aphids also vector pathogens. The aphids excrete honeydew as they feed. Sooty mold often grows on the honeydew. Honeydew and sooty mold block the sorghum stomata, inhibit plant defense mechanisms, and damage harvesting equipment (Hall 1987, Schwartzberg et al. 2013, Armstrong et al. 2015).

Severity of damage to sorghum depends on the amount of infestation by sugarcane aphids. Sugarcane aphids rapidly reproduce by clonal parthenogenesis (Minks et al. 1989). Without treatment, sugarcane aphids can severely reduce sorghum yield potential  $\geq 60\%$  (van Rensburg 1973). The rate and degree of infestation depend on numerous influences. Attributes of a sorghum plant can either impede or promote infestation by aphids (Painter 1951). Positive attributes can be selected to be expressed and therefore controlled by plant breeders. Environmental influences also indirectly determine aphid abundance, but environmental factors are impossible to control. Understanding sorghum resistance to sugarcane aphids and how an aphid reacts to environmental influences is an essential component of control.

### **Statement of the Problem**

Aphids were the first animal to be defined as photoperiodic (Marcovitch 1924). Capable of discerning the difference between day and night, aphids traditionally utilized photoperiod to time sexual reproduction. However, geographically parthenogenetic species do not reproduce sexually. Rendered anholocyclic by latitudinal position, a geographically parthenogenetic species will not traditionally respond to seasonal changes in the length of the photoperiod. The sugarcane aphid is geographically anholocyclic and exclusively reproduces asexually throughout most of its geographical range (Simon et al. 2002, Singh et al. 2004, Bowling et al. 2016).

Regardless of the nontraditional response by aphids, photoperiod is still a density-independent factor of the population dynamics of aphids. Understanding how the photoperiod affects the biology, growth, and development of sugarcane aphids is essential for comprehending population dynamics of the aphid. Sugarcane aphids have been theorized to be influenced by seasonal change and time (van Rensburg 1973b, Singh et al. 2004, Guo et al 2011). However, information regarding how the sugarcane aphid, as well as any anholocyclic species, nontraditionally responds to photoperiod is inadequate. Therefore, this component of the research focuses on evaluating the effects of different photoperiods on the biology, growth, and development of sugarcane aphids, an anholocyclic species, on sorghum.

Irrespective of environmental influences, resistant crop varieties are capable of deterring pests without the use of insecticides. The current methodologies used to discern resistance of sorghum typically involve subjecting sorghum to the pest and observing the severity of damage to the plant (Smith et al. 1994). A predictive method to determine the resistance of sorghum independent of infestation by aphids would greatly speed development of resistant hybrids. A study conducted on the spotted stem borer, *Chilo partellus* (Swinhoe), on sorghum reported a correlation between leaf damage and low sugar °Brix (Muturi et al. 2012). This component of the research focused on the use of Brix refractometry as a potential method to use for quickly determining sorghum resistance independent of infestation and observation of sugarcane aphids.

Brix refractometry is a common method for determining total sugar content in sorghum. Named after Adolf Ferdinand Wenceslaus Brix, Brix is a unit of measurement of refractive index determined by how light refracts in a sucrose solution (Darmstaedter

2013). Calibrated so that pure water is 0 °Brix and pure sucrose is 100 °Brix, a Brix reading reports an estimate of the amount of sucrose present in a sample. However, the liquid drawn from the crushed leaf of a sorghum plant contains multiple forms of carbohydrates and other organic constituents. A Brix refractometer can be used to analyze the refractive index of a solution based on the total soluble solids present, and therefore, Brix refractometry is not a tool used to separate and quantify the constituents of any solution. The °Brix value of a sorghum plant is meant to be interpreted as the total sugar content, not solely the percentage of sucrose (Kawahigashi et al. 2012, Simeone et al. 2017). Brix refractometry is the industry standard for determining the total sugar content in sorghum.

The Germplasm Resources Information Network (GRIN) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) report °Brix values as an indicator of the total sugar content of a given sorghum accession (GRIN 2019, ICRISAT 2019). Kawahigashi et al. (2013) considered Brix as a common and useful parameter for selecting sorghum genotypes on the basis of sugar. Therefore, Brix refractometry is currently a useful and recognized tool in sorghum breeding.

### **Objectives of the Study**

One objective of this research was to assess the biology, growth, and development of sugarcane aphids on sorghum under different photoperiods. In particular, the life history characteristics to be studied included the:

- a. average number of days before the first sugarcane aphid nymph was produced (pre-reproductive period)
- b. average number of days nymphs were produced (reproductive period)

- c. average number of days between the birth of her last nymph and the death of the mother aphid (post-reproductive period)
- d. average number of nymphs produced per mother aphid per day (daily fecundity)
- e. average total number of nymphs produced per mother aphid (total fecundity)
- f. average number of days each sugarcane aphid lived (longevity)
- g. percentage of increase in the abundance of sugarcane aphids (intrinsic rate of increase)
- h. number of days between each sugarcane aphid generation (mean generation time), and

In a second experiment, Brix refractometry was used to measure and compare sugar content in resistant and susceptible hybrids of sorghum. The purpose here was to evaluate the applicability of this method for quickly predicting and detecting sorghum resistance to the sugarcane aphid.

### **Significance of the Study**

Unlike weather, photoperiod is a predictable and constant influence on aphid population dynamics. Photoperiod traditionally is studied in regard to sexual reproduction. However, this study aimed to further the knowledge and understanding of how photoperiod affects sugarcane aphids in addition to sexual reproduction. Understanding how photoperiod influences growth and development of sugarcane aphids will aid in producing integrated pest management strategies, resistant sorghum varieties, and forecasting models for sugarcane aphids. This study also will contribute to the literature and knowledge of: sugarcane aphid biology, population dynamics of the



sugarcane aphid, abiotic influences on sugarcane aphids, and photoperiodic responses of anholocyclic aphids. The results of the photoperiod study could be used for creating predictive models and preemptive aphid control.

Predicting the resistance of a genotype of sorghum independent of infestation by sugarcane aphids would aid plant breeders in selecting aphid-resistant sorghums. Unlike traditional methods for determining resistance, the results of this study could allow plant breeders to determine resistance almost instantaneously in the field. Quickly ascertaining resistance could streamline production of sugarcane aphid-resistant sorghum hybrids. Results of the Brix refractometry study could be used to affect the success and speed of breeding for sorghum resistance to sugarcane aphids. This study will contribute to the literature and knowledge of sorghum breeding, plant physiology, and resistance against sugarcane aphids.

## CHAPTER II

### LITERATURE REVIEW

#### **Sorghum**

Sorghum, *Sorghum bicolor* (L.) Moench, is a monocot in the family Poaceae. Thought to have originated in northeastern Africa, sorghum was first brought to the United States by the slave trade in the 1800's (Kimber 2000). Sorghum is consumed as a staple human food around the world, and the livestock and ethanol industries also use sorghum as a primary feed and fuel source (Rooney 2000). An adaptable plant, sorghum predominantly is grown in the semiarid regions of Africa, China, India, South America, and the United States (Kimber 2000). The versatility of sorghum also has made it part of an elite group of plants that globally provide more than 85% of all human energy (NRC 2006).

Sorghum is a short-day species grown in both temperate and tropical zones, at altitudes from sea level to 3,000 m, and in many types of soil (Kimber 2000). Depending on the lengths of the internode, peduncle, and panicle, and the number of nodes, sorghum can be less than 45 cm or greater than 400 cm tall (Doggett 1988). Some kinds of sorghum have as many as 30 leaves while others have less than 10; however, all sorghum leaves are isobilateral, tapered, and grow in an alternate pattern along a cylindrical stem (Artschwager 1948). Despite a moderate number of leaves, the root system of sorghum can grow as deep as 3 m (Prasad and Staggenborg 2009). At physiological maturity,

sorghum has a panicle inflorescence composed of spikelets on many rachis branches (Kimber 2000). Sorghum is a self-pollinating species but has an outcrossing potential of  $\geq 35\%$  (Karper and Conner 1919, Sieglinger 1921, Quinby et al. 1958). Depending on the genetics and the environment, a panicle can be either compact or loose (Waniska and Rooney 2000). Overall, sorghum is a very variable, adaptable, and versatile crop.

The inherent attributes of sorghum have made it the fifth most important grain crop in the world in terms of acreage and production, and the third most important in the United States of America (Grains 2007, FAO 2018). More than 40,674,113 hectares of sorghum, yielding an average of 1,416.2 kg per hectare, were harvested globally during the 2017 growing season. More than 2 million hectares of sorghum were harvested for grain in 2017 in the United States, with Kansas, followed by Texas, being the leading producers (USDA 2018).

Sorghum is globally impactful. One of the most versatile crops in the world, sorghum is utilized and consumed in a myriad of different ways. Sorghum grain is an excellent gluten-free source of protein and starch for the human diet and possesses phenolic compounds linked to inhibiting the development of tumors (Kulamarva et al. 2009, Prasad and Staggenborg 2009). The overall nutritional quality of sorghum rivals that of maize, *Zea mays* L., for feeding cattle (NAS 2016). Sorghum also is considered economically and industrially useful for ethanol production (Barcelos et al. 2011). Building materials, fiber, and even industrial lubricants and adhesives also are produced from sorghum (NRC 2006). In the United States, sorghum is used primarily for livestock and poultry feed; however, much is exported and/or used for human consumption (USDA 2018). Sorghum can fill many niche markets.

Sorghum is an efficient and a versatile crop. Capable of yielding during drought, sorghum commonly is produced in arid environments where other grain crops cannot be grown. Sorghum because of the C4 photosynthetic cycle is 50% more photosynthetically efficient than rice, *Oryza sativa* L.; wheat, *Triticum aestivum* L.; or potato, *Solanum tuberosum* L. (Kajala 2011). Photosynthetic efficiency, along with the root system and leaf morphology, allows sorghum to use only one-third to one-half the amount of water that maize requires to produce a crop (Pedersen et al. 2000, Rooney 2000). Sorghum can withstand not only drought, but some sorghum genotypes can survive water logging, salty soil, and exceedingly warm temperatures (NRC 2006). Growing a crop of sorghum is a common solution for areas challenged with harsh environments. The versatility of sorghum is one reason why more than 30 countries consider sorghum to be a dietary staple.

Even sorghum, considered to be the sturdiest grain crop, has limitations. Sorghum is environmentally limited by soil pH, altitude, seasonal influences, day length, and frost (Kimber 2000). Other limitations include many insect pests and diseases. Sorghum worldwide is damaged by 120 species of insect pests in 29 families (Young and Teetes 1977). Common insect pests of sorghum in the United States include: sugarcane aphid, *Melanaphis sacchari* (Zehntner); greenbug, *Schizaphis graminum* (Rondani), sorghum midge, *Stenodiplosis sorghicola* (Coquillett); corn earworm, *Helicoverpa zea* (Boddie); fall armyworm, *Spodoptera frugiperda* (J.E. Smith); sorghum webworm, *Nola sorghiella*, Riley; wireworms, families Elateridae and Tenebrionidae; white grub, *Phyllophaga crinita* (Burmeister); chinch bug, *Blissus leucopterus leucopterus* (Say); Banks grass mite, *Oligonychus pratensis* (Banks); and stink bugs, family Pentatomidae

(Teetes et al. 1983, Teetes and Pendleton 2000). Nevertheless, the pests of sorghum can be controlled through integrated pest management.

### **Sorghum Integrated Pest Management**

Humans have been managing pests since the dawn of agriculture. Sulfur, botanicals, and even manipulating natural enemies have been used for more than 1,000 years (Dent 2000). The concept of integrating multiple management techniques to control insects was a well-documented practice throughout history (Norris et al. 2003). The modern ideology of integrated pest management (IPM) is a conglomeration of scientific discovery, innovation, and historical fact (Dent 2000).

The term IPM was adopted in 1972 when the Council on Environmental Quality published a report entitled Integrated Pest Management (CEQ 1972). However, IPM was first conceptualized in 1959. Biological and chemical control tactics were combined into an integrated plan to control spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in California (Stern and van den Bosch 1959). The foundational concept of the plan was to only use chemical control when necessary by virtue of an economic threshold level. An economic threshold is the level at which pest control is enacted before a crop is significantly damaged economically (Kogan 1988). Determining the economic threshold is dependent on factors such as the value of the crop and the cost of controlling the insect (Teetes 2006). The goal of an economic threshold is to ensure that control tactics are economical. The economic loss caused by insect pests totals billions of dollars per year (Holmes 2009, Oliveira 2014, Bradshaw 2016). The more efficient a control tactic, the less economic loss occurs.

However, the first concept of IPM did more than define economic thresholds. Repeated indiscriminant application of insecticide can cause detrimental effects. Integrating biological and chemical control lessened the need for insecticide and ultimately mitigated the risks of detrimental outcomes. The outcomes of arbitrary insecticide application include reduction in abundance of beneficial arthropods, resistance of the primary pest, release of secondary pests, resurgence of the targeted pest, and residues in the environment (Stern and van den Bosch 1959). Ultimately, IPM lessens dependence on pesticides, increases economic profit, and diminishes the environmental impact of pest control. All of this is achieved through applied ecology.

Ecology is the study of organisms and their relationships with biotic and abiotic influences. Agroecology is the study of the relationships in an agricultural production system. An ecosystem in agriculture is called an agroecosystem. A sorghum field is an agroecosystem. Countless influences, both biotic and abiotic, affect sorghum every day. Pest insects are among the influences. Pest insects are undoubtedly negative biotic influences; nevertheless, they are a part of the agroecosystem. Understanding the ecological role of a pest insect as well as its biology is the foundational concept of modern IPM. Without this concept, pest management would be an arbitrary ordeal and would give rise to an unbalanced ecosystem. An ideal IPM strategy would manage pests according to the biology and classification of the pest.

Before classification, however, a pest first must be identified correctly. Many insect identification handbooks are available. Improper control techniques might be used if the pest was identified incorrectly, resulting in unnecessary waste and economic loss. Correctly identifying an insect pest is essential.

Once correctly identified, the pest can be classified. Sorghum pest management strategies are focused on controlling pests that cause significant yield losses. Pests that routinely cause yield loss are classified as key pests (Norris et al 2003). Key insect pests of sorghum are the greenbug and sorghum midge. The defining characteristic of a key insect pest is that it routinely causes severe damage to the sorghum crop in a large geographical area if the insect is not controlled. Other insect pests are classified as secondary and occasional pests.

Secondary pests have the potential to cause significant damage but usually are controlled by natural enemies (Stern and van den Bosch 1959). Indiscriminant use of insecticide against a key insect pest can result in a secondary pest causing economic damage. Secondary pests can rapidly infest a crop if natural enemies were eliminated. An example of a secondary pest of sorghum is the corn earworm.

Minor pests routinely damage a crop, but the damage is insignificant. An example of a minor pest of sorghum is the corn leaf aphid, *Rhopalosiphum maidis* (Fitch). The aphid, although typically abundant in a crop, usually does not cause significant damage (Teetes et al. 1993).

Occasional pests can significantly damage a crop, but are only occasional nuisances. An example of an occasional pest of sorghum is the yellow sugarcane aphid, *Sipha flava* (Forbes). Sorghum infested with as few as five to 10 yellow sugarcane aphids per leaf can easily kill a young sorghum plant (Teetes et al. 1993). However, despite the severity of the yellow sugarcane aphid, it only occasionally occurs and damages sorghum.

Although critical, identifying and classifying insect pests are only preliminary steps. Understanding the biology of an insect and its role in the agroecosystem will ultimately reveal potential control tactics. Five direct control tactics of IPM are: biological, chemical, cultural, physical, and legal controls (Dent 2000). Comprehending the life cycles, behavior, and overall biology of a pest will aid in implementing the correct control tactic. Furthermore, if multiple tactics are integrated, multiple insects can be controlled at once without severely unbalancing an agroecosystem.

The principal objective of IPM is to control pests effectively and economically without harming the environment. The control tactics most often used in sorghum IPM are cultural and chemical. Natural control can be accomplished when cultural control tactics are used (Rechcigl and Rechcigl 1999). Physical and legal control measures are irrelevant when considering the key insect pests of sorghum. Pest control is governed by the value of the crop. Sorghum is a low-value crop, and therefore, pest control must be inexpensive and effective (Elliott et al. 2017). Predominantly cultural and chemical controls are used for sorghum.

Cultural control renders the environment of a pest unfavorable. A preventative control measure, cultural control is considered to be the first line of defense for pest control. The aims of cultural control are to inhibit pest colonization and reproduction, and ultimately keep pests off a crop (Dent 2000). Cultural control conserves beneficial insects, and therefore, implements a biological control component defined as conservation biological control (Debach and Rosen 1991, Waage and Mills 1992).

Cultural control methods usually can be incorporated easily into farming practices (Dent 2000). For example, sorghum commonly is grown in rotation with broadleaf crops



such as cotton, *Gossypium hirsutum* L. (Cronholm et al. 1998). The purpose of rotating sorghum with a broadleaf crop is to mitigate the chances of repeat infestations by pests from the previous year that cannot use the broadleaf crop as a host. Insects that are able to deposit eggs and/or overwinter as adults will emerge in the spring, if the crop has been rotated with an unfavorable crop. Crop rotation is a cultural control tactic that is implemented easily.

Another cultural control tactic is use of resistant crop varieties. Once a sorghum pest discovers a sorghum crop, the pest will ascertain the favorability of the crop. The inherent attributes of a sorghum genotype render the plant either desirable or not desirable to pest insects. A pest insect will colonize susceptible sorghums easier than resistant sorghums (Painter 1951). This is because resistant sorghums are neither desirable nor preferred by pest insects. A resistant sorghum can possess attributes that affect the biology and/or behavior of an insect pest, or can potentially withstand infestation (Painter 1951). A resistant sorghum that affects the biology of a pest has antibiosis; a sorghum that affects the behavior of the pest exhibits antixenosis (Painter 1951, Kogan and Ortman 1978, Teetes 2006). Unlike antixenosis and antibiosis, tolerance is the response by a resistant plant to recover, repair, and/or withstand damage by an insect pest (Painter 1951).

Morphological and physiological characteristics render plants resistant to insects. Morphological characteristics are generally antixenotic and render the outside surface of a sorghum plant unfavorable. For example, a sorghum plant with excess epicuticular wax on the under surface of the leaf is less susceptible to damage by aphids (Pi and Hsieh 1982). Taller sorghums with fewer narrower leaves and that have a waxy lamina also are

resistant to aphids (Mote and Kadam 1984, Mote and Shahane 1994). However, unlike morphological characteristics of a plant, physiological characteristics caused by a pesticide deter feeding by an insect pest.

Sorghum possesses compounds that deter feeding and affect the biology of pests. Tissues of a plant have numerous pesticidal properties (Bruce et al. 1990). Many of the properties affect Aphididae, including but not limited to: tannic acid, benzaldehyde, *p*-hydroxybenzaldehyde, and procyanidin (Dreyer et al. 1981, Hsieh 1988). Upon feeding, aphids ingest the pesticidal constituents of plants that impact the biology of the insect. According to Teetes (1980), results of ingestion include increased mortality and decreased fecundity, longevity, and weight of aphids.

Resistant genotypes defend themselves and/or deter pests independent of insecticides. However, resistant genotypes are most effective when used with other control tactics. The synergistic effect of multiple control tactics is more advantageous than use of a single tactic. Therefore, cultural control cannot be the single solution in a pest management program.

Cultural control reduces the magnitude of pest abundance but fails to eradicate a pest (Norris et al. 2003). The primary objective of cultural control is to mitigate the chances of a pest insect surpassing the economic threshold. Culturally controlled pest insects are often naturally controlled and/or disperse (Rechcigl and Rechcigl 1999, Dent 2000, Norris et al. 2003). However, if pest abundance surpasses the economic threshold level and causes economic damage, a more aggressive tactic should be used.

Chemical control immediately lessens pest abundance. Insecticides, if timely and correctly applied, can reduce pest abundance >90% (Norris et al. 2003). Insecticides,

although effective, will not eradicate pest insects. Approximately 20% of crops in developed countries and  $\geq 40$ -50% in developing countries are destroyed by insect pests despite liberal application of insecticides (Schulten 1975, Elliot 2015). This is because of the deleterious outcomes of repeated application of insecticides (Stern and van den Bosch 1959). The pest, environment, and crop all should be considered before using chemical control (Dent 2000). Pests that economically damage the crop however, must be chemically controlled at the economic threshold level to avoid economic loss. Chemical control should be used only when necessary to avoid resistance and other problems caused by pests.

Sorghum IPM strategies change depending on the pest. Key insect pests of sorghum have been researched. Therefore, IPM strategies for key insect pests of sorghum have been refined to be as effective as possible. Each IPM tactic has been implemented to obtain the best combination. Invasive, occasional, and/or minor pests however, usually have not been thoroughly researched. The sugarcane aphid is considered an invasive, occasional, and/or minor pest of sorghum (Singh et al. 2004, Bowling et al 2016). The aphid was not thoroughly researched at the time of its debut on sorghum in North American.

### **Sugarcane Aphid History**

The sugarcane aphid is a pest of sorghum and sugarcane (Singh et al. 2004). The aphid infests sorghum and sugarcane in Africa, Asia, Australia, the Caribbean, North America, and South America (Zimmerman 1948, Mead 1978, ICRISAT 1985, Passlow 1985, Denmark 1988). Seven genera of Poaceae, including *Sorghum* and *Saccharum*, are classified as hosts of the sugarcane aphid (Raychaudhuri 1974). Despite its wide global

distribution and host range, the aphid was not reported in North America until 1977 (Mead 1978). Identified on sugarcane in Florida, the aphid was observed to infest 16 grass species. A secondary report regarding the first identification in 1977 also noted the impressive host range of the sugarcane aphid and deemed *Sorghum* and *Saccharum* as the most favorable hosts (Denmark 1988).

The sugarcane aphid was not reported again until 1999 (White et al. 2001). The aphid was once again identified on sugarcane, but had dispersed from Florida to Louisiana. The economic importance of the aphid still was not understood. White et al. (2001) alluded to the conclusions of Denmark (1988) and mentioned the ability of the aphid to attack *Saccharum*, *Sorghum*, *Oryza*, *Echinochloa*, *Panicum*, and *Pennisetum*. The conventional consensus of the economic impact of the aphid still was theoretical even after 22 years (Mead 1978, Denmark 1988, White et al. 2001). However, the reports concurred that sorghum was a suitable host for the sugarcane aphid.

The sugarcane aphid was reported on North American sorghum in 2013. The aphid was first detected near Beaumont, Texas, then Louisiana and Mississippi, Oklahoma, and three northeastern states of Mexico during the growing season of 2013 (Villanueva et al. 2014, Bowling et al. 2016). Unlike previous accounts, the aphid was able to overwinter in the northern state of Tamaulipas, Mexico, as well as South Texas where it infested early season sorghum and dispersed northward (Armstrong et al. 2015). This probably was because of the humid subtropical climate and mild winters at both of the regions.

The sugarcane aphid then dispersed as far as Georgia, Tennessee, and Florida, and ultimately was reported in 12 states during the sorghum-growing season of 2014 (Colares

2015b). By the growing season of 2015, the sugarcane aphid was observed infesting sorghum in more than 400 counties in 17 states of the United States, along with many reports in Mexico (Bowling et al. 2015). Compared to 2014, the sugarcane aphid was reported 25% more often and threatened more than 90% of sorghum production in the United States in 2015 (Bowling et al. 2015, USDA-NASS 2015).

### **Sugarcane Aphid Description**

Polyphenism is a phenomenon where two or more discrete phenotypes, without intermediate forms, can be produced from a single genotype (Nijhout 1999, 2003).

Aphids exhibit polyphenism more than any other insect group. Depending on the species, aphids are capable of producing as many as eight discrete phenotypes (Moran 1992). Sugarcane aphids possess polyphenic traits. Multiple, visually distinctive individuals from the same mother can be found commonly in sugarcane aphid colonies. Species with distinctive morphs often are different sizes. The sugarcane aphid is no exception.

Adults are predominantly female and either apterous (non-winged) or alate (winged) (Blackman and Eastop 1984). An adult alate aphid is 1.3-1.84 mm long by 0.56-0.90 mm wide (Raychaudhuri and Banerjee 1974). However, an adult apterous aphid is 1.52-1.66 mm long by 0.44-1.34 mm wide. Alate sugarcane aphids, disregarding their wings, can be compared to large apterous aphids in body shape. However, alate aphids typically have black markings along the dorsal sclerites of the abdomen, as well as hardened black structures at the base of the wings (Raychaudhuri and Banerjee 1974, Blackman and Eastop 1984, Bowling et al. 2016). The aphid can be either of the two forms. However, their color also varies.

Sugarcane aphids vary in color depending on environmental conditions as well as their host plant (Denmark 1988, Teetes et al. 1993, Knutson et al. 2016). White, black, and even pink aphids have been observed; however, they are commonly buff in North America (Teetes et al. 1993). Despite the variable color, they can be identified by their distinctive dark antennae, tarsi, and cornicles at the rear of the abdomen (Raychaudhuri and Banerjee 1974, Villanueva et al. 2014). Their distinctive colors allow sugarcane aphids to be distinguished easily from other aphids that are common on sorghum (Villanueva et al. 2014, Bowling et al. 2016). Correctly identifying the sugarcane aphid is essential for control.

### **Damage by Sugarcane Aphid**

A sugarcane aphid feeds by intracellularly piercing its stylet mouthparts into the vascular tissue and sucking the liquid contents of a plant (Minks et al. 1989). Thereby, damage by sugarcane aphids is attributed to loss of phloem and nutrients (van den Berg et al. 2003). Feeding also causes a significant reduction in nitrogen, phosphorus, potassium, total sugars, and chlorophyll (Singh et al. 2004). The aphid according to Elliott et al. (2017) exhibits no partiality and can be found on leaves of sorghum of any age. Honeydew, the excreted by-product of feeding by an aphid, also can reduce photosynthesis and result in build-up of sooty mold.

A single sorghum plant can host as many as 30,000 aphids (Singh et al. 2004). Exponential increase during the early season, followed by mass dispersal in mid-season, probably because of host degradation, is historically common (van Rensburg and van Hamburg 1975, Singh et al. 2004). Infestation early in the growing season can cause

stunting, chlorosis, and necrosis of sorghum plants. However, outbreaks regularly occur during the flag-leaf to flowering stages of sorghum growth (van Rensburg 1973, Narayana 1975, van den Berg et al. 2003). Late infestations reduce the quality and size of developing sorghum kernels. Nonetheless, sorghum plants are at risk of death when infested, regardless of the growth stage.

If not controlled, sugarcane aphids can severely reduce sorghum yield potential by  $\geq 60\%$  (van Rensburg 1973, Manthe 1992, van den Berg 2002). Zapata et al. (2018) estimated the aphid caused a total economic loss of \$23.33 million dollars in 2014 and \$17.62 million dollars in 2015 in the Rio Grande area of Texas. Elliott et al. (2017) estimated an economic loss of \$47 million dollars in Texas, Oklahoma, and Kansas in 2014. In 2014 and 2015, resistant sorghum genotypes and insecticides had not yet been evaluated against sugarcane aphids in the United States, which probably affected the total economic loss.

The economic threshold level was established at 40 aphids per sorghum leaf regardless of location (Gordy et al. 2019). However, if the environment is favorable, the economic threshold level can be as few as 19 aphids per leaf. Sugarcane aphids are environmentally responsive, and thus, location-specific economic thresholds should be developed. Recently infested sorghum is indistinguishable from non-infested sorghum; therefore, frequent monitoring is necessary (Bowling et al. 2016). However, correctly identifying the aphid is integral for monitoring aphid abundance.

### **Sugarcane Aphid Control**

Sugarcane aphids predominantly are controlled culturally and chemically. Cultural control is used to lessen applications of costly insecticides; however, once the

economic threshold level is reached, insecticides are necessary to avoid severe loss of yield. Data and resources regarding insecticides to effectively control sugarcane aphids are widely available (Bowling et al. 2016, Greenbook 2019).

Cultural control of sugarcane aphids is predominantly agronomic. According to van Rensburg (1979) and Mask et al. (1988), increasing the plant population and early planting would lessen chances of economic damage. Furthermore, maintaining the field by removing refuges of ratooned sorghum and johnsongrass, *Sorghum halepense* (L.) Pers., probably will eliminate local populations of aphids (Singh et al. 2004).

Cultural control of sugarcane aphids also will recruit natural enemies. More than 47 species of natural enemies have been documented to control sugarcane aphids worldwide (Singh et al. 2004). Lady beetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysomelidae and Hemerobiidae), and hover flies (Diptera: Syrphidae) are the most effective natural enemies. Harboring and recruiting beneficial insects can be done inadvertently by using cultural controls and refraining from using insecticide (Rechcigl and Rechcigl 1999, Dent 2000, Norris et al. 2003). According to Colares et al. (2015a), greater rates of predation also can be achieved by increasing species diversity. Crop rotation, strip cropping, and culturally maintaining field margins can increase species diversity. Abundance of sugarcane aphids can be maintained below the economic threshold level if natural enemies are present (van Rensburg 1973, Meksongsee and Chawanapong 1985, Singh et al. 2004). Natural enemies commonly regulate aphids late in the season (van Rensburg and van Hamburg 1975). Cultural control tactics that exploit and harbor natural enemies are immensely effective against sugarcane aphids.



Resistant varieties also can be used. Sorghums resistant to sugarcane aphids have been found in Taiwan (Hsieh and PI 1982, Pi and Hsieh 1982), India (Mote and Kadam 1984; Mote et al. 1985; Singh et al. 2004; Sharma et al. 2013, 2014), Japan (Hagio et al. 1985, Hagio and Ono 1986, Kawada 1995), China (Chang 1981, Chang and Fang 1984), Africa (Teetes et al. 1995), and North America (Armstrong et al. 2015, Bowling et al. 2016, Peterson et al. 2018). Sorghum breeders currently are striving to produce more resistant sorghums that are available commercially.

These concepts have been applied to control several species of aphids on sorghum. Luckily, the sugarcane aphid is responsive to general aphid control; however, species-specific control tactics are more effective. Understanding the biology of the sugarcane aphid is necessary for developing appropriate control tactics.

### **Reproduction and Life Cycle of Sugarcane Aphid**

Aphids were the first parthenogenetic animals identified (Bonnet 1745). Defined as thelytokous parthenogenesis, or simply thelytoky, aphids reproduce clonal daughters from their own nonfertilized eggs independent of genetic recombination (Blackman 1980, White 1984).

A chromosomal mechanism ensures an identical genetic identity between the mother and daughter (Moran 1992). Aphids are capable of bypassing sexual reproduction and investing 100% of their resources into production of clonal daughters (Feigel et al. 2009, Gibson et al. 2016). Although capable of entirely bypassing sexual reproduction, most aphids still sexually reproduce once each year. Bypassing sexual reproduction, even for a short period of time, allows for prodigious rates of increase by telescoping the generations.

Comparable to a matryoshka doll, mature aphid females telescope generations by producing female nymphs that already are gestating embryos (Dixon 1985a, Guerrieri and Digilio 2008). Depending on the favorability of the environment, aphids are capable of exponentially increasing in abundance (Dixon 1985b). Relying exclusively on parthenogenesis, however, can be deleterious.

Aphids that rely solely on thelytokous parthenogenesis to reproduce are defined as anholocyclic. Anholocyclic aphids do not have a sexual phase in their life cycles. Omission of the sexual phase occurs either because of the environment that does not promote sex-inducing conditions, or a permanent genetic mutation (Moran 1992, Simon et al. 2002). Typically, aphids rely on sexual reproduction to produce eggs far more capable of overwintering. This allows the species to remain at a given location despite seasonal change. Therefore, anholocyclic aphids are less likely to survive the winter if the environment becomes unfavorable. Locations that are climatically favorable, however, can harbor anholocyclic aphids throughout the year. Aphids at the locations are capable of reproducing during every season. Although true anholocyclic species are rare, most aphid pests are considered to be geographically anholocyclic (Dixon 1985b, Moran 1992). Geographically anholocyclic species are not necessarily genetically devoid of the ability to reproduce sexually. The environmental stimuli that initiate sexual reproduction are absent; therefore, aphids at these locations will never reproduce sexually. Most aphids, however, are holocyclic and carry out cyclical thelytokous parthenogenesis (Blackman and Eastop 1984, Dixon 1985ab).

Cyclical thelytokous parthenogenesis is characterized as several parthenogenetic generations followed by a single sexual generation produced during an annual life cycle

(Dixon 1985ab, Tagu et al. 2005). Thus, holocyclic aphids benefit from sexual and asexual reproduction. The behavior of the sexual phase of a holocyclic life cycle differs depending on the species of aphid. A host-alternating species will reproduce sexually on a primary perennial plant so the eggs can overwinter successfully (Dixon 1985b, Moran 1992). After diapause, the overwintered eggs will hatch fundatrices that parthenogenetically reproduce alate female offspring that soon disperse onto a secondary host. Non-host alternating species will never disperse off a host plant (Dixon 1985a, Moran 1992, Tagu et al. 2005). Sexual reproduction generally is controlled by a change in photoperiod and temperature (Marcovitch 1924; Lees 1960, 1966; Dixon 1972).

According to Bowling et al. (2016), the sugarcane aphid is an anholocyclic species in North America. Singh et al. (2004) also reported the aphid was anholocyclic throughout most of its range in tropical and subtropical regions. However, tropical and subtropical aphids typically seem to be almost entirely anholocyclic (Blackman and Eastop 1984). Sexual morphs (Wang 1961, Yadava 1966, Setokuchi 1975, David and Sandhu 1976) and eggs (Peña-Martinez et al. 2016) have been reported, and thus eliminating the possibility of an aphid being solely anholocyclic. Because of the reports, the sugarcane aphid is presumed to be anholocyclic because of the environment and not because of a permanent genetic mutation in North America. Therefore, the aphid is geographically parthenogenetic (Vandel 1928, Simon et al. 2002). Because of omission of a sexual phase, the sugarcane aphid is at increased risk of accumulating deleterious mutations (Dixon 1985b, Peng et al. 2016). The sex-inducing conditions that govern the sexual phase of the sugarcane aphid life cycle have yet to be discovered. Although the aphid might not reproduce sexually, it still is responsive to environmental stimuli.

In a favorable environment, sugarcane aphids have the potential to produce as many as 20 generations per year (Singh et al. 2004, Guo et al 2011). In a near-perfect environment, sugarcane aphids might produce  $\geq 60$  generations per year (Chang et al. 1982).

The sugarcane aphid was documented to have a life span of 10 to 37 days, with fecundity of 30 to 100 nymphs during the life time (van Rensburg 1973; Chang et al 1982; Meksongsee and Chawanapoong 1985; Sharma et al. 2013, 2014). Each nymph develops through four stadia during 4.3-12.4 days (van Rensburg 1973a, Chang et al. 1982, Manthe 1992). The number of molts depends on the population density; however, the average number of nymphal molts is four (Manthe 1992). The variability in biology is a testament to dependence of a sugarcane aphid on the environment.

The success of an aphid species depends on the ecosystem (Blackman and Eastop 1984, Dixon 1985a). The favorability of an ecosystem dictates the fitness of an aphid species. Density-dependent and independent factors regulate the abundance of aphids (Watson and Carter 1983, Stiling 1988, Maudsley et al. 1996). Aphids are affected both by density-independent and dependent factors that ultimately will dictate their success.

Abiotic influences that impact aphid fitness regardless of abundance are defined as density-independent factors. Although density-independent factors can be random, dominant factors of aphid fitness are classified as seasonal influences (Shankar 1991, Maudsley et al. 1996, Prakash and Tomar 2010). Seasonal influences are cyclical and have been used historically in a forecasting model to predict abundance of various aphid species (A'Brook 1983, Watson et al. 1983, Prakash and Tomar 2010). Furthermore, seasonal changes in weather influence fitness of sugarcane aphids (van Rensburg 1973,

Mote 1983, Balikai 2001). Historically, temperature and precipitation have had an especially great impact on population dynamics of not only sugarcane aphids but all species of aphids (Marcovitch 1924, Chang et al. 1982, Kodet et al. 1982). Therefore, temperature and precipitation were wrongly considered to be the only driving forces of aphid population dynamics.

Photoperiod is an influential influence on population dynamics. Aphids were the first insect, as well as animal, to be defined as photoperiodic (Marcovitch 1924). A photoperiodic species depends on the metronomic nature of photoperiod to time life cycles and development. Unlike weather, photoperiod is a constant indicator of seasonal change in a non-equatorial environment. Although photoperiod generally is considered to be the primary indicator, temperature also indicates seasonal change (Kodet et al. 1982, Nylin and Gotthard 1998, Tagu et al. 2005, Saunders 2014). Holocyclic species rely on the elongating dark periods (scotophases) of autumn to produce oviparae (Bünning 1936, Saunders 1982, Dixon 1985b, Moran 1992, Saunders 2012). Although typically associated with sexual reproduction, photoperiod also can affect heteroecy, wing production, and polyphenism (Dixon 1971, Lees 1986, Hardie and Vaz Nunes 2001, Tagu et al. 2005). Aphids use photoreceptors to detect change in the photoperiod.

A photoreceptor is a sensory organ used by aphids to discern the difference between light and dark. These photoreceptors are considered to be extra optics, located in the protocerebrum, independent of the eyes and optic lobes (Lees 1964, Hardie and Vaz Nunes 2001, Tagu et al 2005). Because of their location, light must pass through the cuticle as well as any overlying tissues to stimulate the photoreceptors (Hardie et al. 1981). Aphids are not only able to discern the difference but also time their exposure.

Biological clock-counter mechanisms measure the duration of the scotophase in the life cycle of an aphid (Bünning 1936, Lees 1973). The scotophase is defined as the dark phase of a light:dark cycle. Aphids perceive seasonal change by night duration (Hardie and Vaz Nunes 2001, Tagu et al 2005). The length of the scotophase dictates the hormones that promote classical photoperiodic responses such as sexual reproduction. Notwithstanding the other governing factors of sexual reproduction, a scotophase of  $\geq 9$ -10 hours typically induces sexual reproduction in most species of aphids (Trionnaire 2008). The type of clock-counter associated with the response has been greatly scrutinized.

Lees (1973) considered the photoperiodic clock-counter of the vetch aphid, *Megoura viciae* Buckton, to be a non-circadian hourglass mechanism. Hardie and Vaz Nunes (2001) however, later considered the clock-counter of the vetch aphid to be a slowly damping circadian oscillator. Because of numerous biological inconsistencies, a definitive clock model has yet to be discovered for any species of aphid (Saunders et al. 2005, Tagu et al. 2005, Matsuda 2017). Aphids nonetheless respond to changes in photoperiod.

## **CHAPTER III**

### **MATERIALS AND METHODS**

#### **Photoperiod Study**

##### **Plant Material**

The susceptible sorghum hybrid ‘ATx399 x RTx430’, developed by the Texas Agricultural Experiment Station, was used. For each experiment at different photoperiods, six perforated Azalea plastic plant pots with diameter and height of 10.1 by 14.0 cm, respectively, were filled with Miracle-Gro Enriched Potting Mix with Miracle-Gro Plant Food (Miracle-Gro Lawn Products, Inc., Port Washington, NY) and planted with 10 seeds per pot. Each of the six Azalea plastic pots was placed into a clear-plastic saucer (diameter of 12.6 cm and height of 9.2 cm) filled to the brim with water. The pots remained in the saucer until the soil surface was wet. The water was drained as soon as the soil surface was wet. Once germinated, the sorghum seedlings were watered when necessary from the clear plastic saucer. Refraining from surface watering mitigated unnecessary soil disturbances and accumulation of minerals on the soil surface. The seedlings were thinned at the three true-leaf stage to four plants per pot. This was done to reduce competition among plants. The four remaining plants were maintained in the greenhouse until they developed into the seven true-leaf stage.

## **Infestation and Experiments with Sugarcane Aphids**

At the seven true-leaf stage, each experimental plant was infested with eight adult apterous sugarcane aphids ( $F_0$  generation), comparable in age and weight, for a total of 48 aphids per experiment. Aphids were obtained from a pure culture maintained on ATx399 x RTx430 sorghum in a greenhouse. A fine camel-hair brush was used to gently transfer an aphid from the culture into a 2.5-cm<sup>3</sup> plastic clip cage secured onto a leaf of an experimental sorghum plant. An approximately 10-mm-diameter hole was drilled into both sides of the clip cage and covered by organdy cloth attached by hot glue to ensure air flow in the cage. Each clip cage was numbered with a permanent marker. Two clip cages per plant, containing one apterous mother aphid each, were attached to each of the four sorghum plants per pot, for a total of eight clip cages per pot. Six pots were used for each experiment; therefore, a total of 48 aphids in individual clip cages were used per photoperiod in a completely randomized design. A sample size of 48 ensured statistical power and lessened a chance of statistical error. Once infested, the six pots were transferred to a Precision model 818 Microprocessor-Controlled, Low-Temperature, Illuminated Incubator (Precision, Winchester, VA). Three photoperiods of 12:12, 13:11, and 14:10 light:dark hours were used for the study in the incubator. Temperature and humidity were held constant at 30:20 °C (light:dark hours) and 60% relative humidity. Humidity was maintained by hygrometer and water tray in the incubator. Photoperiods were chosen to represent the entire growing season of sorghum at Canyon, Texas.

The original mothers of the  $F_0$  generation of sugarcane aphids transferred from the pure culture were monitored until they each had produced a nymph ( $F_1$  generation).



Once the  $F_1$  generation was born, the birth dates of the aphids were recorded, and the mother aphids of the  $F_0$  generation were discarded. Nymphs of the  $F_1$  generation remained in the clip cages and were allowed to mature. The plant pots were rotated in the incubator each day to ensure that each aphid received an equal amount of light. The process was repeated for each photoperiod.

### **Fecundity and Longevity**

Once mature, aphids of the  $F_1$  generation began producing offspring and were monitored until death. The nymphs produced by each aphid mother of the  $F_1$  generation were removed daily by using a fine camel-hair brush, without disturbing the mother. The pre-reproductive period, reproductive period, post-reproductive period, daily fecundity, total fecundity, and longevity of the mother aphid were evaluated for each photoperiod.

The pre-reproductive period was considered to be the time (days) between the birth of an  $F_1$  generation aphid and production of her first nymph. The reproductive period was the time (days) between production of the first and the last nymphs. The post-reproductive period was the time (days) from when the last offspring was produced until the mother aphid died. Daily fecundity was the number of nymphs produced per aphid per day. Total fecundity was the total number of nymphs produced per aphid. Longevity was the total number of days each aphid lived. All assessments for the photoperiods were of the  $F_1$  generation.

### **Intrinsic Rate of Increase and Mean Generation Time**

The intrinsic rate of increase ( $r_m$ ) was defined as the rate of increase per insect under specified physical conditions in an unlimited environment. Birch (1948) deemed

the intrinsic rate of increase as the appropriate parameter for researching and quantifying insect population dynamics. Wyatt and White (1977) modified the formula of Birch for use in research on aphids and mites. The formula was used for determining the intrinsic rate of increase and the mean generation time.

The Wyatt and White (1977) formula for the intrinsic rate of increase was:

$$rm = 0.738 (\log_e M_d)/d, \text{ where}$$

rm = intrinsic rate of increase

d = pre-reproductive period

Md = number of young produced in a reproductive period equivalent to the pre-reproductive period (d).

The mean generation time, defined as the time interval (days) between two consecutive generations, was calculated for each aphid by using the formula:

$$T_d = d/0.738, \text{ where}$$

Td = mean generation time and

d = pre-reproductive period.

The assumption was used that 95% of the intrinsic rate of increase of an aphid or mite was achieved during twice the amount of time of the pre-reproductive period (d).

### **Statistical Analysis**

All data were analyzed by Statistical Analysis System software (SAS) version 9.4 (SAS Institute, Cary, NC). Averages of data on pre-reproductive period, reproductive period, post-reproductive period, daily fecundity, total fecundity, and longevity were quantified per photoperiod. The intrinsic rate of increase and mean generation time also

were analyzed for each photoperiod. All data were tested for assumptions of normality (Shapiro-Wilks test) and homogeneity of variance (Levine's test) before analysis of variance (ANOVA). The Mixed procedure of SAS was used for ANOVA ( $P \leq 0.05$ ). The least significant difference (LSD) test at  $P = 0.05$  was used to separate means of the data.

### **Brix Refractometry Study**

#### **Plant Material**

Susceptible 'RTx430' and resistant 'RTx2783' sorghum R-lines were used for the study (Peterson et al 1984). A study in a greenhouse with a secondary study in the field were done. The study in the greenhouse consisted of a total of 40 perforated Azalea plastic pots with diameter and height of 10.1 by 14 cm, respectively, filled with Miracle-Gro Enriched Potting Mix with Miracle-Gro Plant Food (Miracle-Gro Lawn Products, Inc., Port Washington, NY). Four seeds of a sorghum line were planted into each of eight labeled pots. The pots were put into a clear-plastic saucer (diameter of 12.6 cm and height of 9.2 cm) filled with water. Each pot remained in the saucer until the surface of the soil was moist. When the soil surface was moist, the water was drained from the saucer. Each pot was watered when necessary during the remainder of the experiment by filling the saucer with water. The seedlings at the three true-leaf stage were thinned to one plant per pot to reduce competition among the plants. The plants were maintained in the greenhouse. The field study was done at the Scott Seed research complex near Amarillo, Texas. A 100-square-foot experimental plot was prepared with 152-cm beds. The previous crop grown in the plot was wheat, *Triticum aestivum* L. The plot was fertilized with cow manure before planting. The plot was planted on 11 May 2019 on 76-

cm rows with 185,000 plants per hectare. A 2-m row of both RTx430 and RTx2783 were planted by using an Almaco two-row cone plot planter. The plot was irrigated in the furrow, when necessary.

### **Brix Values**

At the boot stage, a healthy mature leaf was chosen randomly from each of the 40 plants in the pots in the greenhouse. Each leaf was crushed separately by a hand-held sap press (Spectrum Technologies, Aurora, Illinois). Approximately 5 ml of sap were pipetted from the press into an Atago 3810 PAL-1 Digital Hand-held Pocket Refractometer (Atago, Tokyo, Japan). A °Brix value is meant to be interpreted as the total sugar content (Kawahigashi et al. 2013, Simeone et al. 2017). The Atago 3810 PAL-1 Digital Hand-held Pocket Refractometer was calibrated using distilled water. The values were recorded and the plants discarded to eliminate repeat sampling. The same procedure was used in the study in the field, but only 20 sorghum plants were tested. This was because of detrimental weather early in the season that resulted in a sparse plant stand. The temperature of each sample was recorded to ensure uniform °Brix values.

### **Statistical Analysis**

All data were analyzed by Statistical Analysis System software (SAS) version 9.4 (SAS Institute, Cary, NC). The data on the average Brix measurement also were analyzed by sorghum line. All data were tested for assumption of normality (Shapiro-Wilks test) and homogeneity of variance (Levine's test) before analysis of variance (ANOVA). The Mixed procedure of SAS was used for ANOVA ( $P \leq 0.05$ ). The least significant difference (LSD) test at  $P = 0.05$  was used to separate the means of the data.

## CHAPTER IV

### RESULTS AND DISCUSSION

#### **Pre-reproductive Period**

The pre-reproductive period, the time (days) before sugarcane aphids began to reproduce, increased as the scotophase shortened. At 12:12 light:dark hours, the pre-reproductive period of sugarcane aphids ranged from only 2 to 5 days (Table 1). At 13:11, the pre-reproductive period of sugarcane aphids ranged from 3 to 6 days. At 14:10 light:dark hours, the pre-reproductive period ranged from 3 to 12 days. The number of days of the pre-reproductive period for sugarcane aphids varied more as the scotophase shortened. The coefficients of variation at 12:12, 13:11, and 14:10 photophase:scotophase were 32.9, 21.9, and 27.4, respectively. Thus, the standard deviation of the mean was greatest at 12:12 and least at 13:11 light:dark hours. Photoperiod significantly affected the duration of the pre-reproductive period of sugarcane aphids ( $F_{2,141} = 101.45$ ;  $P < 0.0001$ ).

The pre-reproductive periods of sugarcane aphids exposed to photoperiods of 13:11 and 14:10 light:dark hours were 1.5 and 2.5 times longer, respectively, than aphids exposed to a photoperiod of 12:12 light:dark hours. This was potentially because of a biological mechanism that allowed the aphid to begin to increase in abundance in response to season, with more nymphs produced as the number of hours of light increased during the day.

Van Rensburg (1973) concluded that the abundance of sugarcane aphids probably increased as a function of time rather than host plant quality or age. Photoperiod is a function of time. The development of sugarcane aphids probably is regulated equally by environmental influences such as temperature and humidity, as well as time. The life cycle of a holocyclic aphid probably is regulated by the length of the scotophase (Lees 1973, Hardie and Vaz Nunes 2001, Tagu et al 2005). Therefore, the scotophase probably is the trigger for response to photoperiod. As the scotophase shortened, the pre-reproductive period lengthened (Table 1).

The influence of photoperiod on the length of the pre-reproductive period relative to longevity was statistically significant ( $F_{2,141} = 3.19$ ;  $P = 0.0442$ ). The length of the pre-reproductive period at photoperiods of 12:12 and 13:11 light:dark hours was statistically similar. However, the pre-reproductive period of sugarcane aphids at a photoperiod of 14:10 light:dark hours differed and was significantly longer than that at the other photoperiods (Table 1). Sugarcane aphids exposed to photoperiods of 12:12 and 13:11 light:dark hours spent an average of 23% of their life times during the pre-reproductive period. However, sugarcane aphids reared at a photoperiod of 14:10 light:dark hours spent 33% of their life times during the pre-reproduction period. This indicated that a scotophase of 10 hours lengthened the amount of time spent during the pre-reproductive period by an average of 11%.

### **Reproductive Period**

The amount of time (days) the sugarcane aphids spent producing offspring was not influenced statistically by photoperiod ( $F_{2,141} = 1.55$ ;  $P = 0.2154$ ). The sugarcane

Table 1. Effect of Photoperiod on the Mean ( $\pm$  SE) Length of the Pre-reproductive Period and Percentage of the Pre-reproductive Period Relative to the Longevity of Sugarcane Aphids on Sorghum

Photoperiod	Mean $\pm$ SE	CV
Pre-reproductive period (Days)		
12:12 P:S	2.90 $\pm$ 0.137c	32.82
13:11 P:S	4.20 $\pm$ 0.133b	21.89
14:10 P:S	6.60 $\pm$ 0.260a	27.36
Percentage of lifetime		
12:12 P:S	23.12 $\pm$ 3.82a	114.74
13:11 P:S	23.41 $\pm$ 2.81a	83.17
14:10 P:S	33.78 $\pm$ 3.48b	71.31

Number of observations per photoperiod = 48.

P = photophase (light hours), S = scotophase (dark hours).

CV = coefficient of variation.

aphids reproduced for the same number of days regardless of photoperiod. However, photoperiod influenced the length of the reproductive period relative to longevity ( $F_{2,141} = 7.22$ ;  $P = 0.001$ ). Aphids exposed to photoperiods of 13:11 or 14:10 light:dark hours were statistically similar and spent 50% of their life times in the reproductive period (Table 2).

Aphids exposed to a photoperiod of 12:12 light:dark hours, however, spent an average of 64% of their life times in the reproductive period (Table 2). A photoperiod of 12:12 light:dark hours resulted in a 14% increase in the amount of time, relative to overall longevity, spent during the reproductive period. The significant influence of 12:12 light:dark hours on the amount of time, relative to overall longevity, is inconsequential. Sugarcane aphids exposed to a photoperiod of 12:12 light:dark hours lived an average of 19 days as compared to 23 days (13:11) and 24 days (14:10). The overall effect of photoperiod on the length (days) of the reproductive period of sugarcane aphids was not significant. Therefore, the significance ascertained was because of relative longevity rather than a true increase in the amount of time (days) spent reproducing.

### **Post-reproductive Period**

The photoperiod significantly affected the length (days) of the post-reproductive period ( $F_{2,141} = 15.69$ ;  $P < 0.0001$ ). The average lengths (days) of the post-reproductive periods at photoperiods of 12:12, 13:11, and 14:10 light:dark hours were 2.4, 7.1, and 4.3 days, respectively (Table 3). Unlike other reproductive periods, no linear trend was discerned. Shortening of the scotophase did not result in lengthening of the post-



Table 2. Effect of Photoperiod on the Mean ( $\pm$  SE) Length of the Reproductive Period and Percentage of the Reproductive Period Relative to the Longevity of Sugarcane

Aphids on Sorghum

Photoperiod	Mean $\pm$ SE	CV
Reproductive period (days)		
12:12 P:S	13.66 $\pm$ 0.987	50.04
13:11 P:S	11.81 $\pm$ 0.670	39.35
14:10 P:S	13.93 $\pm$ 1.070	53.55
Percentage of lifetime		
12:12 P:S	64.91 $\pm$ 3.79a	40.49
13:11 P:S	50.12 $\pm$ 2.40b	33.84
14:10 P:S	50.69 $\pm$ 2.96b	40.44

Number of observations per photoperiod = 48.

P = photophase (light hours), S = scotophase (dark hours).

CV = coefficient of variation.

Table 3. Effect of Photoperiod on the Mean ( $\pm$  SE) Length of the Post-reproductive Period and Percentage of the Post-reproductive Period Relative to the Longevity of Sugarcane Aphids on Sorghum

Photoperiod	Mean $\pm$ SE	CV
Post-Reproductive period (days)		
12:12 P:S	2.41 $\pm$ 0.400c	114.78
13:11 P:S	7.14 $\pm$ 0.809a	78.42
14:10 P:S	4.33 $\pm$ 0.517b	82.64
Percentage of lifetime		
12:12 P:S	11.78 $\pm$ 1.79b	105.67
13:11 P:S	26.45 $\pm$ 2.51a	65.71
14:10 P:S	15.52 $\pm$ 1.59b	71.04

Number of observations per photoperiod = 48.

P = photophase (light hours), S = scotophase (dark hours).

CV = coefficient of variation.

reproductive period. The post-reproductive period also was variable. The coefficients of variation at photoperiods of 12:12, 13:11, and 14:10 light:dark hours were 114.78, 78.42, and 82.64, respectively. This indicated that the standard deviation of the mean was greatest at a photoperiod of 12:12 and least at a photoperiod of 13:11 light:dark hours. The variability probably was because sugarcane aphids frequently died before a post-reproductive period.

Irrespective of photoperiod, sugarcane aphids routinely reproduced until the day they died. The large amount of variability, although not inconsequential, was therefore, probably because of a skewed data set resulting in large standard deviations. Many aphids reared at a photoperiod of 12:12 light:dark hours did not have a post-reproductive period and produced nymphs until they died. Therefore, the values of the coefficient of variation were exceedingly large. Photoperiod also significantly affected the length (days) of the post-reproductive period relative to longevity ( $F_{2,141} = 14.46$ ;  $P < 0.0001$ ). The mean percentages of the life time each sugarcane aphids spent during the post-reproductive period at photoperiods of 12:12, 13:11, and 14:10 light:dark hours were 11, 26, and 15%, respectively (Table 3). Aphids exposed to 12:12 and 14:10 light:dark hours were statistically similar. Aphids exposed to 13:11 however, spent <20% more of the life time during the post-reproductive period.

### **Longevity**

Photoperiod notably affected the longevity of the sugarcane aphid ( $F_{2,141} = 7.28$ ;  $P = 0.0010$ ). The average life times (days) of the sugarcane aphid at photoperiods of 12:12, 13:11, and 14:10 light:dark hours were 19.0, 23.2, and 24.9, respectively (Table 4). The sugarcane aphids reared at a photoperiod of 12:12 light:dark hours lived for the fewest

Table 4. Descriptive Statistics on the Longevity (Days) of Sugarcane Aphids on Sorghum at Different Photoperiods

Photoperiod	Mean $\pm$ SE	CV
12:12 P:S	19.02 $\pm$ 0.99b	36.12
13:11 P:S	23.16 $\pm$ 1.10a	33.13
14:10 P:S	24.86 $\pm$ 1.24a	34.39

Number of observations per photoperiod = 48.

P = photophase (light hours), S = scotophase (dark hours).

CV = coefficient of variation.

number of days and were statistically distinctive. However, shortening the scotophase did not further influence longevity. Aphids reared with photoperiods of 13:11 and 14:10 light:dark hours were statistically similar to each other and lived for fewer than 22 days. The coefficient of variation ranged from 33.13 to 36.12 despite a change in photoperiod.

The curves for average daily fecundity were similar across all three photoperiods. The curves exponentially increased, peaked, and then decreased. Photoperiod directly influenced the peak of the curve. The curve for daily fecundity at the photoperiod of 12:12 light:dark hours was steepest and peaked at  $\geq 8$  aphids per day (Fig. 1). The second largest peak ( $>7$  aphids per day) was at a photoperiod of 13:11 light:dark hours. The third steepest peak of  $\geq 6$  aphids per day was at a photoperiod of 14:10 light:dark hours. The values were expressed by inequality symbols because of biological inconsistencies.

Photoperiod influenced the amount of time (days) before climax in the number of nymphs produced. Climax is defined as the day at which the maximum number of nymphs were produced. As the length of the scotophase shortened, the amount of time (days) before climax lengthened. Climax did not occur until Day 6 at a photoperiod of 12:12, Day 9 at 13:11 light:dark hours, and Day 11 at 14:10. The longer amount of time before climax was expected because of the effect of photoperiod on the length of the pre-reproductive period.

The average numbers of sugarcane aphid nymphs produced at the climax for photoperiods of 12:12, 13:11, and 14:10 light:dark hours totaled eight, seven, and six, respectively (Fig. 1). Photoperiod also influenced the most productive period of the sugarcane aphid. The most productive period during the life of the sugarcane aphids occurred during a 4-day period regardless of photoperiod. Photoperiod, however,

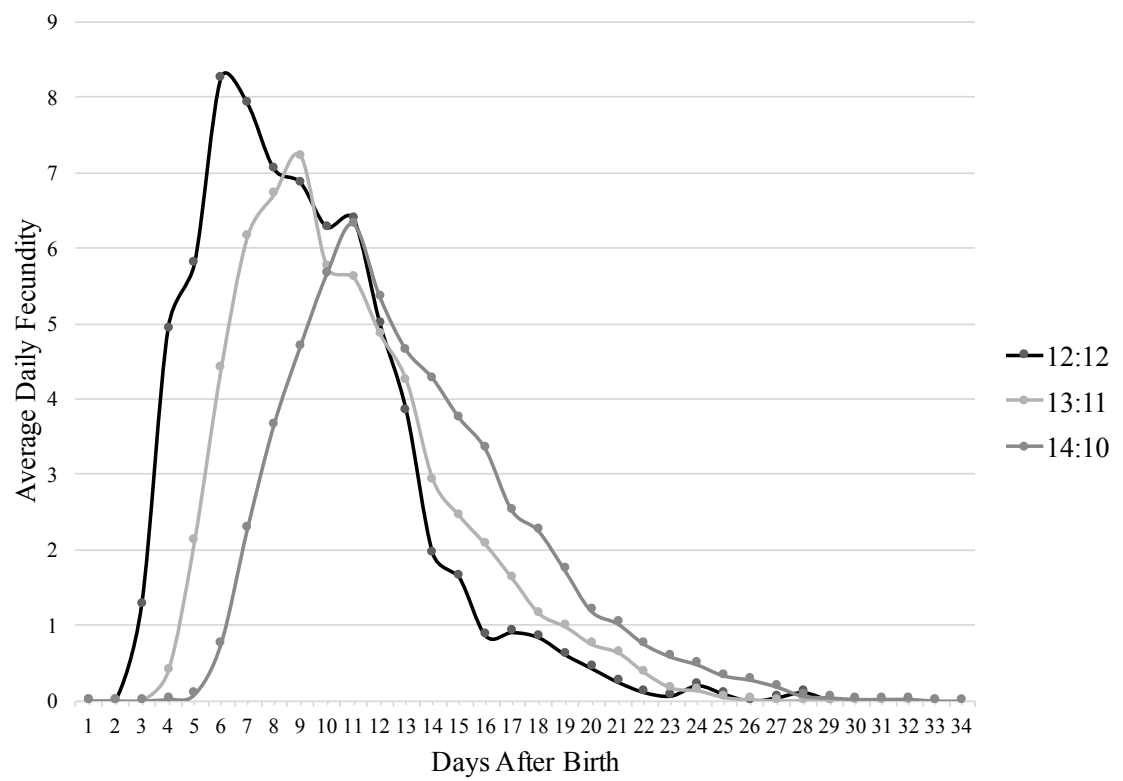


Fig. 1. Average daily fecundity (nymphs) per sugarcane aphid at different photoperiods on sorghum.

influenced the timing and fecundity of the most productive period. The most productive period at photoperiods of 12:12, 13:11, and 14:10 light:dark hours did not occur until Days 5-9, 7-11, and 9-13, respectively. The average numbers of aphids produced during the 4 days totaled 35 at 12:12, 31 at 13:11, and 27 at 14:10 light:dark hours. The curves for reproduction, despite differences in values, were similar in shape.

### **Cumulative Average Daily Fecundity**

The curves of cumulative average daily fecundity were similar at photoperiods of 12:12 and 13:11 light:dark hours (Fig. 2). However, the curve for the 14:10 photoperiod differed from the curves of the other photoperiods. The rate of increases until climax was linear across all photoperiods. The slopes for the rate of increase were similar at photoperiods of 12:12 and 13:11 light:dark hours. The slope at the photoperiod of 14:10 light:dark hours, however, was more gradual. The time when the sugarcane aphids stopped producing nymphs at photoperiods of 12:12, 13:11, and 14:10 light:dark hours was at Day 29, 32, and 39, respectively.

### **Fecundity**

Photoperiod significantly affected the total number of nymphs produced per sugarcane aphid ( $F_{2,141} = 14.69$ ;  $P < 0.0001$ ). As the scotophase shortened, each sugarcane aphid produced fewer nymphs. Subsequently, the total average fecundity of 82.8 nymphs was the greatest at the photoperiod of 12:12 light:dark hours (Table 5). The second greatest average fecundity was at the photoperiod of 13:11 light:dark hours, with an average of 63.0 nymphs. The photoperiod of 14:10 light:dark hours resulted in less total average fecundity of only 56.3 nymphs compared to 82.8 nymphs produced at the

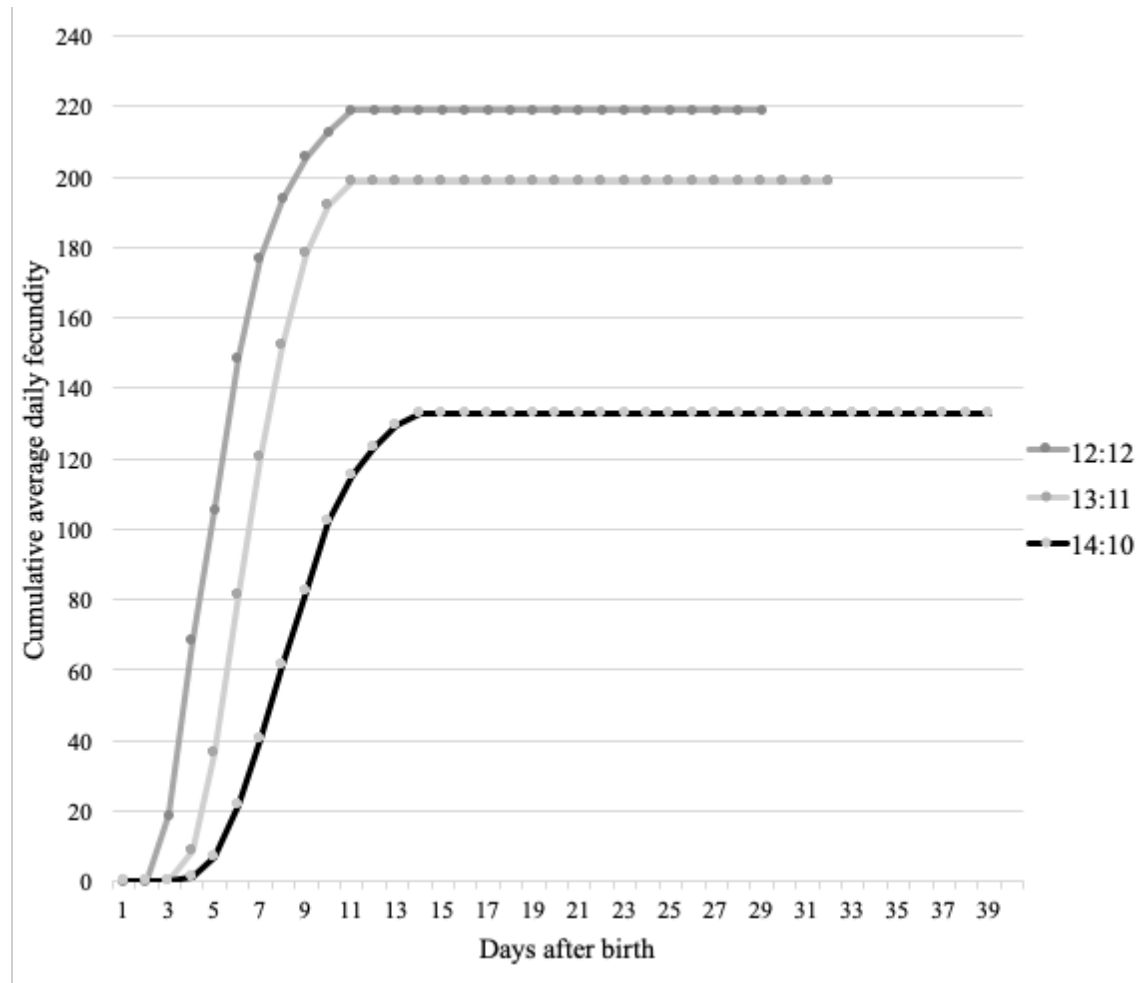


Fig. 2. Cumulative average daily fecundity (nymphs) per sugarcane aphids at different photoperiods on sorghum.



photoperiod of 12:12 light:dark hours. The photoperiod of 12:12 light:dark hours resulted in a total average fecundity that was 25 and 19 nymphs more than at the photoperiods of 14:10 and 13:11 light:dark hours, respectively. Shortening the scotophase reduced the mean total fecundity. The large amount of variation in fecundity among the photoperiods could be attributed to outliers in the data. The data were not transformed or modified and therefore the degree of variation was large.

### **Reproductive Rate**

Reproductive rate, defined as the ratio of total fecundity to the length of the reproductive period, was calculated for each sugarcane aphid at each photoperiod. The reproductive rate was significantly influenced by photoperiod ( $F_{2,141} = 26.89$ ;  $P < 0.0001$ ). The rate of reproduction decreased as the length of the scotophase shortened. Aphids exposed to a photoperiod of 12:12 light:dark hours produced 6.1 nymphs per day (Table 5). The rate is one aphid more per day than at the 13:11 photoperiod and two aphids more than at the 14:10 photoperiod.

### **Mean Generation Time**

Photoperiod greatly affected the mean generation time of the sugarcane aphids on sorghum ( $F_{2,141} = 101.45$ ;  $P < 0.0001$ ). The values gradually increased as the length of the scotophase decreased. Mean generation times were calculated as 3.9 days at the photoperiods of 12:12, 5.7 days at 13:11 light:dark hours, and 8.9 days at the 14:10 photoperiod (Table 6). The time interval (days) between two consecutive generations was greatest at the photoperiod of 14:10 and least at 12:12 light:dark hours. The mean generation time at the photoperiods of 12:12 and 14:10 light:dark hours differed by 5

Table 5. Descriptive Statistics on the Total and Daily Fecundity of Sugarcane Aphids on Sorghum at Different Photoperiods

Photoperiod	Mean $\pm$ SE	CV
Total Average Fecundity per aphid		
12:12 P:S	82.80 $\pm$ 3.90a	50.14
13:11 P:S	63.04 $\pm$ 3.13b	40.47
14:10 P:S	56.25 $\pm$ 4.09c	58.84
Reproductive Rate per aphid per reproductive day		
12:12 P:S	6.06 $\pm$ 0.20a	22.91
13:11 P:S	5.33 $\pm$ 0.22b	27.39
14:10 P:S	4.04 $\pm$ 0.18c	30.94

Number of observations per photoperiod = 48.

CV = coefficient of variation.

Table 6. Descriptive Statistics on the Mean Generation Time of Sugarcane Aphids on Sorghum at Different Photoperiods

Photoperiod	Mean $\pm$ SE
12:12 P:S	3.92 $\pm$ 0.19c
13:11 P:S	5.70 $\pm$ 0.18b
14:10 P:S	8.95 $\pm$ 0.35a

Number of observations per photoperiod = 48.

days. The difference probably was because of significantly longer pre-reproductive periods resulting at the photoperiod of 14:10 light:dark hours.

### **Intrinsic Rate of Increase**

Photoperiod significantly affected the intrinsic rate of increase of sugarcane aphids on sorghum ( $F_{2,141} = 122.18$ ;  $P < 0.0001$ ). The greatest intrinsic rate of increase (31%) occurred at the photoperiod of 12:12 light:dark hours and then gradually decreased as the length of the scotophase decreased (Table 7). The intrinsic rate of increase at the photoperiod of 13:11 light:dark hours (19%) was 12% less than that at the 12:12 photoperiod, while the intrinsic rate of increase at the photoperiod of 14:10 light:dark hours (13%) was 19% less than the intrinsic rate of increase at the photoperiod of 12:12 light:dark hours. The intrinsic rate of increase is calculated as a function of the length of the pre-reproductive period by the number of nymphs produced in the amount of days equivalent to the pre-reproductive period. Therefore, the longer the pre-reproductive period the slower the rate of growth of a population. The pre-reproductive period was significantly affected by photoperiod and gradually increased as the length of the scotophase decreased; the intrinsic rate of increase values reflected the trend.

### **Brix Values**

Brix refractometry was not an applicable method to use to predict resistance to sugarcane aphids at the boot stage of sorghum in a greenhouse ( $F_{1,38} = 0.0016$ ;  $P = 0.8985$ ) nor in the field ( $F_{1,18} = 0.3424$ ;  $P = 0.5656$ ).

The average °Brix of RTx430 and RTx2783 grown in a greenhouse was 2.30 and 2.29%, respectively (Table 8). The average °Brix of RTx430 and RTx2783 grown in the field

Table 7. Descriptive Statistics on the Intrinsic Rate of Increase of Sugarcane Aphids on Sorghum at Different Photoperiods

Photoperiod	Mean $\pm$ SE
12:12 P:S	0.31 $\pm$ 0.00869a
13:11 P:S	0.19 $\pm$ 0.00867b
14:10 P:S	0.13 $\pm$ 0.00845c

Number of observations per photoperiod = 48.

P = photophase (light hours), S = scotophase (dark hours).

Table 8. Descriptive Statistics on the Brix Percentages of Sucrose at the Boot Stage of Two Sorghum Lines Grown in a Greenhouse

Sorghum Line	Mean $\pm$ SE	CV
RTx430	2.30 $\pm$ 0.05%	10.14
RTX2783	2.29 $\pm$ 0.06%	11.82

Number of observations per sorghum line = 20.

CV = coefficient of variation.

was 2.38 and 2.41, respectively (Table 9). The average temperature of each sample, in both the greenhouse and field, was  $20 \pm 1^{\circ}\text{C}$ .

The plants probably were not statistically different because the pedigrees of RTx430 and RTx2783 shared common ancestors. Menze et al. (2004) reported that RTx430 and RTx2783 had a 0.63 coefficient of genetic similarity. The coefficient of genetic similarity was determined by a cluster analysis by 496 P/M AFLPs. Also, RTx430 and RTx2783 are both in the zera-zera working group. Menze et al. (2004) reported sorghums in the zera-zera working group to be 73% genetically similar. Therefore, RTx430 and RTx2783 shared enough similarities for their amounts of sugars to be indistinguishable when compared by Brix refractometry. The total sugar content was not an indicator of the resistance of RTx2783 nor of the susceptibility of RTx430 to sugarcane aphids.

Table 9. Descriptive Statistics on the Brix Percentages of Sucrose at the Boot Stage of Two Sorghum Lines Grown in the Field

Sorghum Line	Mean $\pm$ SE	CV
RTx430	2.38 $\pm$ 0.07%	8.21
RTX2783	2.41 $\pm$ 0.06%	7.74

Number of observations per sorghum line = 10.

CV = coefficient of variation.



## **CHAPTER V**

### **Summary, Conclusion, and Recommendations**

#### **Summary**

At a photoperiod of 12:12 light:dark hours, the sugarcane aphids started producing nymphs when approximately 3 days old, produced for 14 days, and stopped producing nymphs 2 days before dying. A mother aphid lived for an average total of 19 days. Sugarcane aphids at the photoperiod of 12:12 light:dark hours produced an average of 83 aphids during a life time and produced six aphids per day of reproduction. The population growth rate was 31%, indicating that an aphid colony if subjected to this photoperiod would increase at a 31% growth rate. The mean generation time also was approximately 4-days.

At a photoperiod of 13:11 light:dark hours, the sugarcane aphid started producing nymphs when approximately 4 days old, produced nymphs for 12 days, and stopped producing nymphs 7 days before dying. Each aphid lived for an average of 23 days. At a photoperiod of 13:11 light:dark hours, each mother aphid produced an average of 63 nymphs during her life time and produced about five aphids per day of reproduction. The mean generation time was approximately 6 days, with an intrinsic rate of increase of 19% at the 13:11 photoperiod.

At a photoperiod of 14:10 light:dark hours, the sugarcane aphids did not start producing nymphs until they were approximately 7 days old, produced for 14 days, and

stopped producing nymphs 4 days before dying. The aphids had a 25-day life span, during which they produced an average of 56 nymphs total with about four nymphs produced during each day of reproduction. Overall, the population growth rate totaled 13%, with an approximate 9-day mean generation time.

The °Brix values of the two sorghum lines were indistinguishable. Brix refractometry could not be used to distinguish a statistically significant difference between RTx430 and RTx2783 in the greenhouse or the field.

## **Conclusion**

Photoperiod significantly affected the lengths of the pre- and post-reproductive periods, but did not influence the length of the reproductive period of sugarcane aphids on sorghum. Daily and total fecundity, longevity, intrinsic rate of increase, and mean generation time of the sugarcane aphids also were significantly affected by photoperiod. The length of the scotophase affected the degree of effect of the photoperiod on the aphid. The pre- and post-reproductive periods, longevity, and mean generation time all gradually increased as the length of the scotophase decreased. Shortening the length of the scotophase resulted in more days during the life span of the sugarcane aphid, with a larger percentage of life spent during the pre- and post-reproductive periods. Shortening the length of the scotophase, however, did not positively influence the intrinsic rate of increase or the fecundity of the sugarcane aphid. The shorter the duration of the scotophase, the less the number of nymphs produced per aphid.

The greatest fecundity and intrinsic rate of increase resulted from a photoperiod of 12:12 light:dark hours. The fecundity and intrinsic rate of increase decreased as the

length of the scotophase decreased. This might have been because the ancestors of the sugarcane aphid originated near the equator. A geographically parthenogenetic aphid, such as the sugarcane aphid, is incapable of sexually reproducing because of incorrect latitude. Therefore, latitude theoretically also could affect the life span and fecundity. The farther north the sugarcane aphid travels during the sorghum-growing season, the shorter the length of the scotophase and the less reproductive the aphid. However, the aphid still is capable of damaging sorghum.

The most favorable photoperiod for a sugarcane aphid on sorghum was at a photoperiod of 12:12 light:dark hours, demonstrated by the greatest fecundity and reproductive rate, greatest intrinsic rate of increase, and shortest mean generation time. The study also showed that as the length of the scotophase decreased, the aphids produced fewer nymphs and lived longer. This conclusion could be influential in predictive modeling in that aphids as they move north during a sorghum-growing season live longer but produce fewer nymphs. Overall, the sugarcane aphid was most responsive at a photoperiod of 12:12 light:dark hours, perhaps because sugarcane aphids originated near the equator.

Brix refractometry was not an appropriate method to use for detecting nor predicting resistance of RTx2783 nor susceptibility of RTx430 sorghum to sugarcane aphids. This probably was because the sorghum lines shared many of the same ancestors. The mechanism of resistance in RTx2783 could theoretically be ascertained and isolated. However, the two lines shared enough genetic material to cause them to have similar °Brix values. The mechanism of resistance must be unrelated to total sugar content. The potential mechanisms of resistance that could be active, but are undetectable by Brix

refractometry, include but are not limited to: cyanogenic glycosides, hydroxybenzaldehydes, organic acids, and even the surface architecture of the leaf.

### **Recommendations for Future Research**

Recommended future research would be:

1. Calculating the correlation between wind speed and development of alates of sugarcane aphids
2. Evaluating the effect of irrigation on sugarcane aphids on sorghum
3. Evaluating the effect of humidity on sugarcane aphids on sorghum
4. Evaluating the effect of plant population on sugarcane aphids on sorghum
5. Evaluating the sorghum mechanism of resistance against sugarcane aphids

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