THE EFFECTS OF TEMPERATURE ON SUGARCANE APHID, *MELANAPHIS SACCHARI* LIFE HISTORY ON THREE DIFFERENT HOST PLANTS

By

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Abstract: Sorghum, *Sorghum bicolor* (L.) Moench, is one of the most important crops in the world. Since 2013, sugarcane aphid, *Melanaphis sacchari* has become a perennial and significant pest in the southern United States. It can develop on multiple grass hosts but does not appear to survive winter temperatures in the U.S. except in southern Texas. Insects depend on temperature and nutrition to develop and reproduce. The rate of aphid development and reproduction increases as temperature increases until it reaches a maximum temperature where development slows because of metabolic stress. Laboratory experiments were conducted at seven different constant environmental temperatures (5, 10, 15, 20, 25, 30, 35 °C) on three different host plants, sorghum, Johnsongrass, and Columbus grass. Longevity, fecundity, number of nymphs per day, reproductive period, and intrinsic rate of growth were measured. At 5 and 35 °C, reproduction did not occur on any host plant. Longevity was maximum at 15 °C and decreased with increasing temperatures. Reproduction was highest at 25 °C on sorghum and Johnsongrass, and at 20 °C on Columbus grass. The supercooling point (coldest temperature at which survival is possible) was also determined for nymphs, adults, and winged adults of SCA and was found to be between -22 °C and -25 °C. The results of these experiments suggest that alternate host plants support aphid survival but with limited reproduction. Extreme low and high temperatures also affect strongly SCA survival and reproduction.

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CHAPTER I

INTRODUCTION

Sorghum, *Sorghum bicolor* (L.) Moench is one of the most important crops in the world. According to the USDA (2017), the world sorghum production for 2017/2018 will be approximately 59.34 million metric tons. The United States (US) is the world's largest producer of sorghum, with a production of 8,408,000 metric tons. Nigeria, Mexico, India and Sudan are the other top five producers in the world (USDA, 2017).

In the US, sorghum is primarily produced in the region known as the "sorghum belt", spanning from South Dakota to Southern Texas. In 2016, sorghum was planted on 6.7 million acres, with Kansas having the largest planted area followed by Texas, Colorado, Oklahoma and South Dakota. In 2016, according to USDA-NASS, sorghum was planted on 400,000 acres, with harvest from 370,000 acres. Production was 20,350,000 tons, with an average of 55 bushels per acre.

Sorghum cultivation is very important worldwide because the plant can be used in many ways. There are four major uses of sorghum: grain sorghum, forage sorghum, biomass sorghum and sweet sorghum (Queiroz *et al*., 2018; Castro *et al*., 2017). Grain sorghum occurs in many shapes and colors depending on the cultivar and is used for many functions, including animal feed. Forage sorghum can be used for silage, hay, grazing, and what is known as green-chop where it is processed by chopping and feeding it directly to livestock (Bean *et al*., 2013; Pino and Heinrichs, 2017). Biomass sorghum is a different cultivar grown primarily for the production of bioenergy, where it is fermented to produce fuel and other products. Sweet sorghum is grown predominantly for syrup production, alternative sweeteners, biofuel, alcohol for consumption and chemical products (Mercer *et al*., 2011; Maw *et al*., 2017).

Many countries, especially in Africa, use sorghum and its derivatives for human consumption because of its high nutritional value. During processing of sorghum, by-products are also obtained and are becoming part of the human diet. Among the main foods derived from sorghum are flours, oils and beverages, incluiding beer (Dicko *et al*., 2006; Queiroz *et al*., 2017). Because of its characteristics of adapting well to semi-arid climates, the production of sorghum in African countries has increased (Queiroz *et al*., 2017).

Sorghum has long been used in animal feed. In the United States, sorghum has been used to feed cattle, swine, and poultry. The use of sorghum in animal feed includes silage, green chop, hay and pasture, and the manufacture of rations containing grains of sorghum as a major ingredient (Pino and Heinrichs, 2017; Inácio *et al*., 2018). Sorghum for silage is well accepted because it requires less water than maize and produces similar results as those fed with maize (Bean *et al*., 2013).

Increasingly, sorghum has been used for the manufacture of bioethanol. The search for new fuels, especially those that do not originate from fossil sources and that reduce damage to the environment has increased planted acreage, especially in the United States. Ethanol is produced from sweet sorghum juice that is extracted from stalks and has a high concentration of sucrose. After extracting the juice, any leftover raw material can also produce cellulite ethanol through other treatments (Castro *et al*., 2017; Appiah-Nkansah *et al*., 2018).

Some of the characteristics that have made sorghum a major crop across the world is its ability to germinate and grow in low fertility soils and with limited water supply. The ability to grow sorghum with limited rainfall is the main reason it is widely planted in the region today known as the sorghum belt, in the central United States. With irrigation, it is possible to maintain high sorghum yields and achieve positive agronomic results even in arid or semi-arid regions (Pang *et al*., 2018). The genetic improvement of sorghum has allowed cultivation in regions with low rainfall. However, since 2013, the sugarcane aphid (*Melanaphis sacchari*) has threatened sorghum production in the US.

Sugarcane Aphid (*Melanaphis sacchari*)

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) is present in many countries in Africa, Asia, and South America where sugarcane and sorghum are grown. Sugarcane aphid was first documented as a pest of sugarcane; however, many countries have experienced the aphid becoming a sorghum pest. In the US, sugarcane aphids were reported on sugarcane in Florida (Mead, 1978) and Louisiana (Hall, 1987, White *et al*., 2001) but in the last few years it has shown preference for sorghum (Rodríguez-del-Bosque and Teran, 2015).

Sugarcane aphids have been known to feed on a number of grasses including rice, sugarcane, sorghum, millet, Bermuda grass, Johnsongrass and others. Its occurrence on different plants has been reported in different countries and various times of year (Singh *et al*., 2004; Armstrong *et al*., 2015).

Sugarcane aphids (SCA) are pale yellow, gray or tan with dark cornicles, tarsi and antennae (Villanueva *et al*., 2014). Most are females that reproduce asexually, giving birth to several live young, which in a few days become adults with the same ability to reproduce. The nymphal stage consists of four stadia. Depending on the temperature, sugarcane aphids take between 4 and 12 days to become adults (Chang *et al*., 1982).

Adult longevity ranges from 10 to 37 days on average, and females produce 34 to 96 nymphs, depending on temperature and nutrition (Bowling *et al*., 2016). Adult SCA may or may not have wings. When plants begin to deteriorate, and sugarcane aphid densities are high, some adults will develop wings (Singh *et al*., 2004). The sugarcane aphid is not a good flier, but they can be carried by the wind over long distances. It is thought that aphids present in the states north of Texas are transported by air currents from Mexico and southern Texas where these aphids overwinter (Knutson *et al*., 2016).

Usually sugarcane aphids infest the lower leaves of a plant and as colonies grow, the aphids make their way up the plant, and feed on the undersides of the leaf and stem. The aphids feed on the phloem, which is rich in sugar, eliminating excess sugars as honeydew, which accumulates on leaf surfaces. The honeydew causes a shiny appearance and allows sooty mold to grow. By feeding on plant sap, SCA also cause a reddening of leaves, which can lead to the death of leaf tissues (Knutson *et al.*, 2016).

The sugarcane aphid usually reaches its infestation peak on a plant after 2 to 3 weeks, depending on temperature and climatic conditions. After this period, populations begin to decline. Depending on the degree of infestation, sugarcane aphids can cause economic loss either by loss of grain or poor development of the plant and subsequently loss of forage and grain. Sugarcane aphids will eventually infest emerging heads of sorghum, and if left unchecked will reduce anthesis, significantly reducing grain yields. Despite all these aphid-induced injuries, there is no indication that aphids inject any kind of toxin into the plants (Armstrong *et al.*, 2015; Rodriguez-del-Bosque and Teran, 2015).

The SCA utilizes sugarcane where available in the United States, but in 2013, it was detected on sorghum in both Texas and Louisiana (Villanueva *et al*., 2014). From these areas SCA reached Oklahoma and Mississippi. During the winter, the aphids survive in southern Texas, where two crops of sorghum are grown each year. In 2014, it reached sorghum fields in 12 states, and in 2015 it was present in 17 states, which represented 90% of US sorghum acreage (Knutson *et al*., 2016).

Temperature is an important environmental variable for the development, survival and reproduction of aphids. A milder winter allows the SCA to infest sorghum earlier than a colder winter when high mortality occurs. States north of Texas, which have colder winters, have no trace of sugarcane aphid during the winter and early spring. Like other aphid species, these insects are sensitive to low temperatures and are unable to survive or reproduce (Kieckhefer and Elliott, 1989; Armstrong *et al*., 1996).

Kieckhefer and Elliott (1989) observed that the development time of Russian Wheat aphid, *Diuraphis noxia* (Mordvilko), nymphs to adult stage decreased as the temperature increased. Campbell *et al*. (1974) demonstrated that the rate of insect development depends on temperature. Aalbersberg *et al*. (1987) observed that in fluctuating temperatures of 13 to 17.3 °C, female Russian Wheat aphids produced an average of 72.0 to 81.5 nymphs, while Webster and Starks (1987) observed that 31.9 to 50.5 nymphs were produced in fluctuating temperatures of 13.2 to 27.2 °C.

During winter, the temperature in Oklahoma falls below 0 °C. When temperatures drop abruptly, they can reach -22 °C (Jones *et al*. 2008). Insects usually do not survive these temperatures, but some species have physiology that allows survival. Some aphid species have the capacity to withstand low temperatures, allowing them to overwinter in temperate zones by either tolerating being frozen or avoiding freezing through various methods including supercooling the body fluid below the freezing point. Supercooling point (SCP) is defined by the temperature at which internal fluids freeze and indicates the minimum temperature that an insect can survive. Aphids can produce glycerol and other antifreeze compounds, dehydrate, ingest certain substances, or make changes in fatty acids (Powell, 1976; Worland *et al*. 2010). SCP's for some species of aphids have been determined. The greenbug aphid, *Schizaphis graminum* (Rondani) has a mean SCP of -28.98 ± 0.10 (Jones *et al*. 2008) and Russian wheat aphid, has a mean SCP of -27 °C (Armstrong and Nielsen, 2000).

In the spring, SCA appear on sorghum as temperatures begin to rise. For this reason, it is necessary to understand the threshold and optimal temperatures for its survival and reproduction, and what possible mechanisms this aphid uses to overwinter.

Another important issue regarding the survival of sugarcane aphid during periods when sorghum crops are unavailable is to understand how alternate hosts contribute to its survival. Different plants are known to be temporary hosts of these aphids. Among them are mainly Johnsongrass (*Sorghum hapelense* L., Pers.) and Columbus grass (*Sorghum almum* Parodi.) (Armstrong *et al*, 2015).

Johnsongrass is typically considered to be a weed present in all the warm regions of the world and can reduce crop yields. This weed is perennial, 0.5-1.5 m tall, with panicles 10-35 cm long and spikelets in pairs. Johnsongrass differs from other sorghum species by the number of chromosomes (S. halepense n = 20, S. versicolor n = 5 and S. bicolor n = 10). Johnsongrass is known to host insect and disease pests of sorghum (Monaghan, 1979).

Columbus grass is a weed that originated from the natural cross between grain sorghum and Johnsongrass. It was intentionally used as a forage crop in Argentina (Parodi, 1943). It is a perennial plant, 1-2.5 m tall, with leaves up to 50 cm long. This weed causes problems in sorghum crops as a grain contaminant, as well as cross breeding with grain varieties. It also harbors insect pests and diseases of sorghum (Eberlein, 1987).

Management of the Sugarcane Aphid in Sorghum

Scouting for sugarcane aphids should begin when plants have 4 to 5 leaves. When SCA are found in a field, sampling should be done once or twice a week. If they reach the action threshold, insecticide must be applied to protect the crop (Knutson *et al*. 2016).

Scouting for aphids consists of walking at least 25 feet into the field and examining plants for SCA on the underside of leaves. A total of 60 to 80 plants around the field need to be checked as well as sites near Johnsongrass (Knutson *et al*. 2016).

When the population of SCA is increasing rapidly, the use of insecticides may be needed to prevent honeydew buildup and yield loss before harvest. There are two available insecticides to control SCA, Sivanto 200SL and Transform WG, which can manage SCA on both grain and forage sorghum. There are other products that have been used for SCA but they have not been as effective as Sivanto and Transform WG (Villanueva *et al*. 2014; Knutson *et al*. 2016). Insecticide seed treatment also controls SCA and other insects for approximately 4 to 6 weeks after planting (Knutson *et al.* 2016).

An alternative method to control SCA is utilizing resistant varieties of sorghum against these aphids that exhibit tolerance, antibiosis, or antixenosis (Armstrong *et al.* 2016). Tolerant cultivars can withstand or recover from insect damage. Another way resistance is expressed is by antibiosis where SCA cannot survive or reproduce as well as it can on a susceptible hybrid. When the rate of population increase is lower, natural enemies also play an important role controlling the number of aphids (Knutson *et al*. 2016).

Objectives

1. Compare survival and reproduction of Sugarcane aphid on sorghum, Johnsongrass and Columbus grass at different temperatures.
2. Determine the supercooling point for the sugarcane aphid.

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CHAPTER II

The effects of temperature on sugarcane aphid, *Melanaphis sacchari* life history, on three different host plants.

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Abstract

Sugarcane aphid (SCA) has become a severe pest across much of the sorghum belt. It can develop on multiple grass hosts but does not appear to survive winter temperatures in the U.S. except in southern Texas. Survival and reproduction by insects is a result of exposure to appropriate nutrition and temperatures at which metabolic processes are maintained. The rate of aphid development and reproduction increases as temperature increases until it reaches a maximum temperature where development slows because of metabolic stress. A series of laboratory experiments were performed where clonal SCA were housed at seven different constant environmental temperatures (5, 10, 15, 20, 25, 30, 35 °C) on one of three host plants, sorghum, Johnsongrass, or Columbus grass. Longevity, fecundity, number of nymphs per day, reproductive period, and intrinsic rate of growth were measured. At temperatures below 10 °C and above 30 °C, reproduction did not occur on any host plant. Longevity was maximum at 15 °C and decreased with increasing temperatures. Optimal temperatures for intrinsic rate of increase was between 15 °C and 25 °C on all host plants but maximum fecundity differed by host plant and was greatest on sorghum. The supercooling point (coldest temperature at which survival is possible) was also determined for nymphs, adults, and winged adults of SCA and was found to be between -22 °C and -25 °C. The results of these experiments suggest that SCA can use alternate hosts for survival and reproduction, but both low and high temperatures limit its biology. Higher temperatures may trigger dispersal, while low temperatures eliminate SCA in most of the United States.

Key words: Winter survival, host plant, population dynamics, alternate host

Introduction

The sugarcane aphid (SCA), *Melanaphis sacchari* (Zehntner) is a pest of sorghum, *Sorghum bicolor* (L.) Moench, in many parts of the world and since 2013 became a major pest of the crop in the United States (Knutson *et al*. 2016, Nibouche *et al*. 2018). SCA was first introduced to the United States (U.S.) in the 1970s from Hawaii and was known as a pest only of sugarcane *Saccarum officinarum* L. The clonal lineage that attacks sorghum was recently introduced to North America from either Africa or Asia (Nibouche *et al*. 2018). Populations of the SCA have also been observed utilizing other hosts including sugarcane *Saccarum officinarum* L., Johnsongrass *Sorghum halepense* (L) Pers., and Columbus grass *Sorghum almum* Parodi especially in the southern U.S. (Nibouche *et al*. 2015).

Based on colonization patterns and absence of SCA in most areas until after sorghum has reached advanced growth stages, SCA appears to only survive in the extreme southern U.S. (Michaud *et al*. 2018). In southern Texas, sorghum is grown twice per year and temperatures do not reach freezing. Understanding cold tolerance is important to predict whether seasonal crop pests are able to overwinter. The ability of these pests to overwinter will influence management decisions (Worland *et al.* 2010).

Insects are generally classified as freeze tolerant or freeze avoidant, although these designations represent endpoints on a spectrum (Somme 1982). Freeze tolerant insects are able to withstand freezing by controlling where ice forms in their tissues. These insects generally freeze at temperatures close to 0 °C. Ice, is nucleated in the extracellular fluids in a controlled manner. In contrast, freeze avoidant insects use physiology and behaviors to lower freezing points and/or find thermal refugia. These insects lower the freezing point of their extracellular fluids up to -30

°C and survive in a supercooled state. The ability to supercool is accomplished through the accumulation of cryoprotectants and removal of ice nucleators from their body (Somme, 1982; Armstrong and Nielsen, 2000; Worland *et al*. 2010).

The supercooling point (SCP) provides an indication of the lowest temperature at which an insect can survive. The SCP is determined by finding the temperature at which the insect freezes and is recorded as a sudden increase in temperature from the release of the latent heat of fusion. SCP is influenced by body size, feeding status, and the quality and quantity of nucleating agents (Powell, 1976; Somme, 1982; Jones *et al*. 2008).

Effects of Temperature on SCA survival and reproduction

Because environmental temperatures influence nearly every aspect of insect physiology, weather strongly influences the population dynamics of insect pests. For aphids, heavy rains can wash them off host plants and reduce populations; while winds can spread them over long distances allowing them to reach new areas (Lowe, 1966; Knutson *et al*. 2016). Temperature is the most important abiotic factor affecting the development and reproduction of aphids (Aalbersberg *et al*. 1987, Ozder and Saglam, 2013). Aphid development rate is directly related to the temperature. When these insects are exposed to temperatures below the threshold for development, survival is possible, but growth and maturation are not (Campbell *et al*., 1974).

As temperatures increase, insect development rate increases from the threshold of development to an almost linear relationship where increasing temperatures speed development rates in a predictable manner. However, when temperatures become too warm, the rate of development decreases because of physiological stress. These temperature limits are often species-specific (Campbell *et al*, 1974). Previous work on aphids by Ozder and Saglam (2013) showed that development time of the common lime aphid, *Eucallipterus tiliae* (L.) and the crapemyrtle aphid, *Tinocallis kahawaluokalani* (Kirkaldy) decreased as temperature increased. Kieckhefer and Elliot (1989) also observed that temperature affected the development time of Russian wheat aphid *Diuraphis noxia* (Mordvilko). In contrast, development time of the oleoander aphid, *Aphis nerii* Boyer de Fonscolombe species increased as temperature increased. In this experiment the effects of temperatures ranging from 20 to 27 °C were evaluated (Ozder and Saglam 2013). Similar experiments have not been previously reported for SCA.

The goals of this research were to determine the effects of temperature on SCA survival, development, and reproductive biology. The SCP was also determined. In addition, performance on three different host plants was tested across all experimental temperatures. The hypotheses tested were 1) SCP would be similar for all life stages; 2) a temperature threshold and thermal maximum could be determined for SCA, and 3) that SCA would respond similarly to temperature regardless of host plant used.

Materials and Methods

All experiments were conducted at the United States Department of Agriculture – ARS lab in Stillwater, Oklahoma between February 2017 and July 2018. Sugarcane aphids used in this experiment are part of the stock colony maintained at the USDA-ARS lab. They were originally collected from a field in Matagorda, Texas in 2013 and kept on the susceptible sorghum variety RTx7000 (Armstrong *et al*. 2016). This colony is transferred every two weeks to new seedling plants and kept on a greenhouse bench at temperatures ranging from 21 °C to 31 °C.

Determination of supercooling point (SCP)

To determine the supercooling point of sugarcane aphids, a Sable Systems International TC-2000 was connected to a computer running the RealTerm 2.0 program, which captured and stored data from the thermocouple. To carry out the SCP measurement, a thermocouple chamber was constructed using a 2.5 cm by 20 cm pyrex test tube with a stopper. A metal dowel was attached to the stopper in order to slide it up and down inside the test tube. A hole was drilled in the middle of the stopper and a 1-m-long copper constantan 30-gauge thermocouple wire was extended through it and taped to the metal dowel.

Dry ice was mixed with alcohol in a 300 ml beaker to obtain an extreme low temperature (-50 °C). The test tube was placed in the middle of that mixture in the beaker. Each aphid was placed singly onto the tip of the thermocouple using petroleum jelly to secure it. The aphid was then lowered into the test tube. As the stopper was moved, the aphid was exposed to lower and lower temperatures. Temperature measurements were taken every 0.2 seconds until the supercooling point was detected.

Twenty aphids of three different life stages, nymphs 24 to 48 hours old, and nymphs older than 48 hours, winged adults were tested. Aphids were not acclimated prior to being tested, as previous research has shown that acclimation had no effect on SCP in other aphid species (Armstrong and Nielsen, 2000).

Effects of temperature on life history

To evaluate survival and reproduction of sugarcane aphids at different temperatures, a susceptible sorghum variety, KS 585, and two known alternate hosts, Johnsongrass and Columbus grass, were used. Johnsongrass seeds were obtained directly from a research station in Stillwater where this grass grows naturally. Columbus grass seeds were purchased from Turner Seeds Company located in Breckenridge, Texas.

Two sorghum seeds were planted in cone-tainers (model SC10, S7S greenhouse supply, Tangent, Oregon 97389) 5 cm diameter x 20 cm tall in a rich three-layer media of potting soil, fritted clay and sand (from bottom to top respectively). When the plants reached the three-leaf stage, the most vigorous one was maintained and the other one removed. To protect the plants, a clear plastic cylinder was placed over them. All tubes had three holes covered with polyester fine mesh netting for ventilation. The seeds of Johnsongrass and Columbus grass had a different treatment to germinate. First, they were scarified to remove the outer shell of the seed. Then they were placed on a moist paper towel, which was folded and placed inside a ziploc bag. The bag was placed in a growth chamber at a temperature of 27 °C. After 3 days inside the growth chamber, the seeds germinated and were transferred to a pot with potting soil where they remained for another 5 days in the same growth chamber. After this period, they were transplanted into the cone-tainers, in the same ways as the sorghum trials.

When the plants reached the three-leaf stage, 10 replicates were placed on a tray. Using a camel hair brush, one SCA nymph was placed on a leaf of each plant and covered with the tube. Each tray was then placed in a growth chamber, previously set at a constant experimental temperature, with a photoperiod of 14:10 (L: D) hours and humidity of approximately 65%.

Replicate treatments were grown at 5, 10, 15, 20, 25, 30 and 35 °C. Each day, each plant was evaluated and observed to determine if the aphids had reproduced. When there were new nymphs on the plants, all nymphs were counted and removed from the plant, leaving only the original female. This process was performed every day until the original female died. When the original female was missing for three consecutive days, she was also considered dead.

Statistical analysis

Data from SCP experiments were summarized in SAS 9.3. Differences among life stages were tested using PROC Mixed Analysis of Variance. All data from the experiments to quantify the effects of temperature, including aphid longevity, fecundity, pre-reproductive and reproductive periods, were analyzed using PROC Mixed Analysis of Variance (SAS 9.3, SAS Institute 2010) followed by a LSMEANS test when significant differences were detected.

Results

The SCP was determined by exposing aphids to cold temperatures and measuring when the exothermic reaction of ice crystallizing occurred (Figure 1). All life stages of aphids had mean SCP at temperatures below -20 °C. Nymphs, 24 to 48 hours old, had the lowest supercooling point of -25.8 °C, while nymphs older than 48 hours had a SCP of -22.2 °C. Winged adults had a SCP of -23.7 °C (Table 1). The SCP temperature were significantly different among life stages tested with nymphs between 24 and 48 hours old capable of surviving the coldest temperatures.

SCA responds in different ways when exposed to varying temperatures, as well as when exposed to constant temperature but to different grass hosts. At cold and hot temperatures (5 °C and 35 °C), aphids did not survive long on the different grasses nor did they reach the adult reproductive stage. On Columbus grass, no aphids reached the adult stage at 10 °C but some aphids had low reproduction on sorghum and Johnsongrass (Tables 3-5). Overall, the aphids' longevity was higher on sorghum than on Johnsongrass and Columbus grass (Fig. 2). At 15 °C, the aphids had the greatest longevity on sorghum and Johnsongrass, but the greatest longevity on Columbus grass was observed at 20 °C (Tables 3-5).

Fecundity of aphids increased as temperatures increased across all grasses. The most progeny on sorghum and Johnsongrass was observed at 25 °C while on Columbus grass, reproduction was greatest at 20 °C. Across temperatures from 15 °C to 30 °C, SCA females produced significantly more offspring (55.6 nymphs per female) on sorghum than on the other grasses, where mean fecundity on Johnsongrass and Columbus grass resulted in 39.5 and 30.4 nymphs per female (Figure 3).

The pre-reproductive period of aphids on all three grasses decreased with increasing temperature. The reproductive period for aphids that reached maturity on sorghum and Johnsongrass was longest when they were exposed to 15 °C while the longest reproductive period for Columbus grass was at 20 ºC (Tables 3, 4, and 5). The relationship between pre-reproductive period and the number of progeny produced in the same amount of time (md) varied by grass species and was longest at 15 °C for sorghum and Columbus grass (Tables 3 and 5). For Johnsongrass, md was similar for all temperatures above 10 °C Table 4). The intrinsic rate of increase (rm) of SCA increased as temperature increased for both sorghum and Johnsongrass; however, on Columbus grass rm peaked at 25 °C (Table 3, 4 and 5).

Discussion

Supercooling point (SCP)

Results obtained in the SCP experiment demonstrated that SCA can withstand different temperatures depending on life stage. This difference in freezing point has been shown to be directly related to aphid size (Armstrong and Nielsen, 2000). Winged adults, though older, are smaller than 48-hour-old nymphs, and larger than 24-48 hour-old nymphs. Larger aphids likely contain more haemolymph and thus may be the most susceptible to freezing.

Aphids have been classified as freeze avoidant and some species accumulate cryoprotectants and eliminate nucleating agents (Powell, 1976; Jones *et al.* 2008). However, in these experiments, the aphid colonies were never exposed to cold or freezing temperatures and thus were unlikely to have accumulated cryoprotectants. The fact that aphids feed directly on the phloem makes them consistently void of ice nucleators in all life stages, likely influencing their resistance to freezing (Armstrong and Nielsen, 2000).

Although SCA originates from tropical regions, this species can withstand extremely cold temperatures. This ability appears to be inherent in aphid species independent of their origin (Somme, 1982). Armstrong and Nielsen (2000), when evaluating SCP for Russian wheat aphid adults, detected a mean SCP of -26.94 °C. Jones *et al.* (2008), evaluating the SCP for the adult greenbug, detected a mean SCP of -25.98 °C. Other species of aphids have a mean SCP between -20 and -25 °C (Table 2).

Effects of temperature on life history

Survival and reproductive capability of SCA was strongly affected by temperature and host plant. Aphids lived longer at 5 °C and 35 °C temperatures when inhabiting sorghum than they did on either Johnsongrass or Columbus grass (Tables 3-5). The threshold temperature that would allow SCA to become adults and reproduce appears to be between 5 °C and 10 °C and is similar for other aphid species (Conti *et al.* 2010). Temperatures above 30 °C also appear to impact SCA survival. High temperatures can be lethal because they directly affect the somatic tissue of aphids including the developing embryos within them and can also affect the nutritional quality of the plant (Daniels, 1967; Campbell and Mackauer, 1977; Hayakawa *et al*. 1990; Asin and Pons, 2001). Columbus grass is the most tropical host tested but SCA performance was poorest on this host, even at higher temperatures, suggesting the potential for secondary plant compounds or nutritional limitations of this host.

Reproduction did not occur at the lowest or highest temperatures on any grass. At 15 °C, low rates of reproduction occurred on both sorghum and Johnsongrass but not on Columbus grass. At 15 °C, aphids reared on sorghum and Johnsongrass showed the longest life span and reproductive period, in addition to having a high fecundity level. The longevity of several aphid species has been reported to be longest at around 15 °C (Michels and Behle, 1989; Kieckhefer and Elliott, 1989; Hayakawa et al., 1990; Conti *et al*. 2010). Above 15 °C, SCA longevity decreased except for the aphids on Columbus grass, where they had the greatest longevity at 20 °C. This difference was not significant and additional research on the interaction between SCA and Columbus grass is needed to determine the reason that the relationship between temperature and longevity differed for this host.

Although the increase in temperature decreases the longevity of aphids, this is generally not a major factor in reproductive rate of aphids because most nymphs are produced early in the adult's lifetime. Therefore, it is more advantageous for aphids to have a high reproductive rate at a higher temperature even when it shortens their lifespan (Hayakawa *et al*. 1990; Girma *et al*. 1990; Diaz and Fereres, 2005). At higher temperatures, SCA reproduced an average of about 2 nymphs per female per day between 20 and 30 ^{o}C. Interestingly, SCA produced fewer nymphs per day on Columbus grass, except at 20 ^{o}C (Tables 3-5). This difference is also reflected in the intrinsic rate of increase which increased with increasing temperatures for two of the hosts, but not Columbus grass.

Based on these experiments, the optimum temperature for longevity, fecundity and development of the sugarcane aphid is between 20 and 25 °C. Several species of aphids have similar reproductive and survival characteristics at these temperatures. In a constant temperature experiment, the currant-lettuce aphid, *Nasonovia ribisnigri* (Mosley), had a high survival rate between 16 °C and 24 °C, with no mortality of nymphs occurring above 20 °C. The optimum temperature for reproduction and survival of this species is recorded at 24 °C (Diaz and Fereres, 2005). Wood and Starks (1972) observed that for greenbug, *Schizaphis graminum* (Rondani), on a susceptible sorghum variety, the optimal temperature for reproduction was between 21.1° C and 26.7 °C. Walgenbach *et al.* (1988) also observed that greenbug fecundity peaked at around 20.8 ° C, whereas development rate increased until 26 °C for this species. The Asparagus aphid, *Brachycorynella asparagi* (Mordvilko) had its highest fecundity at 23 °C (Hayakawa *et al.* 1990). The Bird cherry-oat aphid, *Rhopalosiphum padi*, showed the highest fecundity at 25 °C (Asin and Pons, 2001).

SCA differs from most other species of aphids by being able to survive and reproduce in a constant temperature of 30 °C. Although there is a decrease in longevity and in the total number of nymphs produced, survival and fecundity were greater when compared to other species of aphids. At 30 °C, SCA took between 5 and 6 days to reach the adult stage and reproduce. On sorghum and Johnsongrass, the intrinsic rate of increase was highest at 30 °C. Comparing the effects of temperature on aphids reared on different hosts, Johnsongrass is a good alternate host in the absence of sorghum. Most life history characteristics when compared to the results obtained on sorghum. With the increase in temperature, longevity decreased but reproductive rate increased, and at 25 °C, the largest number of nymphs was produced (Table 4).

It is noteworthy that the SCA colony is maintained on susceptible sorghum; however, the variety of sorghum used in these trials was different than the one used to maintain the aphids. It is expected that aphids have preferred hosts and in the absence of these hosts, they may use other less desirable plants. Starks *et al*. (1973) evaluated the preference of two different biotypes of greenbug (Biotype B and C) in relation to three sorghum varieties (OK-8, Deer and Piper) and concluded that longevity and fecundity was lower for both biotypes on the nonpreferred cultivars.

Columbus grass proved not to be as good of an alternate host as Johnsongrass. Besides SCA having lower numbers for all variables when compared to sorghum and Johnsongrass, no reproduction occurred at temperatures 5, 10 and 35 °C. Temperature and quality of food influence development, survival, and reproduction of aphids (Aalbersberg *et al*., 1987; Campbell *et al*., 1974; Singh and Singh, 2015). It is likely that nutritional qualities influence SCA on Columbus grass. Columbus grass occurs naturally in the extreme southern United States and it is likely that cooler temperatures used in these trials affected the plant quality or synthesis of photosynthate needed for SCA nutrition. Even at 15 °C, SCA did not have as long a life span as those on sorghum or Johnsongrass. SCA exposed to 20 °C had a longevity similar to those at 15 °C, but fecundity was greater. Above 20 °C, aphid longevity and fecundity decreased, and when comparing these variables on Columbus grass to sorghum and Johnsongrass, the results were lower.

Temperature determines the physiological state of insects and consequently is the key variable that regulates survival, fecundity, and population density. Although insects are not exposed to constant temperatures in the environment, this type of study offers valuable insights into population dynamics of a species and shows that SCA does not survive in northern areas of the sorghum belt even in the presence of Johnsongrass. Information on how temperature influences the life cycle of an insect pest is essential to understanding its biology and developing IPM strategies (Diaz and Fereres, 2005; Ozder and Saglam, 2013). In the absence of sorghum, scouting should be conducted on Johnsongrass in the spring to limit population build up and further dispersal to sorghum by the SCA.

Figure 1. Generalized plot showing the point when ice formed and the latent heat of crystallization was released.

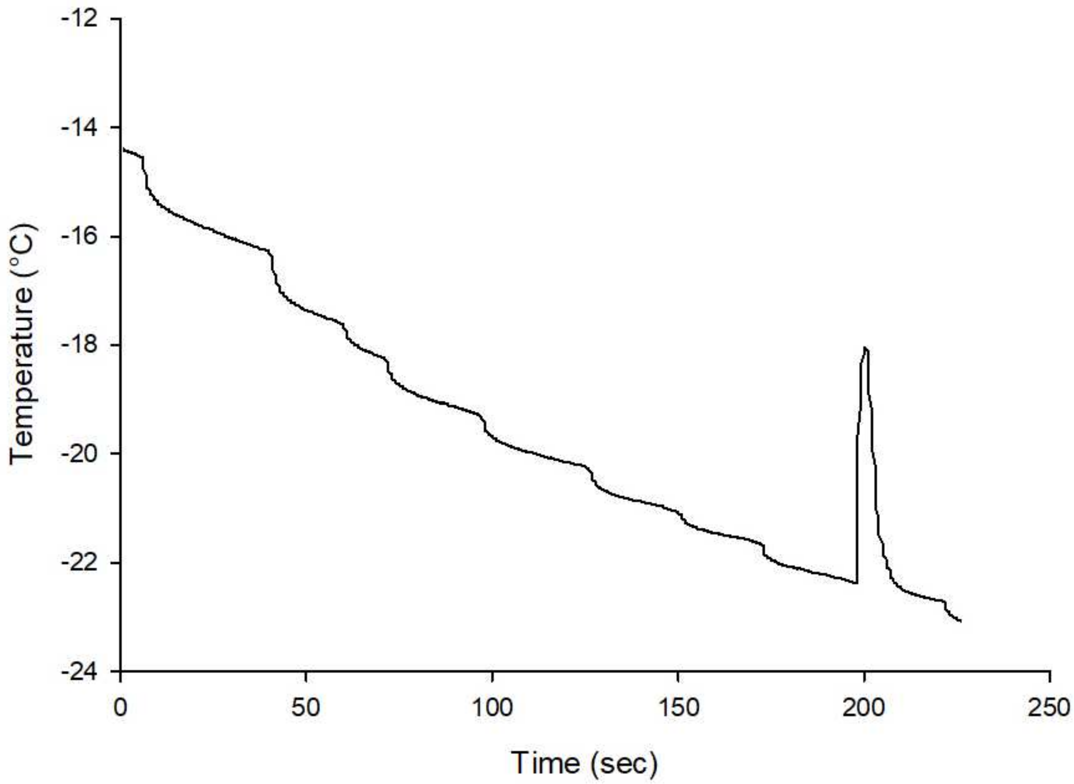

Figure 2. Longevity (Mean ± 1 S.E.) of sugarcane aphid on three different grasses across 15-25 ºC). Longevity was significantly reduced on Columbus Grass (Kruskal-Wallis ANOVA, $P < 0.05$).

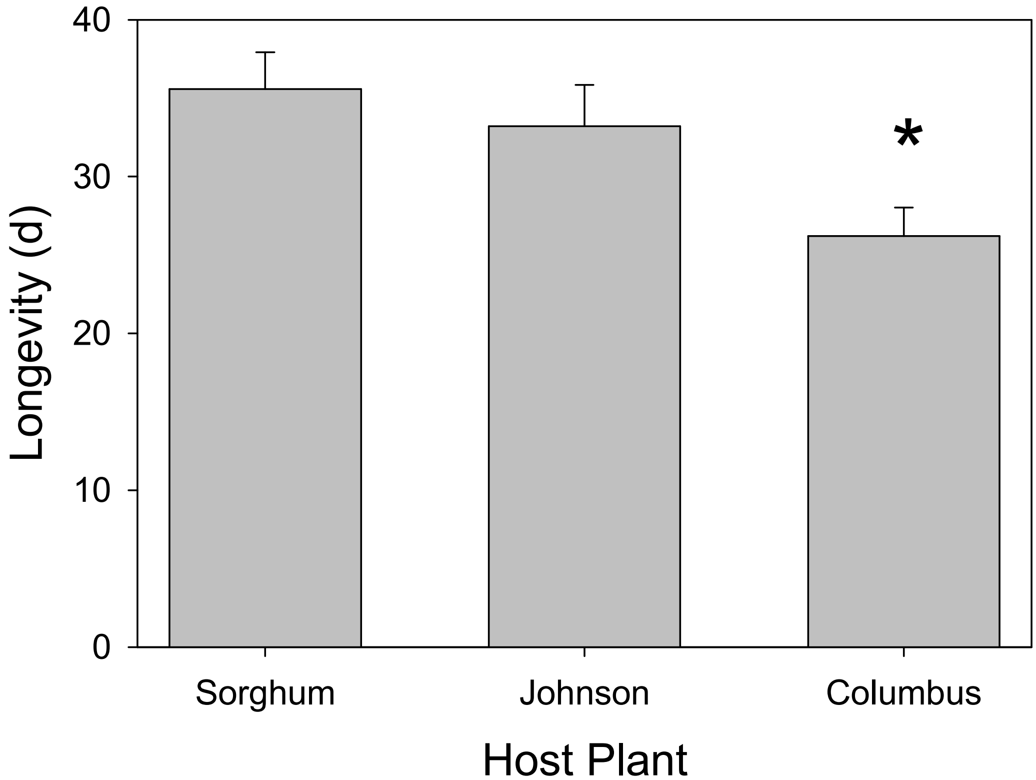

Figure 3 Reproduction (Mean ± 1 S.E.) of sugarcane aphid on three different grasses across 15-30 ^{o}C). Reproduction was significantly reduced on Columbus Grass and JohnsonGrass (Kruskal-Wallis ANOVA, $P < 0.05$).

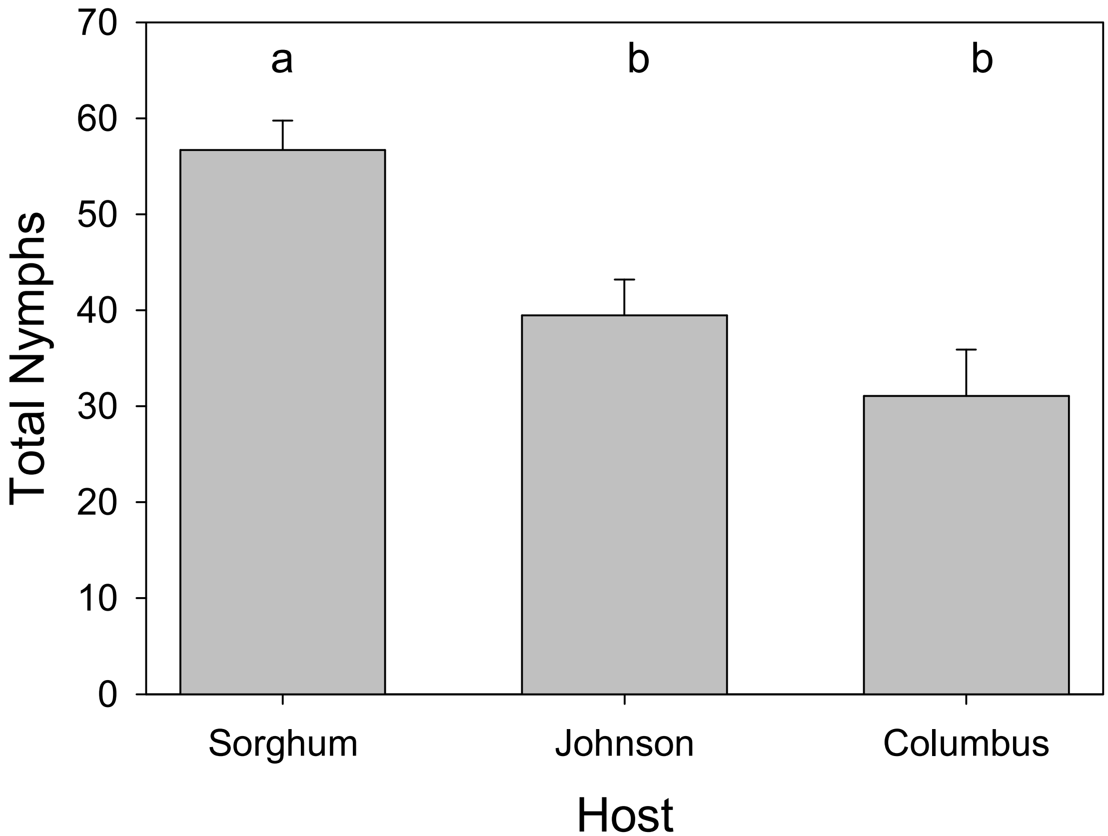

Table 1. Mean (± S.E.) for supercooling points for three different age classes of sugarcane aphids (N = 20 per trial).

Sugarcane aphid age class	Supercooling point (± S.E.)
Winged Adults	-23.7 ± 0.41b
24-48h nymphs	-25.8 ± 0.24 c
Older than 48h nymphs	-22.2 ± 0.41 a

Overall model df 2, 57; F = 25.24, P>F = <0.0001.

Means followed by the same letters are not significantly different; LSMeans P<0.05.

Table 2. Coldest reported SCPs for adults of aphid species.

Species	SCP (°C)	Reference
Aphis glycenes Matsumura	-24.9	McCornack et al. 2005
Megoura crassicauda Mordvilko	-24.5	Asai et al. 2002
Myzus persicae (Sulzer)	-24.2	Bale et al. 1988
Sitobion avenae (F.)	-24.2	Knight et al. 1986
Acyrthosiphon pisum (Harris)	-23.7	Asai et al. 2002
Aphis fabae Scopoli	-23.6	O'Doherty 1986
Elatobium abietinum (Walker)	-15.7	Powell 1974

Table 3. Mean (± 1 S.E.) longevity and number of offspring produced for sugarcane aphids at different experimental temperatures in Sorghum.

Temperature (°C)	Longevity (d)	Fecundity	Nymphs/ day	Rp	d	md	rm
5	8.8 ± 1.41 a	-	-	-	-	-	-
10	31.2 ± 2.9 b	3.66 ± 2.66 a	0.08 ± 0.05 a	5.00 ± 4.00 a	33.3 ± 1.33 a	3.66 ± 2.66 a	-
15	47.4 ± 4.64 c	58.1 ± 7.24 b	1.2 ± 0.10 b	26.1 ± 2.46 b	14.5 ± 1.38 b	34.8 ± 6.94 b	0.15 ± 0.01 a
20	30.5 ± 1.91 b	61.5 ± 4.47 b	2.03 ± 0.13 c	20.6 ± 1.57 bc	7.3 ± 0.44 c	29.8 ± 3.94 b	0.33 ± 0.00 b
25	28.8 ± 2.21 b	67.3 ± 4.84 b	2.38 ± 0.16 c	18.8 ± 2.48 c	6.0 ± 0.33 cd	27.3 ± 4.15 b	0.40 ± 0.02 c
30	16.9 ± 1.85 d	39.8 ± 4.69 c	2.35 ± 0.42 c	10.8 ± 1.59 a	5.1 ± 0.10 d	23.5± 1.64 b	0.45 ± 0.01 d
35	8.7 ± 1.15 a	-	-	-	-	-	-

Means followed by the same letters are not significantly different; LSMeans $P<0.05$.

Longevity = df= 4, 36, F = 15.39 P > F = 0.0001

Fecundity = df= 4, 29.8, F= 11.67 P > F = 0.0001

Nymphs per day = df= 4, 38, F= 28.45 P > F = 0.0001

Rp (Reproductive period) = df 4, 38, F= 10.13 P > F = 0.0001

d (Pre-reproductive period) = df= 4, 38, F= 103.38 P > F = 0.0001

md (Number of progeny produced in a reproductive period equal to the pre-reproductive period) = df= 4, 38, F= 3.06 P > F = 0.0278

rm (Intrinsic rate of increase) = df= 3, 36, F= 62.71 P > F = 0.0001

Table 4. Mean (± 1 S.E.) longevity and number of offspring produced for sugarcane aphids at different experimental temperatures in Johnson grass.

Temperature (°C)	Longevity (d)	Fecundity	Nymphs/day	Rp	d	md	rm
5	3.90 ± 1.08 a	-	-	-	-	-	-
10	36.8 ± 7.42 bc	8.2 ± 2.46 a	0.14 ± 0.04 a	12.0 ± 3.05 a	39.4 ± 1.43 a	8.2 ± 2.46 a	0.04 ±0.01 a
15	44.3 ± 4.34 c	39.5 ± 5.35 b	0.90 ± 0.10 ab	24.7 ± 3.27 b	14.9 ± 0.67 b	25.6 ± 4.11 b	0.16 ± 0.01 b
20	31.6 ± 4.18 bd	40.5 ± 9.65 b	1.20 ± 0.25 b	14.7 ± 2.69 a	11.7 ±0.88 c	21.9 ± 4.44 b	0.20 ± 0.03 b
25	23.7 ± 2.56 de	48.3 ± 7.11 b	2.22 ± 0.36 c	15.0 ± 2.06 a	6.8 ± 0.49 d	25.8 ± 4.30 b	0.36 ± 0.04 c
30	14.6 ± 1.36 e	32.7 ± 6.95 b	2.00 ± 0.35 c	7.4 ± .088 a	5.4 ± 0.29 d	23.8 ± 3.71 b	0.42 ± 0.04 c
35	4.2 ± 1.16 a	-	-	-	-	-	-

Means followed by the same letters are not significantly different; LSMeans $P<0.05$.

Longevity: df= 6, 54 F= 17.81 P>F= 0.0001

Fecundity: df= 4, 30.4 F= 4.79 P>F= 0.0041

Nymphs/day: df= 4, 30.4 F= 11.00 P>F= 0.0001

Rp (Reproductive period) : df= 4, 39 F= 6.54 P>F= 0.0004

d (Pre-reproductive period): df= 4, 32.1 F= 245.97 P>F= 0.0001

md (Number of progeny produced in a reproductive period equal to the pre-reproductive period): df= 4, 30.3 F= 2.93 P>F= 0.0371

rm (Intrinsic rate of increase): df= 4, 29.9 F= 22.97 P>F= 0.0001

Table 5. Mean (± 1 S.E.) longevity and number of offspring produced for sugarcane aphids at different experimental temperatures in Columbus grass.

Temperature (°C)	Longevity (d)	Fecundity	Nymphs/day	Rp	d	md	rm
5	3.1 ± 0.18 a	-	-	-	-	-	-
10	13.9 ± 2.84 bc	-	-	-	-	-	-
15	27.0 ± 3.83 d	8.5 ± 4.18 a	0.33 ± 0.14 a	11.0 ± 1.96 ac	21.3 ± 0.95 a	12.1 ± 5.48 a	0.07 ± 0.05 a
20	31.4 ± 2.05 d	62.9 ± 7.41 b	2.00 ± 0.20 b	18.7 ± 1.61 b	10.5 ± 0.80 b	34.3 ± 4.64 b	0.25 ± 0.04 b
25	20.2 ± 2.41 b	36.1 ± 10.42 c	1.74 ± 0.45 b	12.2 ± 1.70 a	7.55 ± 0.84 c	21.5 ± 4.56 ab	0.33 ± 0.04 b
30	13.4 ± 1.58 c	16.8 ± 5.05 ac	1.20 ± 0.31 ab	6.0 ± 1.70 c	7.11 ± 0.84 c	14.1 ± 4.14 a	0.28 ± 0.04 b
35	3.7 ± 0.63 a	-	-	-	-	-	-

Means followed by the same letters are not significantly different; LSMeans $P<0.05$.

Longevity: df= 6, 63 F= 23.02 P>F= 0.0001

Fecundity: df= 3, 36 F= 11.30 P>F= 0.0001

Nymphs/day: df= 3,32 F=5.02 P>F= 0.0058

Rp (Reproductive period): df= 3, 31 F= 9.99 P>F= 0.0001

d (Pre-reproductive period): df= 3, 23.9 F= 51.71 P>F= 0.0001

md (Number of progeny produced in a reproductive period equal to the pre-reproductive period): df= 3, 31 F= 4.78 P>F= 0.0075

rm (Intrinsic rate of increase): df= 3, 31 F= 6.21 P>F= 0.0020

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CHAPTER III

NOT TOO COLD NOT TOO HOT: THE EFFECTS OF TEMPERATURE ON SUGARCANE

Sugarcane aphid (SCA), like any other insects, needs a good quality food source and adequate temperatures that provide enough heat to support metabolism and mechanism. The sugarcane aphid has shown a preference for sorghum in recent years causing substantial economic losses to sorghum growers since 2013. It was first discovered on sorghum in south Texas and gradually spread across the North American sorghum belt. Since 2013, SCA has become a perennial pest.

At present, SCA does not overwinter in states north of Texas including Oklahoma, and throughout the winter, there is no trace of these aphids in the field. However, most aphids are freeze avoidant insects, with an ability to supercool. In the present work, SCA was able to cool to -4 °F as winged adults and down to -13 °F for nymphs.

Based on laboratory studies, SCA cannot survive for more than a week if temperatures drop below 41 °F. When exposed to these constant temperatures, nymphs are not able to develop and reach the adult stage. However, when the temperature is 50 °F or greater, SCA exhibits a longer life span and some may even reproduce. Although infesting sorghum at this average temperature, SCA will not be able to increase to the point of causing economic damage to the crop. Nevertheless, sorghum growers should be aware of increasing populations because with an average temperature of 50 °F, aphids can live up to 31 days, and as temperatures rise, populations will increase. At 59 °F, SCA has its longest life span on sorghum, living for up to 47 days. In this temperature range, SCA requires about two weeks to reach the adult stage, and thereafter begins to reproduce. The total number of nymphs produced by each female at this temperature averages 58. However, the reproductive period of the aphids at this temperature is also long, with fewer nymphs produced per day.

At 68 °F, SCA has a higher reproductive rate and can reach economic thresholds quickly. At this temperature, the time the nymphs take to reach the adult stage is only 7 days. Although the life span of SCA declines to an average of 30 days, the total number of nymphs produced increases to 61 nymphs per female. The females' reproductive period is shorter, but the number of nymphs produced per day is higher. This means that at 68 °F, females take less time to start reproducing and when they start, they produce more nymphs per day. Thus, the ability to infest sorghum and increase the population is higher than at lower temperatures.

Above 59 °F, the life span of SCA decreases with increasing temperature. However, the ability of the population to increase rapidly rises with temperature. At 77 °F, the total number of nymphs produced is the greatest. This temperature seems to be optimal for population increase. The number of nymphs produced per day at this temperature is higher than at all other temperatures and the nymphs take only 6 days to reach the adult stage and begin reproduction.

At 86 °F, the life span and fecundity of SCA falls dramatically. However, nymphs take only 5 days to reach adult stages, but their reproductive period is shorter when compared to lower temperatures. However, they still have a high production of nymphs per day, which also gives them a greater capacity to reach economic thresholds.

As with most species of aphids, SCA is sensitive to high temperatures and at 95 °F, they survive for an average of 8 days. However, nymphs do not reach the adult stage and therefore do not reproduce at temperature at this temperature. Sorghum growers can expect aphid populations to decline when average temperatures for a period of time are in the range of 95 °F.

In the absence of sorghum in the field, SCA may use alternate hosts such as Johnsongrass or Columbus grass. Although these grasses exhibit some similarities with sorghum, the performance of SCA on both is different.

In general, Johnsongrass is a good alternate host. It provides the nutrients needed for the aphids to survive and maintain their populations. However, when comparing it to sorghum, SCA does not have similar population increases. SCA did not survive for long and could not reproduce at temperatures of 41°F and 95 °F on Johnsongrass.

SCA on Johnson grass showed a greater longevity at 59 °F (44 days) as well as those on sorghum, but fecundity was lower and the number of nymphs per day / female was less than one. Fecundity was higher at 77 °F, producing an average of 44 nymphs; the number of nymphs per day / female was also similar to sorghum (2.22). At 86 °F, SCA had shorter periods to reach the adult stage, shorter life span, and lower total nymphs than in lower temperatures, but high reproductive rate, which indicates an ability to infest other plants quickly.

Johnsongrass appears to be a good alternate host in the absence of sorghum. On the other hand, Columbus grass while also an alternate host does not appear to support SCA population growth. Perhaps because of nutritional deficiency, SCA has lower longevity and fecundity on Columbus grass when compared to sorghum and Johnsongrass. On Columbus grass, the aphids did not survive for long or reproduce at temperatures of 41, 50 and 95 °F. Longevity, which was around 30 days on both sorghum and Johnsongrass at 50 °F, was only 14 days on Columbus grass.

At 59 °F, aphids survived for 27 days but reproduction was significantly reduced. For Columbus grass, the best temperature for longevity and reproduction was 68 °F. Aphid longevity peaked at 31 days and fecundity was a maximum of 63 nymphs / female. However, even with this improvement in temperature, when compared to sorghum and Johnsongrass, the aphids on Columbus grass survived less, reproduced less, and took longer to develop. This demonstrates that Columbus grass does not represent a preferred host for SCA populations to reach high numbers.

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